Periodontitis-induced Systemic Multi-organ Aging Mediated by the NF-κB Signalling Pathway

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Objective: *To investigate the causal role of periodontitis in inducing distal-organ aging and elucidate the mechanism between periodontitis and multi-organ aging by assessing the role of NF-κB signalling in mediating cellular senescence.*

Methods: *A ligature-induced periodontitis mouse model was established to systematically examine the expression profiles of senescence markers, including senescence-associated secretory phenotype (SASP) regulatory genes (Il1b, Il6, Mmp13), telomere length, and cellular senescence markers (p16^INK4a, p21^CIP1/WAF1 and β-galactosidase) in peripheral blood, bone marrow, aorta, heart, thymus and uterus. To further clarify the mediating role of the NF-κB signalling pathway in periodontitis-induced multi-organ aging, an intervention study was conducted using the specific NF-κB inhibitor BAY 11-7082.*

Results: *The results revealed significantly elevated expression of SASP-related genes in the peripheral blood of periodontitis mice compared to controls (P < 0.01). Various classic molecular hallmarks of aging, such as upregulated expression of SASP genes, shortened telomeres and increased p16 expression, were also observed in multiple distal organs/tissues. Further mechanistic studies revealed that selective inhibition of the NF-κB signalling pathway significantly attenuated the expression of these aging phenotypic markers (P < 0.01), indicating that this pathway plays a critical role in mediating periodontitis-induced systemic aging.*

Conclusion: *This study provides direct experimental evidence that periodontitis can promote systemic multi-organ aging via activation of the NF-κB signalling pathway, establishing a theoretical basis for developing therapeutic interventions.*

Keywords: *biological aging, NF-κB signalling pathway, periodontitis, senescence-associated secretory phenotype (SASP)*

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Periodontitis is a prevalent chronic inflammatory disease, the incidence of which increases with age, thus posing a major global health care challenge in the current era of rapidly aging populations worldwide.[1,2] As a typical example of oral-systemic disease association, periodontitis not only leads to the destruction of periodontal supporting tissues but is also associated with the progression of biological aging. Aging is considered a complex process characterised by systemic multi-organ functional decline, manifested as decreased cellular function, accumulation of molecular damage and increased inflammation.[3-6] During cellular senescence, telomere shortening restricts the replicative potential of cells,[7-9] while elevated β-galactosidase (GLB1) activity and increased expression of p16 INK4a (p16) and p21 CIP1/WAF1(p21) result in proliferative arrest, which are key molecular features of senescence.[10-12] Senescent cells also secrete a distinct senescence-associated secretory phenotype (SASP), including various cytokines, chemokines, proteases and other signalling molecules, which contribute to a self-perpetuating

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cycle of inflammation, thereby accelerating the aging process.[13-15]

Periodontal tissues from patients with periodontitis exhibit elevated levels of senescence-associated markers. These senescent cells can secrete various pro-inflammatory cytokines (e.g., IL-6 and IL-8) into the bloodstream, leading to significantly increased levels of SASP factors in peripheral circulation.[16] During the pathological progression of periodontitis, activation of the NF-κB signalling pathway in periodontal ligament stem cells promotes the secretion of classical SASP-associated proinflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) and matrix metalloproteinase-1 (MMP-1).[12,17,18] These cytokines not only exacerbate local inflammation but may also enter the systemic circulation to trigger inflammatory cascades in distal organs via NF-κB signalling, ultimately causing tissue and organ functional decline.[19-21] Activation of NF-κB also leads to increased expression of the senescence markers p53 and p21 in the nuclei of periodontal ligament stem cells. As key components of the SASP, TNF-α, IL-6, IL-1β and MMP-1 are persistently expressed and can activate inflammatory signalling pathways such as NF-κB, p38 and MAPK. This reaction creates a positive feedback loop that further amplifies the highly organised inflammatory response.[20,21] Both clinical and animal studies have reported increased levels of senescence markers and inflammatory mediators within periodontal tissues along with the presence of senescent cells, SASP and key senescence markers (p16, GLB1),[22,23] as well as elevated systemic inflammatory markers and oxidative stress.[3,24] In a pro-inflammatory microenvironment, both the composition and function of immune cell populations undergo significant senescence-like alterations. Increased oxidative stress and leucocyte mitotic activity can lead to accelerated telomere shortening and replicative senescence of immune cells. Previous studies have shown that patients with periodontitis exhibit significantly shortened telomere length in leucocytes, along with impaired neutrophil chemotaxis, phagocytic activity and bactericidal capacity. Periodontitis may thus induce systemic immunosenescence by disrupting the initiation and maintenance of both innate and adaptive immune responses.[25,26]

However, most published studies to date have focused only on the local effects of periodontitis in the oral cavity, and there is a lack of systematic elucidation of its impact on distal-organ aging and the underlying molecular mechanisms involved. To address this critical gap within the scientific literature, we established a ligature-induced periodontitis mouse model and comprehensively examined the expression profiles of SASP regulatory genes, inflammatory mediators, telomere length and senescence markers across multiple distal organs and tissues, including peripheral blood, bone marrow, aorta, heart, thymus and uterus. This study thus focused on the role of the NF-κB signalling axis in linking periodontitis with multi-organ aging, aiming to delineate the molecular mechanisms by which periodontitis accelerates aging. This study offers new insight into the oral-systemic connection from the perspective of aging biology. The findings may help establish a theoretical framework for targeting periodontitis-associated aging through modulation of inflammatory signalling pathways, thereby providing a theoretical basis for developing targeted interventions, and contributing to the development of novel therapeutic strategies in both dental and systemic age-related diseases.

