Received: 27 March 2022 | Revised: 14 May 2022 | Accepted: 12 June 2022

DOI: 10.1111/jcpe.13684

ORIGINAL ARTICLE

Journal of Clinical Periodontology WILEY

Long non-coding RNA SNHG5 mediates periodontal inflammation through the NF-κB signalling pathway

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Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 82071142, 82071119

Abstract

Aim: We investigated the role of long non-coding RNAs and small nucleolar RNA host gene 5 (SNHG5) in the pathogenesis of periodontitis.

Materials and Methods: A ligature-induced periodontitis mouse model was established, and gingival tissues were collected from patients with periodontitis and healthy controls. Inflammatory cytokines were detected using quantitative reverse transcription-polymerase chain reaction and western blotting analyses. Direct interactions between SNHG5 and p65 were detected by RNA pull-down and RNA immunoprecipitation assays. Micro-computed tomography, haematoxylin and eosin staining, and immunohistochemical staining were used to measure periodontal bone loss.

Results: SNHG5 expression was down-regulated in human and mouse periodontal tissues compared to that in the healthy controls. In vitro experiments demonstrated that SNHG5 significantly ameliorated tumour necrosis factor α-induced inflammation. Mechanistically, SNHG5 directly binds to the nuclear factor-kappa B (NF-κB) p65 subunit and inhibits its translocation, thereby suppressing the NF-κB signalling pathway activation and reducing the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing three inflammasome expression. Locally injecting si-SNHG5 aggravated the periodontal destruction.

Conclusion: This study revealed that SNHG5 mediates periodontal inflammation through the NF-κB signalling pathway, providing a potential therapeutic target for periodontitis treatment.

KEYWORDS
inflammation, NF-κB, NLRP3, periodontitis, SNHG5

Clinical Relevance

Scientific rationale for study: Periodontitis is an oral disease characterized by inflammatory responses to elevated subgingival pathogen levels. The long non-coding RNA small nucleolar RNA host gene 5 (SNHG5) regulates inflammation in diseases such as chronic obstructive pulmonary disease, acute respiratory distress syndrome, and endometrial fibrosis. However, its role in periodontal pathogenesis remains unclear.

Principal findings: SNHG5 expression was down-regulated in human and mouse periodontal tissues. SNHG5 knockdown increased the expression of inflammatory cytokines, including interleukin (IL)-1β, IL-6, and IL-8 in vitro, whereas SNHG5 overexpression ameliorated inflammation

in human periodontal ligament cells. SNHG5 directly interacts with the nuclear factor-kappa B (NF-κB) p65 subunit, inhibits its translocation, and suppresses the NF-κB signalling pathway activation. SNHG5 knockdown enhanced NF-κB expression downstream of nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3), whereas SNHG5 overexpression has the converse effect. Additionally, locally injecting si-SNHG5 aggravates periodontal inflammation in vivo.

Practical implications: Targeting the SNHG5/NF-κb/NLRP3 axis may potentially aid in periodontitis treatment.

1 | INTRODUCTION

Periodontitis is an oral disease characterized by the inflammatory responses to elevated subgingival pathogen levels causing damage to the periodontal tissues and ultimately resulting in tooth loss (Ratheesh et al., 2018; Renn et al., 2018). Periodontitis is the most common cause of tooth loss in adults worldwide. Severe periodontitis may increase the risk of systemic diseases such as diabetes, cardiovascular disease, and rheumatoid arthritis, thereby adversely affecting the systemic patient health (Genco & Van Dyke, 2010; Lalla & Papapanou, 2011). Therefore, improving our understanding of the biological mechanisms underlying periodontal pathogenesis is necessary to improve treatment methods.

Various pro-inflammatory cytokines, including tumour necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, and the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome are involved in the inflammatory periodontal process (X. Huang et al., 2015; Isaza-Guzman et al., 2017; Garcia-Hernandez et al., 2019). Activating the transcription factor nuclear factor-kappa B (NF-κB) is an important inflammatory stimulus in the pathogenesis of periodontitis (Kim et al., 2012). When the NF-κB signalling pathway is activated, the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα), is degraded by the immunoproteasome. The p50/p65 subunit then translocates to the nucleus and binds to specific promoter sequences of target inflammatory genes, including NLRP3 and IL-1β (Visekruna et al., 2006; Wu et al., 2015). The NF-κB and NLRP3 inflammasomes play an important role in the inflammatory stages of periodontitis (Jimi et al., 2019; Aral et al., 2020). However, the specific regulatory mechanisms of the NF-κB and NLRP3 inflammasomes during periodontitis progression remain unclear.

Long non-coding RNAs (lncRNAs) comprise more than 200 nucleotides but do not possess any coding abilities; however, they are involved in large-scale biological processes (Rinn & Chang, 2012). A portion of lncRNA is closely related to the NF-κB signalling pathway, which regulates inflammation. For example, lncRNA Gm4419 activates the NF-κB pathway by directly interacting with the p50 subunit (Yi et al., 2017). Some lncRNAs are abnormally expressed in periodontitis, playing a crucial role in its pathogenesis, such as the Fab-1, YGL023, Vps27, and EEA1 (FYVE); Rho guanine nucleotide exchange factor (RhoGEF); and pleckstrin homology (PH) domain-containing 5-antisense RNA 1 (FGD5-AS1), H19, and papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) (H. Chen, Lan, et al., 2019; Liu et al., 2019; Guo et al., 2020; Zhou et al., 2020). However, the exact roles of these key differentially expressed lncRNAs in periodontal pathogenesis and the regulation of NF-κB activation remain largely unknown.

The lncRNA, small nucleolar RNA host gene 5 (SNHG5), is 524 bp in size and is located on chromosome 6q14 in humans. SNHG5 has drawn increasing attention because of its role in various cancers (Damas et al., 2016; Ju et al., 2018; L. Chen, Gong, & Huang, 2019; Xin et al., 2019). SNHG5 promotes osteosarcoma progression by sponging the miR-212-3p/SGK3 axis (Ju et al., 2018). SNHG5 also stabilizes target transcripts by blocking their degradation by Staufen-1 (STAU1), promoting tumour cell survival in colorectal cancer (Damas et al., 2016). Our previous study suggested that SNHG5 promotes osteogenic differentiation of bone marrow mesenchymal stem cells via the miR-212-3p/growth differentiation factor 5/suppressor of mothers against decapentaplegic pathway (Han et al., 2022). We measured a group of lncRNAs in human gingival tissues from healthy controls and patients with periodontitis. The results demonstrated decreased SNHG5 expression in gingival tissues from patients with periodontitis. Therefore, we hypothesized that SNHG5 is involved in the pathogenesis of periodontitis. In this study, the expression profile of SNHG5 in mouse periodontal tissues was determined. The regulatory relationship between SNHG5 and the NF-κB signalling pathway and in vitro effects of SNHG5 on human periodontal ligament cells (hPDLCs) with TNFα stimulation were studied to evaluate the regulatory role of SNHG5 in periodontal inflammation, providing a potential therapeutic target for periodontitis treatment.