Materials and methods

Animal models

All animal experimental procedures were approved by the Ethics Committee of Peking University (ethics approval number LA2023304). Female C57BL/6J mice (8 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology. After one week of acclimatisation, mice were randomly divided into two groups: control and periodontitis. In the periodontitis group, mice were anaesthetised with 1% (w/v) pentobarbital sodium and placed in a supine position. A 5-0 non-absorbable silk ligature was tied around the cervical region of the bilateral maxillary second molars at the gingival margin to induce chronic periodontitis, which was maintained for 14 days.[27] In the control group, mice underwent sham procedures under anaesthesia. After 14 days, bilateral maxillae were dissected, fixed in 4% (w/v) paraformaldehyde for 24 hours, and scanned using a micro-computed tomography (microCT) system to confirm successful establishment of the periodontitis model. Mice were monitored throughout the 1-month experimental period, and ligatures were replaced whenever needed to maintain sustained inflammation. To inhibit the NF-κB signalling pathway, mice in both the control and periodontitis groups were further divided into two groups after 14 days of modelling. The treatment groups received intraperitoneal injections of the NF-κB inhibitor Bay 11-7082 dissolved in saline (15 mg/kg; S1523-2 mg, Beyotime, Shanghai, China) every other day for 2 weeks.[28] Control mice were administered equivalent volumes of physiological saline.

Sample collection

One month after ligature placement, mice from both the control and periodontitis groups were anaesthetised with 1% (w/v) pentobarbital sodium, and blood was collected via enucleation using ethylenediaminetetraacetic acid (EDTA)-coated tubes. The mice were then euthanised and major organs were harvested, including the maxilla, brain, heart, liver, kidneys, spleen, thymus, uterus, bone marrow and aorta. Samples were stored according to experimental requirements for subsequent analyses.

Starting from day 14 of periodontitis modelling, both the NF-κB inhibitor and vehicle control groups received intraperitoneal injections for 14 consecutive days. After treatment, blood and multiple organ samples were collected as described above for molecular and histological analyses.

MicroCT scanning and analysis

After euthanasia, bilateral maxillae were fixed in 4% (w/v) paraformaldehyde for 24 hours and scanned with microCT. The scanning parameters were voltage 80 kV, current 500 μA, spatial resolution 6 μm, exposure time 1500 ms, rotation step 0.4 degrees and scan angle 360 degrees. Images were reconstructed using a filtered back projection algorithm and processed with NRecon software (Bruker microCT, version 1.7.4.2; Bruker, Billerica, MA, USA).

Ribonucleic acid (RNA) isolation and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Peripheral blood and homogenised tissues were lysed with 1 ml TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), mixed with 200 μl anhydrous chloroform and centrifuged, then ribonucleic acid (RNA) precipitation was performed using isopropanol. The RNA pellet was washed with 75% (v/v) ethanol, dried and resuspended in 20-50 μl DEPC-treated water. RNA concentration was measured with NanoDrop 8000 (Thermo Fisher Scientific). 1 mg RNA was reverse transcribed into cDNA using a commercial kit (TaKaRa, Shiga, Japan) according to the manufacturer's instructions.

The expression of SASP-regulated genes was entrusted to Wcgene Biotech (Shanghai, China) for testing, using a mouse SASP polymerase chain reaction (PCR) array (Wcgene Biotech). Other RT-qPCR assays were performed using SYBR Green Master Mix (Roche, Grenzach-Wyhlen, Germany) with specific primers on a real-time PCR system (Thermo Fisher Scientific). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilised as an internal housekeeping control. The volume of the reaction system was 20 μl. The PCR conditions were 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Relative gene expression was calculated using the ΔΔCt method.

RT-qPCR assay was used to determine telomere length. An 84-bp synthetic DNA oligonucleotide consisting of 14 repeats of the TTAGGG telomeric sequence was used as the telomere standard. In parallel, a single-copy gene standard was prepared using an oligonucleotide corresponding to mouse 36B4, which encodes the acidic ribosomal phosphoprotein P0. Standard curves were established for telomere and 36B4 amplification. The reaction system and PCR condition were the same as described above.[29]

Tissue embedding, sectioning and immunofluorescence

After 1 month, tissues were collected and fixed in 4% (w/v) paraformaldehyde for 24 hours, dehydrated in 75% (v/v) ethanol and paraffin embedded. Sections (5 μm thick) were cut using a microtome (Leica, Wetzlar, Germany) and mounted on slides. Immunofluorescence was performed using a NEON TSA 5-color kit (Histovabio, Beijing, China). Slides were dewaxed, rehydrated and subjected to antigen retrieval. After 30 minutes of blocking at room temperature, various primary antibodies including anti-GLB1/beta-galactosidase rabbit polyclonal antibody (Histovabio, [HA500021]; 1:100), anti-p16 ARC recombinant rabbit monoclonal antibody (Histovabio, [SR34-02]; 1:100), and anti-p21 rabbit monoclonal antibody (Abcam, Cambridge, UK [EPR18021]; 1:200) were added and incubated overnight at 4°C. The following day, the slides were incubated with PolyHRP-conjugated secondary antibodies and fluorophores, followed by nuclear staining with DAPI for 10 minutes. Coverslips were then affixed. Fluorescence signals were visualised using a digital scanner (Pannoramic SCAN II, 3DHISTECH, Budapest, Hungary) and quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Protein extraction and western blotting

Tissues were lysed in RIPA buffer with 1% (v/v) protease inhibitors (Solarbio, Beijing, China), homogenised with an ultrasonic disruptor (Yiheng Instruments, Shanghai, China), and placed on ice for 30 minutes. The supernatants were then collected by centrifugation, and protein concentrations were measured using a BCA assay kit (Thermo Fisher Scientific). The protein samples were

diluted to a final concentration of 1-2 μg/μl.

Appropriate quantities of protein samples were mixed with 6× SDS-PAGE loading buffer (Solarbio) and denatured by boiling at 100°C for 5 minutes. SDS-PAGE gels consisting of 10% to 12% resolving gel and 5% stacking gel were prepared. The denatured protein samples and pre-stained protein molecular weight markers (Thermo Fisher Scientific, USA) were loaded into each well. Electrophoresis was performed at 80 V through the stacking gel, followed by separation at 120 V through the resolving gel until the target proteins were adequately resolved. Proteins were then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Darmstadt, Germany).