2 | MATERIALS AND METHODS

2.1 | Cell culture

This study was approved by the Ethics Committee of Peking University School of Stomatology (PKUSSIRB-201837096). This study utilized hPDLCs to explore the role of SNHG5 in periodontitis in vitro. hPDLCs is reportedly involved in inflammatory responses and bone resorption, which are two major features of periodontitis (Saito, Rosol, et al., 1990; Saito, Saito, et al., 1990; Hosokawa et al., 2019; W. Huang et al., 2019). hPDLCs were obtained and characterized according to previously published protocols (Han et al., 2020). Briefly, periodontal ligament tissues were scraped from premolar teeth extracted from

four healthy individuals (mean age, 15 years) for orthodontic treatment. All patients and their parents provided written informed consent. The periodontal ligament tissues were then dissected, digested, and cultured in α-modified Eagle medium (α-MEM; Gibco, USA) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco) at 37°C with 5% carbon dioxide. Primary cells began to migrate outwards from periodontal ligament tissues after 7 days. They were passaged and expanded to ensure that there were sufficient cells for the experiments. Cells at passages 4–8 were used.

2.2 | Animals

Eight-week-old male C57 black 6 (C57BL/6) mice were purchased from WeiTong LiHua Co. (Beijing, China). The animals were kept under specific-pathogen-free conditions and administered a standard laboratory diet and water. All animal experimental procedures were approved by the Peking University Animal Care and Use Committee (LA2019315).

2.3 | Gingival biopsy collection

Human gingival tissues were obtained from patients diagnosed with periodontitis ($n = 12$; mean age, 32.4 ± 3.2 years) and healthy controls receiving gingivectomy or crown lengthening during orthodontic or prosthodontic treatment ($n = 10$; mean age, 31.1 ± 2.8 years). All protocols related to patients were approved by the Ethics Committee of the Peking University School of Stomatology, Beijing, People's Republic of China (PKUSSIRB-201950166), and all patients provided written informed consent. After tissue collection, four control and six periodontitis biopsies were fixed in 4% paraformaldehyde for 24 h for histological analysis. The other biopsies were submerged in TRIzol reagent (Invitrogen, USA) for RNA extraction and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Periodontal examination, including periodontal probing depth (PD) and bleeding index (BI) using a Williams periodontal probe at six sites on each tooth, was performed to determine clinical periodontal status. Criteria were formulated according to previous studies (Garaicoa-Pazmino et al., 2019). Briefly, samples meeting the criteria of BI ≤1, PD <3 mm, no attachment loss, >20 teeth, and no alveolar bone loss on radiographic examination (the distance between the cemento-enamel junction [CEJ] and alveolar bone crest < 2 mm) were included in the control group. Samples meeting the criteria BI ≥2, PD >6 mm, clinical attachment loss >3 mm, and obvious bone loss observed in the radiographic examination were included in the periodontitis group. All patients conformed to the following criteria: (1) no smoking, maxillofacial surgery, radiotherapy, or chemotherapy history; (2) no systemic diseases that might influence periodontal conditions; (3) no anti-inflammatory medication intake during the past 3 months; (4) no periodontal treatment during the past 6 months; and (5) no pregnancy or breastfeeding. Gingival biopsies were collected from the buccal side and were approximately 4 mm × 4 mm in size.

2.4 | Immunofluorescence staining of p65 in hPDLCs

Immunofluorescence staining of p65 was performed according to a previously described method (Han et al., 2020). Briefly, cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 at room temperature for 10 min. The cells were then incubated with 5% normal goat serum at room temperature for 40 min, followed by incubation with primary p65 antibody (1:100; Abcam) at 4°C overnight. The cells were then incubated with secondary antibodies in the dark at room temperature for 1 h. As hPDLCs with SNHG5 lentivirus infection expressed green fluorescent protein, TRITC-conjugated secondary antibody (1:200; ZSGB-Biotech, China) was used for the SNHG5 overexpression group (1:200; ZSGB-Biotech), and FITC-conjugated secondary antibody (1:200; ZSGB-Biotech) for the SNHG5 knockdown group. 4′,6-diamidino-2-phenylindole (Servicebio, China) was used to counterstain the nuclei. Staining was visualized using a confocal laser-scanning microscope (Carl Zeiss).

2.5 | RNA pull-down assay

The BersinBioTM RNA pull-down kit (BersinBio, China) was used to detect the interactions between SNHG5 and proteins. The biotin-labelled probe was purchased from GenePharma (China). The probe solution (4 μg) was denatured at 90°C for 2 min and incubated with pre-cooled RNA structure buffer to form the RNA secondary structures. Streptavidin magnetic beads were then incubated with the mixture at room temperature for 30 min. The cells were lysed at 4°C and centrifuged at 15,000*g* for 15 min. The supernatant fractions were then collected and mixed with the probe-bead mixture. RNase inhibitor (5 μl), ethylenediaminetetraacetic acid (5 μl), and ethylene glycol-bis (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (2.5 μl) were added, followed by incubation for 2 h and elution at 37°C for 2 h. The supernatant fractions were transferred to a new tube for western blotting analysis.

2.6 | RNA immunoprecipitation

The RNA immunoprecipitation (RIP) assay was conducted in hPDLCs using the BersinBioTM RNA RIP kit (BersinBio) according to the manufacturer's instructions. Briefly, the magnetic beads were incubated with antibodies against p65 (Abcam, USA) and IgG control (from the kit). The cells were collected and lysed using the RIPA lysis buffer. The lysate was then immunoprecipitated with bead–antibody complexes. The coprecipitated RNAs were used for cDNA synthesis and qRT-PCR evaluation. The results were normalized to those of the input group.

2.7 | Statistical analysis

Statistical analysis was performed using SPSS v.19.0 (IBM, Chicago, IL). Data are expressed as mean ± standard deviation (SD) of at least