The blotted membranes were then blocked in 5% (w/v) skimmed milk for 1 hour at room temperature, and then incubated at 4°C overnight with primary antibodies against NF-kB p65 (recombinant rabbit monoclonal antibody [SZ10-04; 1:1000]; HuaBio, Woburn, MA, USA) and Phospho-NF-kB p65 (S536) (recombinant rabbit monoclonal antibody [PSH10-58; 1:1000]; Huabio). After washing, the membranes were incubated with HRP-conjugated secondary antibodies (1:5000) at room temperature for 1 hour, followed by incubation with enhanced chemiluminescence (ECL) substrate solution (eECL, Thermo Fisher Scientific) for 30 seconds. Signal detection and imaging were carried out with the ChemiDoc MP system (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Data analysis was conducted using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Normality was assessed using a Shapiro-Wilk test with Lilliefors correction. For pairwise comparisons among the four independent groups with or without NF-κB inhibition, unpaired *t* tests were used for normally distributed data with equal variance; otherwise, a Mann-Whitney U test was applied. A two-sided *P* value < 0.05 was considered statistically significant.

Results

Establishment of periodontitis mouse model and multi-organ sample collection

Two weeks after ligature placement, the mice were euthanised and bilateral maxillae were collected to confirm the successful establishment of a periodontitis model (Fig 1a). As shown in the microCT images, mice in the periodontitis group exhibited significantly reduced alveolar bone height compared to the controls. Bone resorption reached the middle third of the roots of the second molars, and displacement of the first molars was observed, thus confirming the successful establishment of the murine periodontitis model (Fig 1b). At 1 month after establishment of the periodontitis model, multi-organ samples were collected from both the periodontitis and control groups, including brain, heart, aorta, thymus, liver, kidney, spleen, uterus, bone marrow and peripheral blood. Similarly, after 14 days of intraperitoneal treatment with the NF-κB inhibitor, samples from both the treated and vehicle control mice were collected and stored for further analysis (Fig 1a).

Periodontitis upregulates SASP gene expression in peripheral blood

To investigate the impact of periodontitis on SASP in distal organs, RT-qPCR was used to examine the expression of 90 known SASP regulatory genes in peripheral blood. The gene expression profile of SASP was significantly altered in the periodontitis group (Fig 2). Compared to controls, seven SASP factors were significantly upregulated ($P < 0.05$), while one gene, namely plasminogen activator inhibitor-1 (PAI-1), was significantly downregulated ($P < 0.05$). The upregulated genes mainly encode pro-inflammatory cytokines including IL-6, IL-1β, MMP-13 and TNF, which play key roles in the inflammatory and tissue remodelling responses. These results suggest that periodontitis may drive systemic inflammation and disrupt tissue homeostasis by specifically perturbing SASP gene expression.

Periodontitis induces senescence-like phenotypes in multiple organs

Based on the detection of SASP factors in peripheral blood and previous research findings, the present authors selected key SASP regulatory genes strongly associated with periodontitis such as *Il6*, *Il1b* and *Mmp13*,[23,30-32] and assessed their expression across multiple organs using RT-qPCR. In addition, telomere length, a key marker of cellular senescence, was evaluated, and the expression levels of established senescence markers, including p16, p21 and GLB1, were examined. Senescence phenotypes were observed in the heart, aorta, thymus, bone marrow and uterus, but not in the brain, liver, kidney or spleen.

Firstly, the present authors examined the presence of senescence phenotype in the cardiovascular system. In the heart, *Il1b* expression was significantly increased to 21.22-fold higher than the control ($P = 0.03$; Fig 3a), while there were no significant changes in *Il6* and