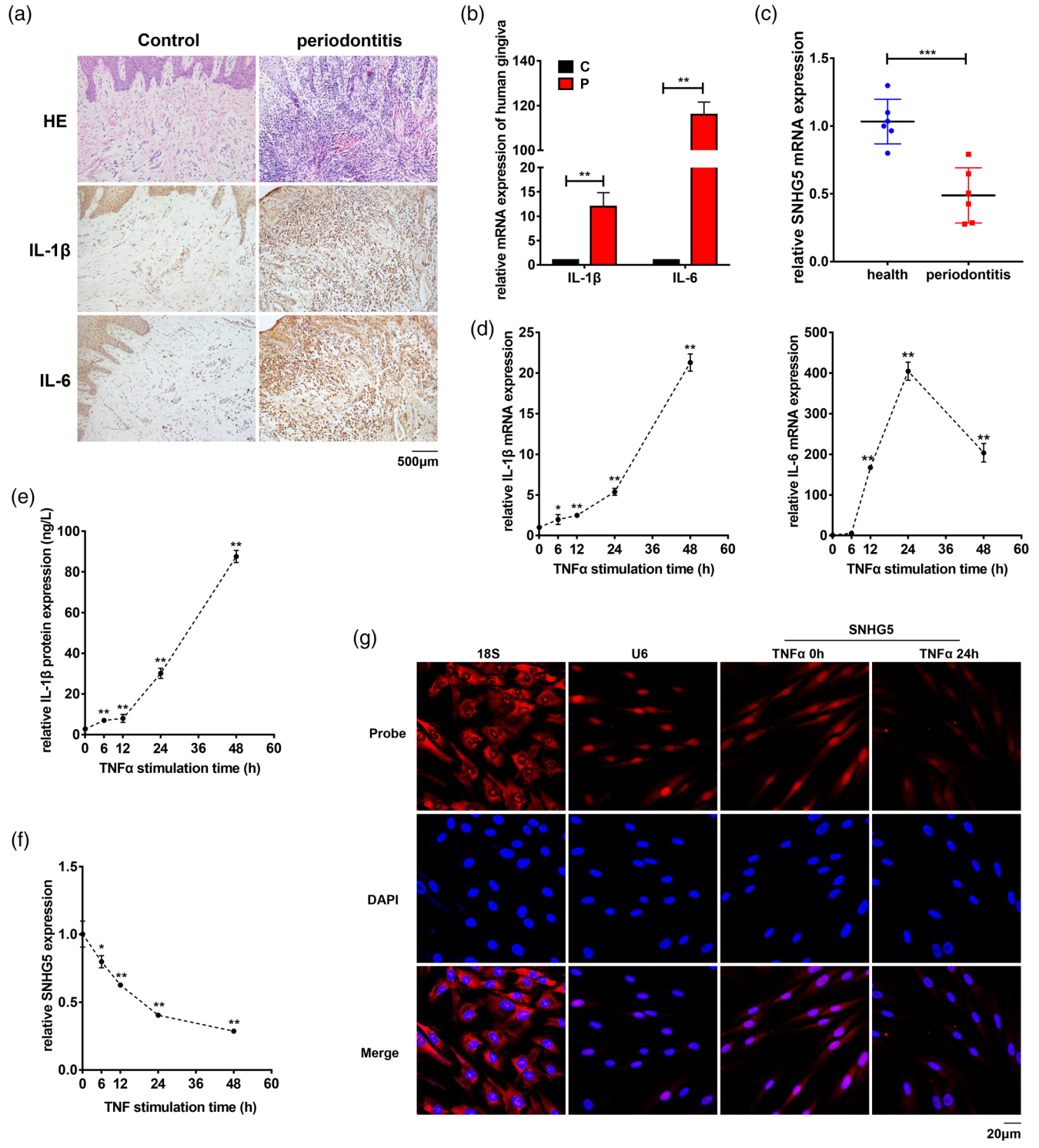

FIGURE 1 SNHG5 is down-regulated in human periodont tissues and TNFα-induced inflammation in hPDLCs. (a) H&E staining and immunohistochemistry staining of IL-1β and IL-6 in the gingivae of healthy and periodontitis. (b) qRT-PCR results show the mRNA expression of IL-1β and IL-6 in the gingivae of healthy and periodontitis. GAPDH was used for normalization relative to the control group. (c) qRT-PCR results show the RNA expression of SNHG5 in the gingivae of healthy and periodontitis ($n = 6$/group). GAPDH was used for normalization relative to the control group. (d) qRT-PCR analysis depicting the expression of inflammatory cytokines, including IL-1β and IL-6, at different time points after TNFα stimulation. GAPDH was used for normalization relative to the control group. (e) ELISA results depicting the IL-1β production at different time points after TNFα stimulation. (f) qRT-PCR results demonstrating the SNHG5 expression at different time points after TNFα stimulation. GAPDH was used for normalization relative to the control group. (g) FISH images of 18S (the first column), U6 (the second column), and SNHG5 (the third and fourth column). A probe of human 18S was used as the positive control for cytoplasmic RNA, and U6 was used as the positive control for nuclear RNA (*$p < .05$; **$p < .01$; ***$p < .001$). ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence in situ hybridization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, haematoxylin and eosin; hPDLCs, human periodontal ligament cells; IL, interleukin; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SNHG5, small nucleolar RNA host gene 5; TNFα, tumour necrosis factor α

three independent experiments. Parametric data underwent two-tailed unpaired Student's *t*-tests for two-group comparisons. One-way analysis of variance was used for multiple group comparisons. The Mann–Whitney test was used for two-group comparisons of non-parametric data; the Kruskal–Wallis test for multi-group comparisons; and Tukey's multiple comparisons test for post hoc tests. Differences were considered statistically significant at $p < .05$.

The following methods are described in detail in the supplementary materials:

- qRT-PCR.
- Western blotting.
- Transfection with RNA oligoribonucleotides.
- Lentivirus infection.
- Cell fractionation assay.
- Ligature-induced periodontitis model and associated interventions.
- Micro-computed tomography (micro-CT) analysis.
- Haematoxylin and eosin (H&E) and immunohistochemical staining.
- Fluorescence in situ hybridization (FISH) assay.
- Enzyme-linked immunosorbent assay (ELISA).

3 | RESULTS

3.1 | SNHG5 is down-regulated in the human periodo tissues

The expression of SNHG5 in periodontitis-affected samples and healthy controls was analysed to determine its differential expression in the gingival tissues of patients with periodontitis and healthy controls. H&E staining revealed a significantly higher inflammatory cell infiltration in the gingival tissues of patients with periodontitis (Figure 1a). Immunohistochemistry revealed higher IL-1β and IL-6 expression in periodontal tissues than in healthy controls (Figure 1a). qRT-PCR results further confirmed that mRNA expression of IL-1β and IL-6 was significantly increased in human periodontit tissues (Figure 1b), whereas SNHG5 expression was significantly decreased (Figure 1c).

3.2 | SNHG5 is down-regulated due to TNFα-induced inflammation in hPDLCs

To investigate whether lncRNA SNHG5 is affected by an inflammatory environment, we measured its expression in TNFα-treated hPDLCs (Shindo et al., 2014; Hosokawa et al., 2017; Hosokawa et al., 2019; X. Li et al., 2019). TNFα is a common and crucial inflammatory factor in developing periodontal inflammation and alveolar bone resorption and is a direct NF-κB signalling pathway activator (W. Li et al., 2010). We observed that the mRNA expression of IL-1β and IL-6 were significantly upregulated in a time-dependent manner (Figure 1d). The protein expression of IL-1β and IL-6 was also significantly upregulated (Figures 1e and S1). The SNHG5 expression was significantly decreased by TNF-α stimulation (Figure 1f). Additionally, FISH assay demonstrated that SNHG5 was mainly located in the cytoplasm and nucleus of hPDLCs, and TNFα stimulation significantly reduced SNHG5 expression in the nucleus and cytoplasm (Figure 1g).