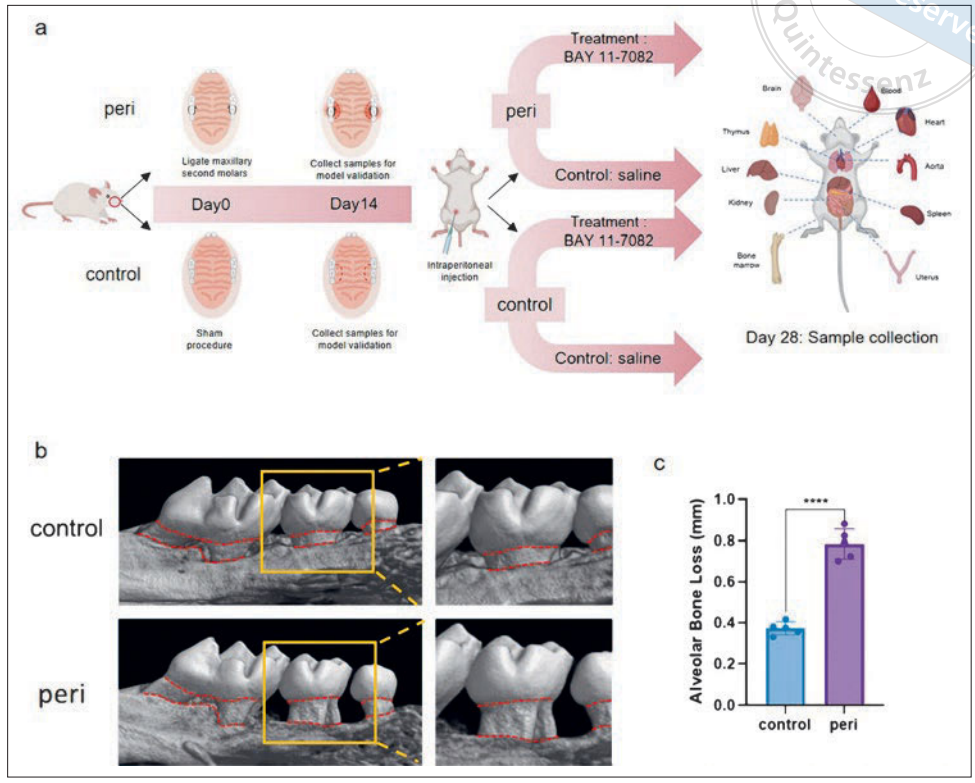

Fig 1a to c Establishment of the periodontitis mouse model and multi-organ sample collection. Schematic representation and timeline of the experimental design and sample collection **(a)**. MicroCT results of maxillary bone in the control (n = 5) and periodontitis mice (n = 5), showing the third, second and first molars from left to right. Red dashed lines indicate the positions of the cemento-enamel junction (CEJ) and alveolar bone crest (ABC), respectively. The vertical distance between the CEJ and ABC represents alveolar bone loss **(b)**. Comparison of alveolar bone loss height between the periodontitis and control groups **(c)**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. ns, not significant. Error bars represent the standard deviation (SD).

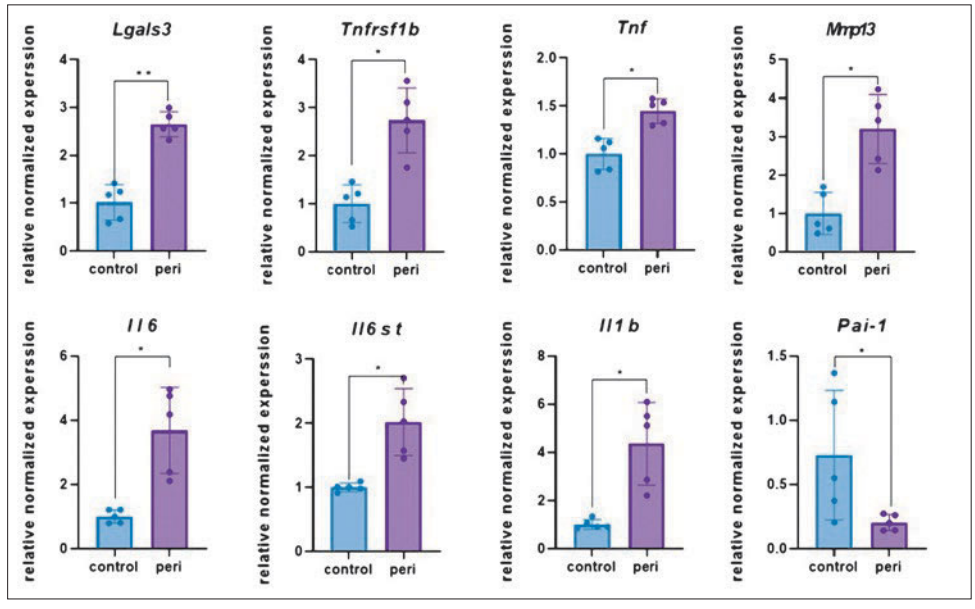

Fig 2 Upregulation of SASP regulatory genes in the peripheral blood of periodontitis mice. Comparison of SASP gene expression profiles between the periodontitis (n = 5) and control (n = 5) groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. ns, not significant. Error bars represent the standard deviation (SD).

Mmp13 expression. Telomere lengths also displayed no significant difference between the periodontitis and control groups (Fig 3b). The immunofluorescence analysis showed that p16 protein expression was significantly increased, whereas p21 and GLB1 expression levels remained unchanged (Fig 3c and d). Similar to the result observed in heart tissue, *Il1b* expression in the aorta was also significantly upregulated (Fig 4a), while the telomere length was significantly shortened (Fig 4b). A significant increase in p16 expression was also observed (Fig 4c and d), but no significant changes in p21 or GLB1expression.

In addition to the cardiovascular system, significant age-related changes were also observed in the immune system (thymus and bone marrow). In the thymus of periodontitis mice, *Il1b* expression was significantly

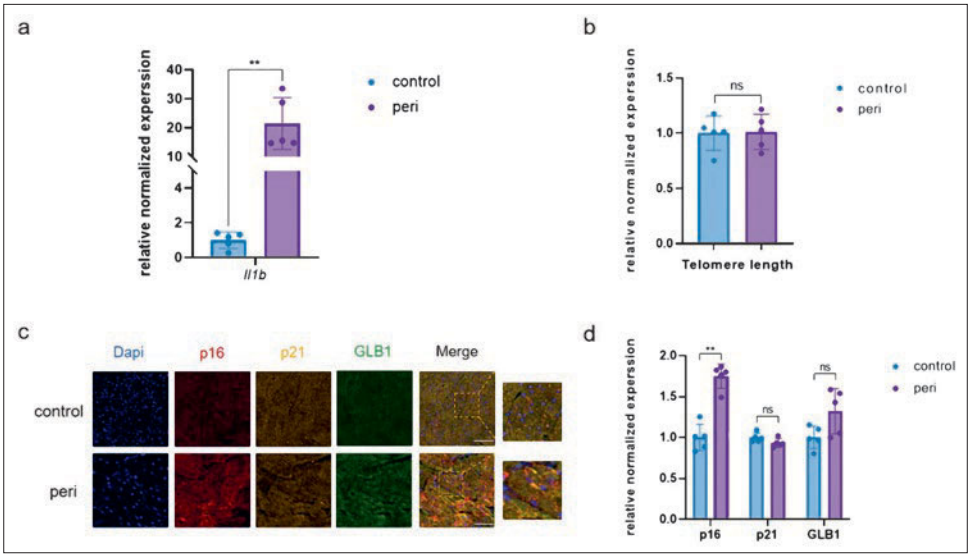

Fig 3a to d Senescence-associated phenotypes observed in the heart of periodontitis mice. RT-qPCR analysis of *Il1b* expression in heart tissue (n = 5) **(a)**. Relative telomere lengths assessed by RT-qPCR (n = 5) **(b)**. Immunofluorescence staining of senescence markers p16, p21 and GLB1in heart tissue (n = 5) **(c)**. Quantitative analysis of fluorescence signal intensities using ImageJ software **(d)**. Scale bar 50 μm. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