3.3 | SNHG5 is down-regulated in the mice periodontal inflammation models

Mouse models of periodontitis were constructed to explore the SNHG5 expression in response to periodontal inflammation. Micro-CT and H&E staining results showed increased distances between the CEJ and alveolar bone crest and bone loss in the ligature-induced periodontitis group (Figure 2a,b). Immunohistochemical staining results demonstrated higher expression of IL-1β around the periodontal tissues in the periodontitis group (Figure 2b). FISH assay confirmed that ligature-induced periodontitis infection decreased SNHG5 expression in mouse periodontal tissues (Figure 2c). Moreover, the qRT-PCR results demonstrated that the SNHG5 expression was significantly decreased in the periodontal tissues compared with that in the control, whereas inflammatory cytokines (IL-1β, IL-6, and IL-8) were significantly increased (Figure 2d).

3.4 | SNHG5 reduces the TNFα-induced inflammatory responses in hPDLCs

To further investigate the role of SNHG5 in TNFα-mediated inflammation of hPDLCs, SNHG5 was overexpressed in hPDLCs by lentiviral transduction, and siRNA targeting SNHG5 was used to knock down SNHG5. qRT-PCR results demonstrated that SNHG5 was increased in the SNHG5 lentivirus transduction group and significantly decreased in the si-SNHG5-transfected group (Figure S2). The lentivirus transduction efficiency was also confirmed by fluorescent staining (Figure S3). IL-1β, IL-6, and IL-8 expression increased after SNHG5 knockdown in non-inflammatory and inflammatory environments and decreased after SNHG5 overexpression (Figure 3a,b). These results indicate that SNHG5 regulates the expression of inflammatory cytokines and modulates TNFα-induced inflammatory response in hPDLCs.

3.5 | SNHG5 inhibits NF-κB p65 subunit translocation and suppresses NF-κB signalling pathway activation

NF-κB is a critical link between inflammation and periodontal pathogenesis. We speculated that SNHG5 might be involved in the NF-κB signalling pathway to regulate periodontitis. Western blotting results showed that knockdown of SNHG5 significantly increased the phosphorylation of p65 in hPDLCs stimulated with TNFα, whereas overexpression of SNHG5 markedly decreased p65 phosphorylation (Figure 4a,b). Moreover, immunofluorescence staining demonstrated

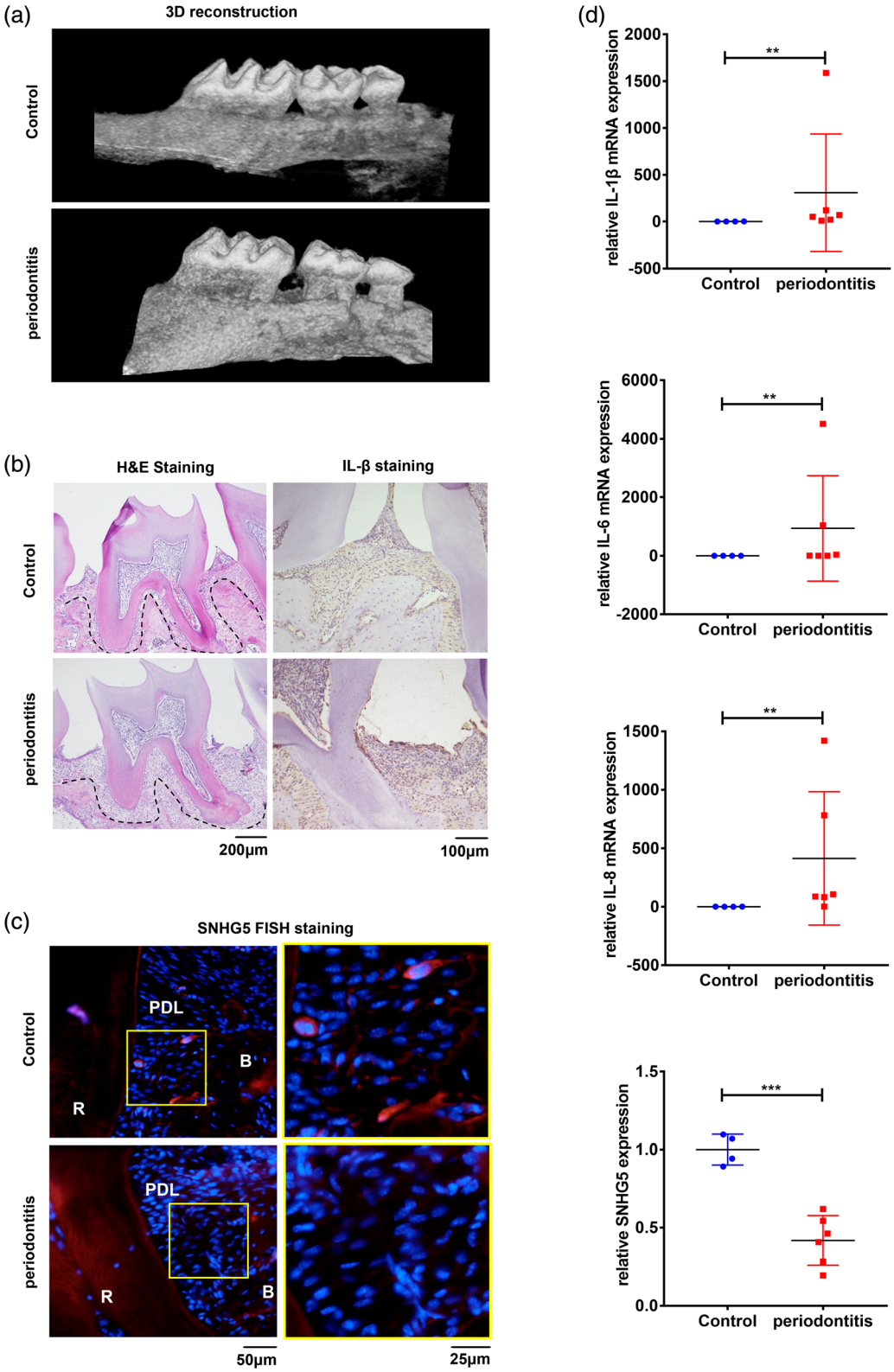

FIGURE 2 SNHG5 is down-regulated in the periodontal inflammation mice models. (a) Images of reconstructed 3D micro-CT images in the periodontal tissues of healthy and periodontitis mice. (b) Images of H&E and immunohistochemistry staining of IL-1β in the periodontal tissues of healthy and periodontitis mice. (c) FISH staining of SNHG5 in the periodontal tissues of healthy and periodontitis mice. (d) qRT-PCR results show the mRNA expression of IL-1β, IL-6, IL8, and SNHG5 in the gingivae of healthy ($n = 4$) and periodontitis ($n = 6$) mice. GAPDH was used for normalization relative to the control group (**$p < .01$; ***$p < .001$). FISH, fluorescence in situ hybridization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, haematoxylin and eosin; IL, interleukin; micro-CT, micro-computed tomography; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SNHG5, small nucleolar RNA host gene 5

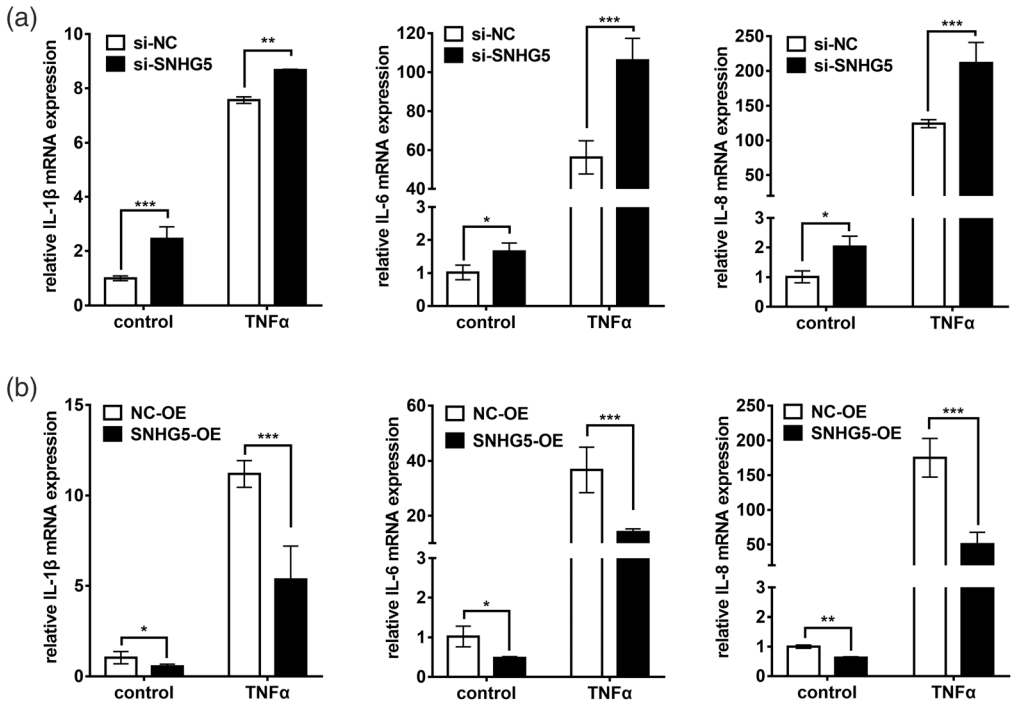

FIGURE 3 SNHG5 reduces TNFα-induced inflammatory response in hPDLCs. (a) Effects of SNHG5 knockdown on the expression of inflammatory cytokines with or without TNFα stimulation for 24 h in hPDLCs by qRT-PCR in the si-NC, si-SNHG5, si-NC+TNFα, and si-SNHG5+TNFα groups. GAPDH was used for normalization relative to the control group. (b) Effects of SNHG5 overexpression on the expression of inflammatory cytokines with or without TNFα stimulation for 24 h in hPDLCs by qRT-PCR in the NC-OE, SNHG5-OE, NC-OE+TNFα, SNHG5-OE+TNFα groups. GAPDH was used for normalization relative to the control group (*$p < .05$; **$p < .01$; ***$p < .001$). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hPDLCs, human periodontal ligament cells; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SNHG5, small nucleolar RNA host gene 5; TNFα, tumour necrosis factor α

that knockdown of SNHG5 promoted the NF-κB p65 subunit translocation from the cytoplasm to the nucleus in TNFα-induced hPDLCs, whereas SNHG5 overexpression significantly inhibited TNFα-induced p65 nuclear translocation in hPDLCs (Figure 4c,d). Western blotting results indicated a significant increase in p65 nuclear translocation in the SNHG5 knockdown group after 30 min of TNF-α stimulation in hPDLCs (Figure 4e).

3.6 | SNHG5 reduces NF-κB expression downstream NLRP3 and mediates the inflammatory response via the NF-κB/NLRP3 inflammasome signalling pathway in hPDLCs

The NLRP3 inflammasome plays a crucial role in the process of periodontitis (Isaza-Guzman et al., 2017), and NF-κb is a key NLRP3-inflammasome activator by inducing pro-IL-1β and NLRP3 expression (Schroder & Tschopp, 2010). SNHG5 knockdown enhanced NLRP3 expression with and without TNFα stimulation for 24 h, whereas SNHG5 overexpression reduced NLRP3 mRNA expression (Figure 5a). Moreover, SNHG5 knockdown led to increased NLRP3 protein expression after 24 h of TNFα stimulation than in the si-NC group, and SNHG5 overexpression significantly decreased NLRP3 protein expression after TNFα stimulation (Figure 5b). Next, we explored whether SNHG5 mediated inflammation via the NF-κB signalling pathway by using the NF-κB signalling pathway inhibitor, 4-methyl-N1-(3-phenyl-propyl)-benzene-1,2-diamine (JSH-23). The results revealed that JSH-23 significantly reduced the mRNA expression of TNFα-stimulated inflammatory cytokines during SNHG5 knockdown or overexpression (Figures 5c and S4). These data suggest that SNHG5 mediates inflammatory responses through the NF-κB/NLRP3 inflammasome signalling pathway in hPDLCs.

3.7 | SNHG5 directly interacts with the NF-κB p65 subunit

As SNHG5 is mainly localized in the cytoplasm and significantly inhibits the NF-κB p65 subunit translocation, we hypothesized that SNHG5 might function by interacting with the NF-κB complex. The RIP assay, using p65 antibodies, demonstrated a significant amount of SNHG5 RNA enrichment with p65 (Figure 5d). RNA pull-down assays were performed using in vitro-generated biotinylated full-length SNHG5 transcripts and western blotting demonstrated that SNHG5 specifically precipitated p65 in the SNHG5 group but not in the SNHG5-AS group (Figure 5e), suggesting that SNHG5 binds directly to the NF-κB p65 subunit.