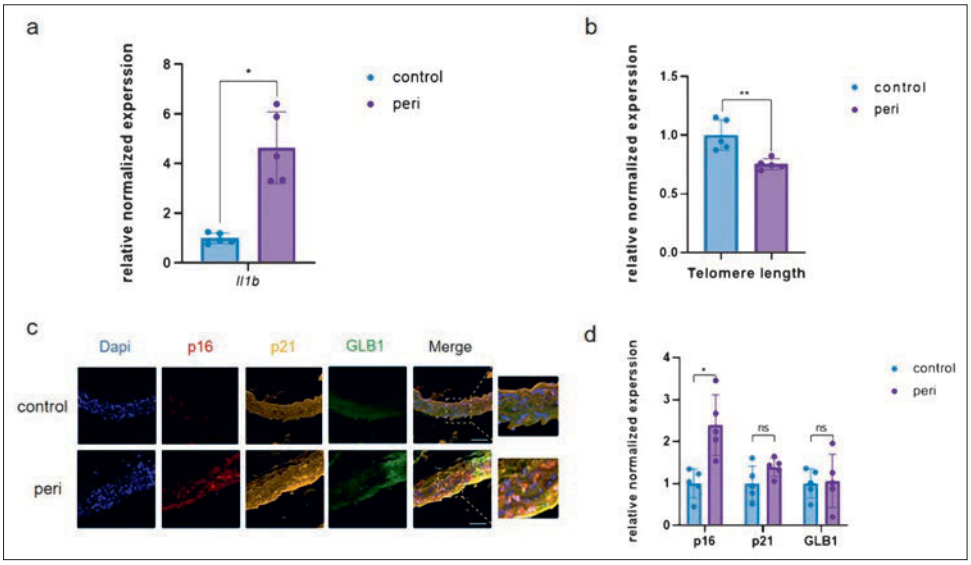

Fig 4a to d Aging-related phenotypes observed in the aorta of periodontitis mice. RT-qPCR analysis of *Il1b* expression (n = 5) **(a)**. Relative telomere lengths assessed by RT-qPCR (n = 5) **(b)**. Immunofluorescence staining of senescence markers p16, p21 and GLB1in aorta tissue (n = 5) **(c)**. Quantitative analysis of fluorescence signal intensities using ImageJ software **(d)**. Scale bar 50 μm. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

upregulated (Fig 5a), while there were no significant changes in the expression of other SASP regulatory genes (*Il6* and *Mmp13*). Notably, the telomere length in thymic cells of the periodontitis group was significantly shorter, reaching only 0.59-fold of that in the control group ($P = 0.025$; Fig 5b). Immunofluorescence analysis further revealed that p16 expression was significantly elevated in the thymic tissue of periodontitis mice (Fig 5c and d), whereas the expression levels of p21 and GLB1 did not differ significantly with respect to the controls.

Within the bone marrow, expression of both *Il1b* and *Mmp13* was significantly increased in periodontitis mice (Fig 6a), while other SASP-related genes displayed no significant changes. Similar to the thymus, bone marrow cells in the periodontitis group exhibited significantly shortened telomeres (Fig 6b). Additionally, immunofluorescence staining indicated that p16 expression was significantly upregulated in the bone marrow of periodontitis mice (Fig 6c and d), whereas p21 and GLB1 expression levels remained comparable to those in the control group.

With regard to the reproductive system, the present authors focused on examining age-related changes in uterine tissue. Within the uterus, *Il6IL-6* and *Mmp13* expression levels in the periodontitis group were significantly increased (4.8-fold and 3.88-fold, respectively; Fig 7a), telomeres were shortened by 0.63-fold ($P = 0.017$; Fig 7b), and p16 was upregulated (3.01-fold, $P = 0.001$; Fig 7c and d), as compared to the

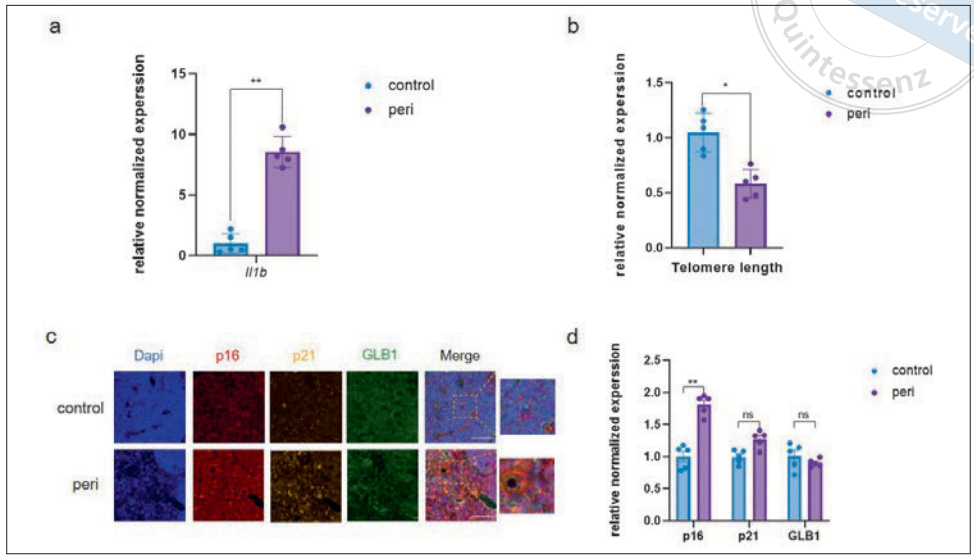

Fig 5a to d Aging-related phenotypes observed in the thymus of periodontitis mice. RT-qPCR detection of *Il1b* expression (n = 5) **(a)**. Relative telomere lengths assessed by RT-qPCR (n = 5) **(b)**. Immunofluorescence staining of senescence markers p16, p21 and GLB1in aorta tissue (n = 5) **(c)**. Quantification of fluorescence intensity using ImageJ **(d)**. Scale bar 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