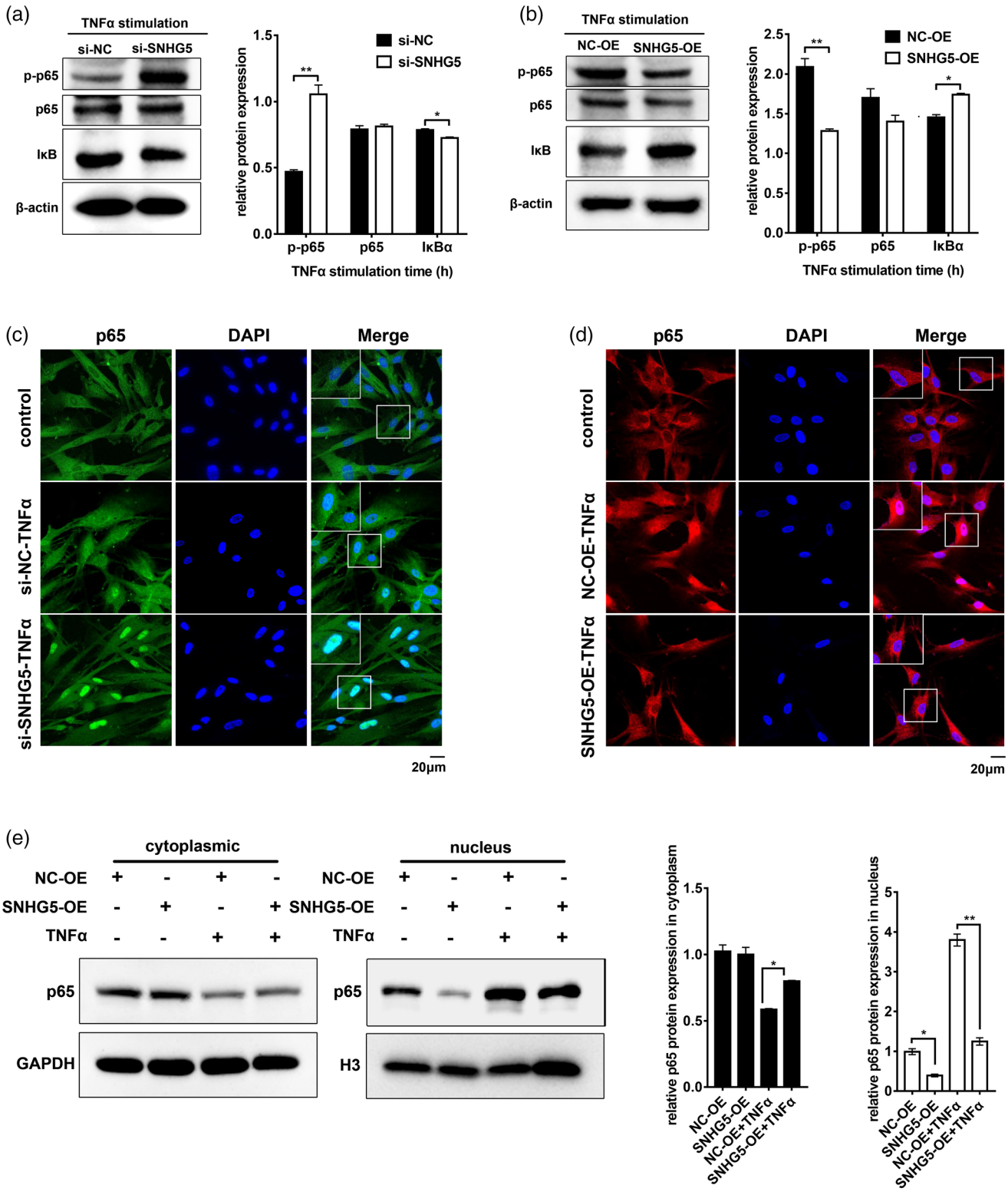

FIGURE 4 SNHG5 inhibits the translocation of the NF-κB p65 subunit and suppresses the activation of the NF-κB signalling pathway. (a,b) Western blotting analyses show the effects of SNHG5 knockdown or overexpression SNHG5 on the activation of NF-κB pathways induced by TNFα in hPDLCs. Histograms show the quantification of band intensities. β-Actin was used for normalization relative to the si-NC group (c,d). Confocal microscopy shows the effects of SNHG5 on p65 nuclear translocation in TNFα-treated hPDLCs. (e) Cytoplasmic extracts were blotted using the p65 antibody with GAPDH as the loading control in hPDLCs. Nuclear extracts were blotted using the p65 antibody with H3 as the loading control in hPDLCs. Histograms show the quantification of band intensities (*$p < .05$; **$p < .01$). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hPDLCs, human periodontal ligament cells; NF-κb, nuclear factor-kappa B; SNHG5, small nucleolar RNA host gene 5; TNFα, tumour necrosis factor α

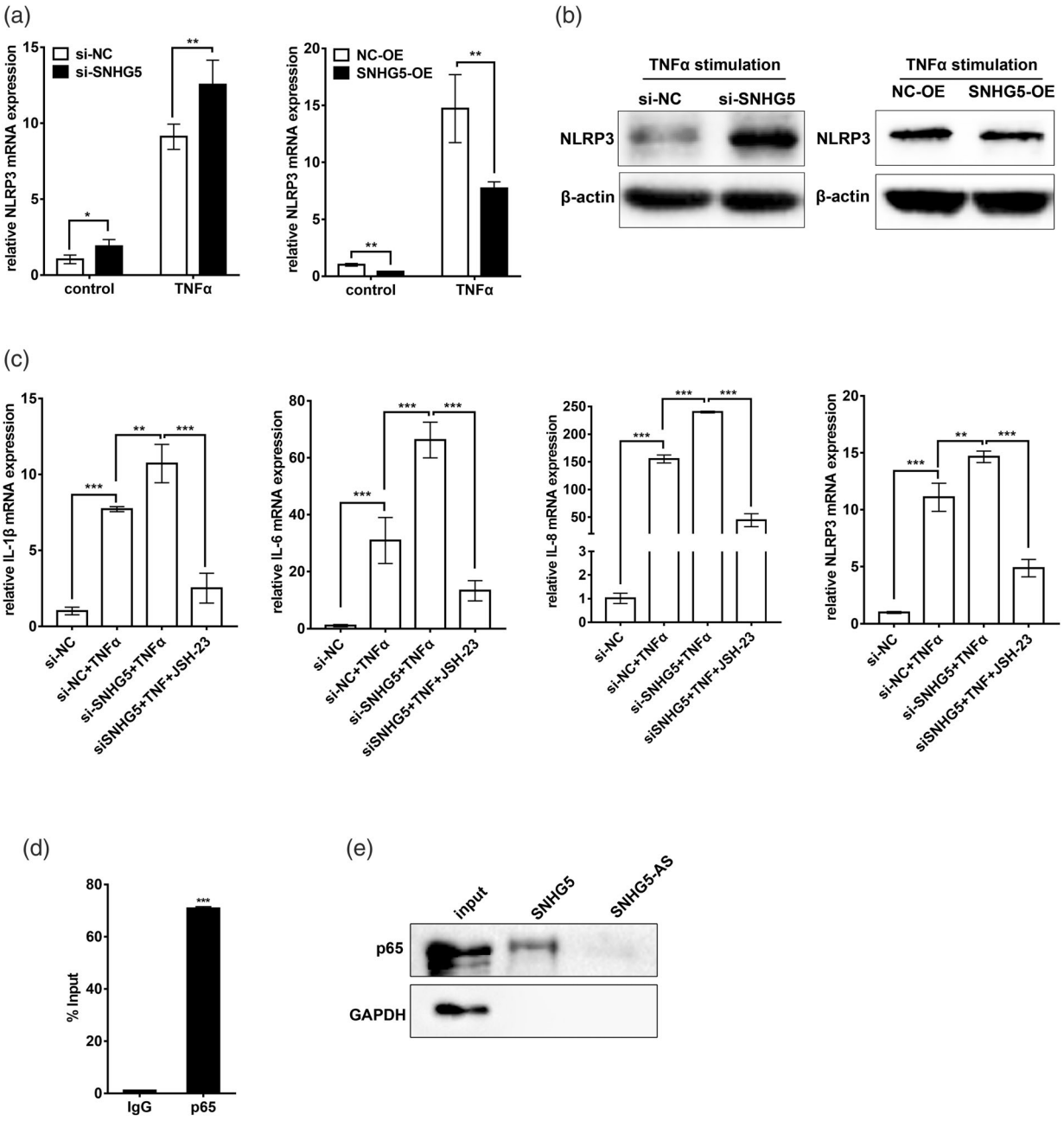

FIGURE 5 SNHG5 mediates inflammatory response through the NF-κB/NLRP3 inflammasome signalling pathway and directly interacts with the NF-κb p65 subunit in hPDLCs. (a) Effects of SNHG5 knockdown or overexpression on the NLRP3 mRNA expression with or without TNFα treatment in hPDLCs by qRT-PCR. (b) Effects of SNHG5 knockdown or overexpression on the NLRP3 expression in hPDLCs after TNFα stimulation for 24 h by western blotting analysis. (c) Effects of SNHG5 knockdown on the mRNA expression of inflammatory cytokines treated with TNFα and NF-κB inhibitor JSH-23 in hPDLCs by qRT-PCR analysis. GAPDH was used for normalization relative to the control group. (d) RT-PCR was used to measure the RNA enrichment in the RIP assay using the anti-p65 antibody in hPDLCs. IgG was used as the non-specific control antibody. (e) The specific association of SNHG5 with the p65 protein was validated through RNA pull-down, followed by western blotting. GAPDH was used as the negative control (*$p < .05$; **$p < .01$; ***$p < .001$). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hPDLCs, human periodontal ligament cells; NF-κb, nuclear factor-kappa B; NLRP3nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RIP, RNA immunoprecipitation; SNHG5, small nucleolar RNA host gene 5; TNFα, tumour necrosis factor α

3.8 | SNHG5 knockdown aggravates in vivo periodontal inflammation

To further validate the potential role of SNHG5 in inflammatory periodontitis, we established a mouse periodontitis model by locally injecting si-SNHG5 or si-NC. Micro-CT reconstruction demonstrated that local injection of si-SNHG5 aggravated ligature-induced periodontitis compared with the si-NC group (Figure 6a). The si-SNHG5 injection resulted in significantly severe bone loss, with a lower bone volume/tissue volume ratio and more severe