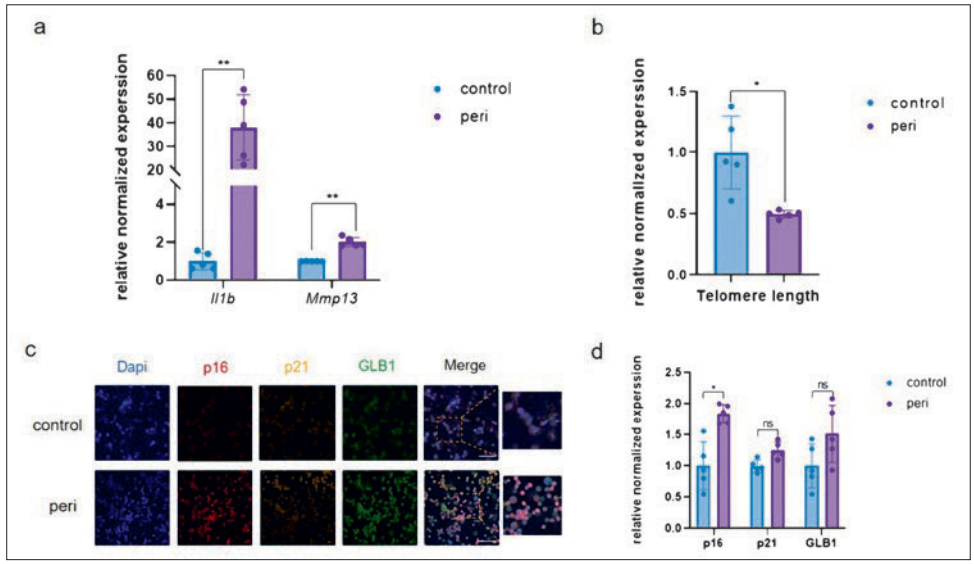

Fig 6a to d Aging-related phenotypes observed in the bone marrow of periodontitis mice. RT-qPCR detection of *Il1b* and *Mmp13* expression (n = 5) **(a)**. Relative telomere lengths assessed by RT-qPCR (n = 5) **(b)**. Immunofluorescence staining of senescence markers p16, p21 and GLB1 in aorta tissue (n = 5) **(c)**. Quantitative analysis of fluorescence signal intensities using ImageJ software **(d)**. Scale bar 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

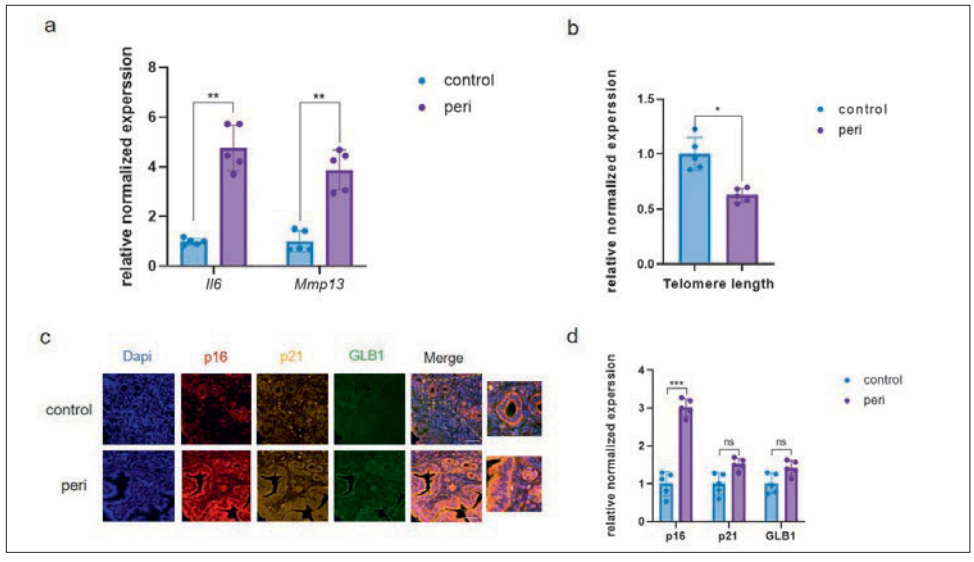

Fig 7a to d Aging-related phenotypes observed in the uterus of periodontitis mice. RT-qPCR detection of *Il6* and *Mmp13* expression (n = 5) **(a)**. Relative telomere lengths in uterine cells assessed by RT-qPCR (n = 5) **(b)**. Immunofluorescence staining of p16, p21 and GLB1 (n = 5) **(c)**. Quantitative analysis of fluorescence signal intensities using ImageJ software **(d)**. Scale bar 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

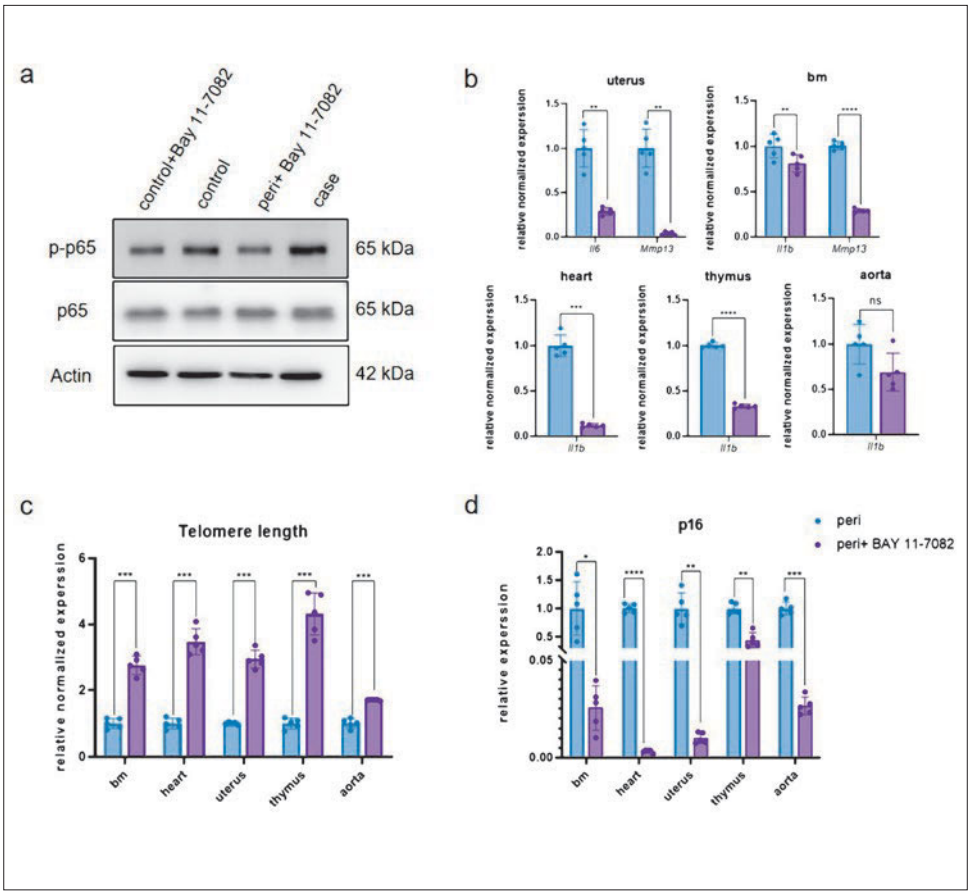

Fig 8a to d Reversal of aging phenotypes in periodontitis mice following NF-κB inhibition with Bay 11-7082. Western blot analysis showed that the phosphorylation levels of p65 (p-p65) in peripheral blood were decreased in both the control and periodontitis mice after treatment with Bay 11-7082, as compared to the untreated groups (n = 5) **(a)**. RT-qPCR analysis of SASP regulatory gene expression across multiple distal organs in periodontitis mice before and after NF-κB inhibitor treatment (n = 5) **(b)**. RT-qPCR detection of telomere repeat sequence levels in multiple distal organs of periodontitis mice before and after NF-κB inhibition (n = 5) **(c)**. RT-qPCR analysis of the senescence marker p16 in multiple distal organs of periodontitis mice before and after NF-κB inhibitor treatment (n = 5) **(d)**. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. ns, not significant. Error bars represent the standard deviation (SD).

control group. However, no significant changes were observed in p21 and GLB1expression levels.

Taken together, periodontitis not only alters the SASP profile in blood but also induces aging-like molecular features in distal organs, particularly within the cardiovascular (heart, aorta), immune (bone marrow, thymus) and reproductive (uterus) systems.

Inhibition of the NF-κB signalling pathway reverses periodontitis-induced senescence

The SASP-regulated genes (*Il6*, *Il1b*, *Mmp13*) and p16 are known to be downstream targets of the NF-κB signalling pathway and play central roles in inflammation and aging.[4,33] The present authors therefore hypothesise that periodontitis may induce distal-organ aging via activation of the NF-κB signalling pathway, based on its central role in cellular stress responses, inflammation and immune regulation.

To test this hypothesis, an intervention study was conducted using the specific NF-κB inhibitor BAY 11-7082 (Beyotime, S1523-2 mg). BAY 11-7082 inhibits IκBα phosphorylation, thereby preventing its degradation and subsequently blocking NF-κB nuclear translocation and activation.[34] To validate the effectiveness of BAY 11-7082 in suppressing the NF-κB pathway, the authors assessed the phosphorylation of p65, a key subunit of the NF-κB complex, the phosphorylation of which is considered a hallmark of NF-κB activation.[35] Western blot analysis demonstrated that after 2 weeks of BAY 11-7082 treatment, the phosphorylation level of p65 in peripheral blood was significantly reduced in the treated group (Fig 8a), confirming effective inhibition of NF-κB pathway activation.

In periodontitis mice treated with BAY 11-7082, the expression of SASP regulatory genes downstream of the NF-κB signalling pathway, including *Il6*, *Il1b* and *Mmp13*, was decreased across multiple organs compared to the untreated counterparts (Fig 8b). Moreover, the telomere length was longer in the treated group (Fig 8c), while the expression of the senescence marker p16 was also significantly downregulated following NF-κB inhibition (Fig 8d). These findings thus confirmed that the NF-κB signalling pathway plays a critical regulatory role in periodontitis-induced multi-organ aging and that pharmacological inhibition of this pathway can partially reverse the aging phenotypes associated with periodontitis.

Discussion

This study demonstrates that periodontitis induces significant upregulation of SASP-related gene expression in the peripheral blood and multiple organs of mice. Female C57BL/6J mice were chosen as model animals to avoid the inhibitory effects of androgens on NF-κB activation.[36] Notably, the expression of inflammatory mediators *Il6*, *Il1b* and *Mmp13* were elevated in peripheral blood, bone marrow, thymus, aorta, heart and uterus, which suggests that periodontitis may drive multi-organ aging by disrupting the functions of distal organs through circulatory inflammation.[37,38] It was reported that IL-6 accumulation in periodontal tissues can spill over into the bloodstream and affect haematopoiesis by promoting myeloid cell expansion and inhibiting lymphocyte function,[39] thereby contributing to immunosenescence. Likewise, IL-1β and MMP-13, which are secreted by gingival fibroblasts and osteoclasts during periodontal inflammation,[31,40,41] may activate immune cells and induce chronic low-level inflammation, ultimately disrupting tissue homeostasis and accelerating aging.[4] The present findings corroborate previous studies that highlight chronic inflammation as a key driver of aging, with SASP factors acting along the inflammation-aging axis.[42] They therefore provide direct in vivo evidence that local periodontal inflammation can induce multi-organ senescence via systemic SASP activation.

Furthermore, this study showed that periodontitis mice displayed shortened telomeres and increased p16 expression in various distal organs and tissues. When telomeres reach a critically short length, the replicative potential of cells becomes limited, leading to replicative senescence. This process activates the DNA damage response, which in turn induces the upregulation of senescence-associated factors such as p16 and p21, ultimately promoting the entry of cells into a senescent state.[12] This senescence response is regulated by two major tumour suppressors, p53 and pRB, which serve as central interacting pathways in the control of cellular aging.[43,44] Previous studies have shown that the p16 protein acts upstream of pRB to establish growth arrest, and its expression is modulated by inflammatory signals, which are closely associated with SASP signalling.[45,46] Elevated p16 levels can initiate telomere-dependent cellular senescence, inhibit cellular proliferation and further lead to irreversible cell cycle arrest within tissues.[11] In this study, the authors observed significant telomere shortening in the thymus, aorta and uterus of periodontitis mice. These findings thus suggest that the chronic inflammatory environment induced by periodontitis may accelerate telomere attrition, thereby promoting p16 upregulation and further driving the aging of target organs. However, murine telomeres are significantly longer than those in humans, and this methodological consideration may affect direct clinical translatability of these particular findings.

Organ-specific aging effects were also observed in this study. In line with previous findings, the present authors collected multiple distal organs that are functionally implicated in immunosenescence and inflammaging, including the brain, thymus, heart, aorta, liver, spleen, kidney, bone marrow and uterus, to examine age-related changes.[47] Senescence-like alterations were observed in all organs except the brain, liver, spleen and kidney. In the bone marrow, the chronic inflammatory environment induced by periodontitis may lead to a decline in haematopoietic stem cell (HSC) function. Haematopoietic stem and progenitor cells (HSPCs) exhibit increased expression of growth factors and cytokine receptors, such as those for IL-1β and IL-6,[48,49] leading to reduced self-renewal capacity, overexpression of pro-inflammatory cytokines and disruption of immune homeostasis.[50] These changes reflect senescence-like alterations in the bone marrow microenvironment, which may further compromise systemic immune balance. The present authors also observed telomere shortening and increased p16 expression in the thymus, indicating that periodontitis may promote replicative senescence in thymic tissue, thus impairing T-cell development and contributing to immune aging.[39] In the uterus, significant telomere shortening and upregulation of SASP gene expression were observed, potentially reflecting inflammation-induced structural and functional damage.[51,52] Previous studies have shown that chronic inflammatory states may lead to endothelial dysfunction, arteriosclerosis and accelerated myocardial aging in the cardiovascular system,[37] thereby enhancing NF-κB-mediated gene expression and amplifying the inflammatory senescence response.[53] In this study, the present authors further validated this mechanism through animal experiments and elucidated a potential biological pathway by which periodontal inflammation contributes to the aging of the cardiovascular system.

NF-κB signalling plays a critical regulatory role in chronic inflammation and aging. In the present study, NF-κB pathway activation was evidenced by increased p65 phosphorylation and SASP factor expression in periodontitis mice. Pharmacological inhibition with BAY 11-7082 significantly reduced these senescence phenotypes, confirming the key role of NF-κB role in

modulating periodontitis-induced multi-organ aging. BAY 11-7082 treatment effectively reduced mRNA levels of key aging markers across multiple organs. Previous studies have demonstrated that periodontitis can activate the NF-κB signalling pathway through multiple mechanisms,[54-56] and such activation can induce the expression of SASP factors, thereby establishing a positive feedback loop between inflammation and senescence.[19-21] However, there was no direct evidence confirming that periodontitis can induce biological aging via the NF-κB signalling pathway. The present findings thus provide new evidence of the NF-κB signalling pathway acting as a bridge between periodontitis and aging, further supporting its role as a key regulator of periodontitis-induced senescence. These results also suggest that the NF-κB signalling pathway may serve as a potential therapeutic target for interventions aimed at mitigating periodontitis-associated aging.

Despite these findings, some limitations remain. This study was conducted in a ligature-induced mouse model, and additional data are needed to verify whether similar aging effects occur in humans. Although BAY 11-7082 reversed aging phenotypic markers, its long-term safety and off-target effects warrant further investigation. Future studies should also explore the roles of other inflammatory pathways, such as p38 MAPK and JAK-STAT, in periodontitis-induced aging.

Periodontitis is highly prevalent in the elderly and often coexists with aging-related diseases such as cardiovascular disease, diabetes and osteoporosis. The present findings reveal that periodontitis promotes senescence-like changes in multiple organ systems, suggesting that periodontitis may act as a systemic driver of aging and related comorbidities. These findings underscore the clinical importance of periodontal health in the context of aging. Effective control of periodontitis may not only reduce the risk of systemic comorbidities but also potentially delay the overall aging process in older adults. Importantly, inhibiting the NF-κB signalling pathway significantly reduces these aging phenotypes, highlighting a potential therapeutic target. These results emphasise the clinical value of maintaining periodontal health as part of integrated strategies for healthy aging, offering significant clinical relevance and translational opportunities.

Conclusion

This study demonstrated that periodontitis can induce significant upregulation of SASP factors in peripheral blood and multiple organs, which is accompanied by telomere shortening and increased p16 expression. Activation of the NF-κB signalling pathway appears to be a key underlying mechanism. Hence, these findings enhance understanding of the link between periodontitis and biological aging and provide a theoretical basis for future therapeutic interventions targeting periodontitis-associated aging processes.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Sheng Chen LO contributed to the experiments, methodology, manuscript draft and editing; Dr Yi Fan WANG contributed to the methodology and project administration; Dr Xu Liang DENG contributed to the study conceptualisation, supervision and critical revision of the manuscript.

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