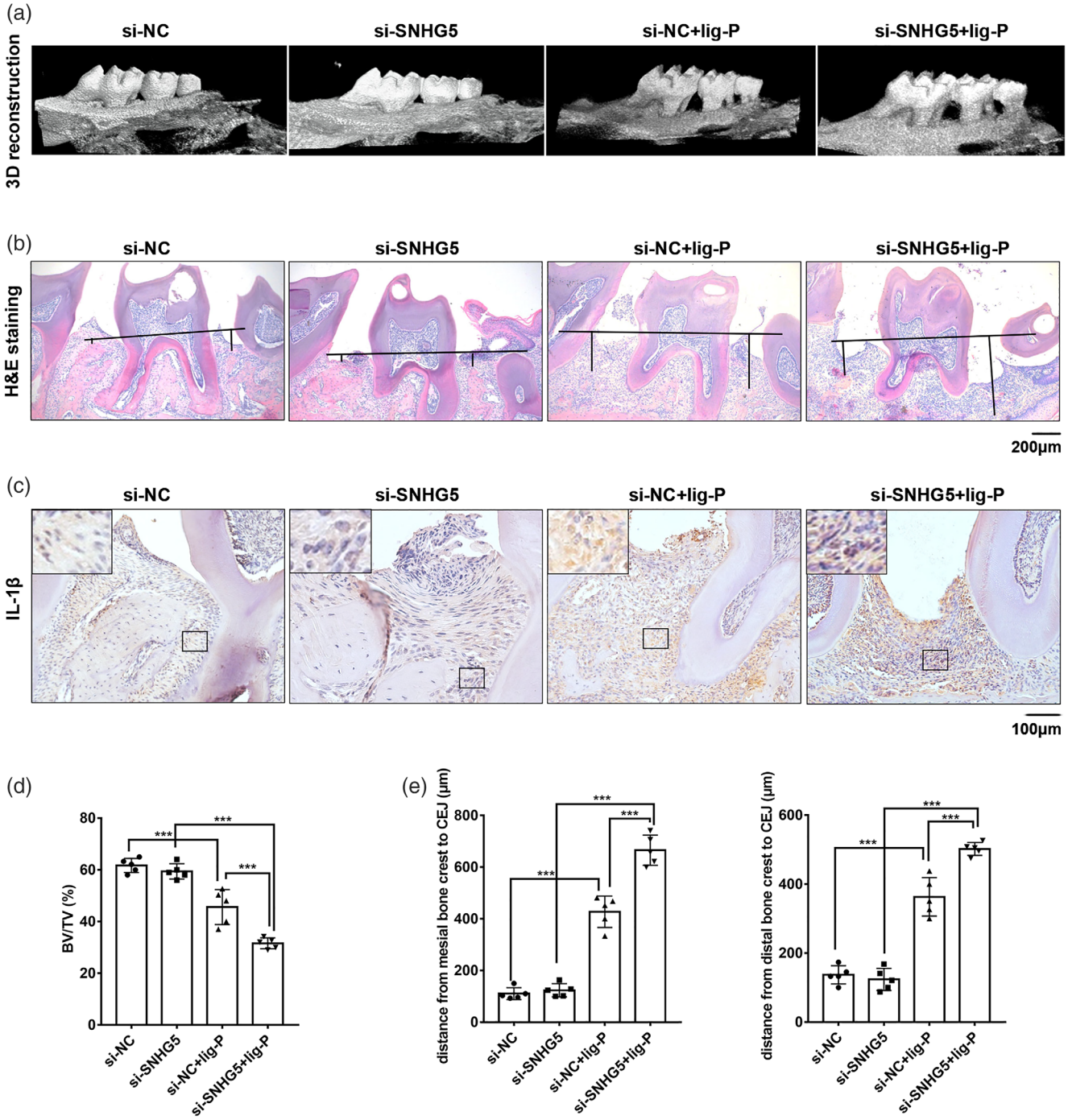

FIGURE 6 SNHG5 knockdown aggravates the periodontal inflammation in vivo. (a) Reconstructed 3D micro-CT of si-NC local injection group (si-NC), si-SNHG5 local injection group (si-SNHG5), ligature-induced periodontitis with si-NC local injection group (si-NC+lig-P), and ligature-induced periodontitis with si-SNHG5 local injection group (si-SNHG5+lig-P). (b,c) H&E and immunohistochemistry staining of IL-1β in the si-NC, si-SNHG5, si-NC+lig-P, and si-SNHG5+lig-P groups. (d,e) BV/TV analysis of the si-NC, si-SNHG5, si-NC+lig-P, and si-SNHG5+lig-P groups ($n = 5$). (e) The distances of the mesial and distal bone crest to the CEJ in the si-NC, si-SNHG5, si-NC+lig-P, si-SNHG5+lig-P groups (***$p < .001$). CEJ, cemento-enamel junction; IL, interleukin; micro-CT, micro-computed tomography; SNHG5, small nucleolar RNA host gene 5

inflammation than in the si-NC group (Figure 6d). H&E staining and software analysis demonstrated that the distances from the CEJ to the alveolar bone crest of the mesial and distal sides were significantly larger in the si-SNHG5 injection group than in the control (Figure 6b,e). Immunohistochemical staining results showed that the IL-1β expression was significantly higher in the si-SNHG5 treatment group than in the si-NC group in periodontitis mice (Figure 6c).

4 | DISCUSSION

lncRNAs play a critical role in multiple biological processes including chromatin modification (Kanduri, 2011), RNA processing (Gong & Maquat, 2011), and structural scaffolds (Clemson et al., 2009). Several lncRNAs are reported to be involved in the pathogenesis of periodontitis. For instance, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) regulates the production of inflammatory cytokines in the lipopolysaccharide-stimulated human gingival fibroblasts by sponging miR-20a and activating the toll-like receptor 4 pathway (Pignatelli et al., 2020). However, the role of lncRNAs in periodontal pathogenesis remained unclear. We found that the expression of SNHG5 was decreased in the gingival tissues of periodontitis patients than in healthy controls. In vitro experiments demonstrated that TNFα-induced inflammation decreases the expression of SNHG5, indicating that SNHG5 may be involved in the pathogenesis of periodontitis.

Few studies have reported on the role of SNHG5 in the regulation of inflammation; however, one study demonstrated that SNHG5 influences inflammatory genes by serving as a competing endogenous RNA via the miR-132/PTEN axis (Shen et al., 2020). In our experiments, IL-1β, IL-6, and IL-8 expression increased after SNHG5 knockdown and decreased after SNHG5 overexpression in non-inflammatory and inflammatory environments, suggesting that SNHG5 inhibits inflammation, which is consistent with its role in chronic obstructive pulmonary disease (Shen et al., 2020). Our previous experiments showed that YY1 promotes SNHG5 expression by directly binding to the promoter region of SNHG5 (Han et al., 2022). Similarly, studies have reported that YY1 is involved in inflammatory regulation (Nakayama et al., 2019; Sun et al., 2021). Another study elucidated that mRNA expression of YY1 was significantly decreased in the spinal cord cells of bilateral chronic constriction injury rats (Sun et al., 2021). Therefore, the association between inflammation and reduced SNHG5 expression may be owing to reduced YY1 expression. However, in the present study, we did not aim to explore upstream mechanisms, and there may be other regulatory mechanisms, such as DNA methylation, waiting for exploration in the future.

The NF-κB signalling pathway is the central mediator of various processes, including inflammation, periodontitis, and atherogenesis (Nichols et al., 2001). Dong et al. (2018) revealed that osteopontin promotes bone destruction in periapical periodontitis by activating the NF-κB signalling pathway. The present study demonstrated that overexpression of SNHG5 reduced TNFα-induced NF-κB signalling pathway activation, whereas SNHG5 knockdown enhanced the NF-κB signalling pathway activation. Immunofluorescence staining and cell fractionation assays revealed that SNHG5 knockdown promoted the NF-κB p65 subunit translocation from the cytoplasm to the nucleus in TNFα-induced inflammatory hPDLCs.

The NF-κB pathway induces numerous inflammatory chemokines, cytokines and their precursors, including pro-IL-1β and NLRP3. *Porphyromonas gingivalis*-derived lipopolysaccharide induces the p65 subunit binding to the NLRP3 inflammasome promoter in murine

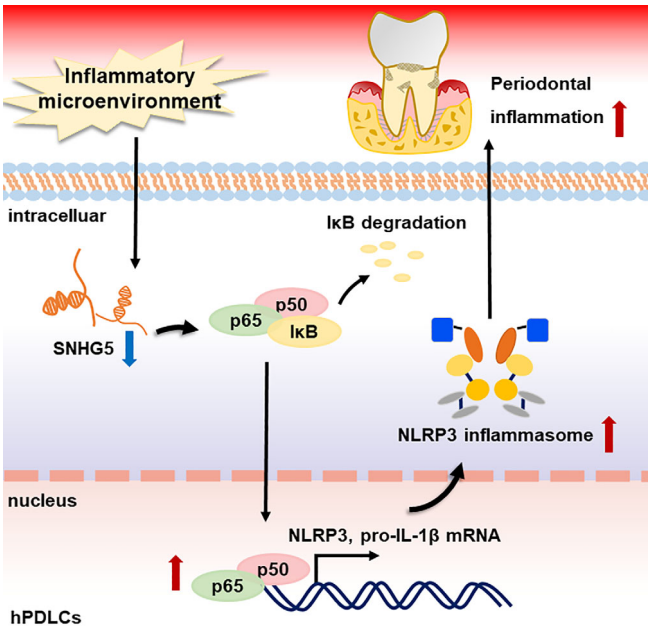

FIGURE 7 Schematic model of the mechanism that SNHG5 mediates periodontal inflammation through the NF-κB signalling pathway. NF-κb, nuclear factor-kappa B; SNHG5, small nucleolar RNA host gene 5

macrophages, which is important for the priming and assembly of inflammasomes (Schroder & Tschopp, 2010; Qiao et al., 2012). The NLRP3 inflammasome is a novel inflammatory factor responsible for the maturation of IL-1β to induce inflammation, which is important in the inflammatory process of periodontitis (Isaza-Guzman et al., 2017; Garcia-Hernandez et al., 2019). In the priming step of NLRP3 inflammasome activation, the activated NF-κB p65 sequence binds to the promoter region of NLRP3 and IL-1β mRNA, upregulating NLRP3 inflammasome transcription (Bauernfeind et al., 2009). Notably, we found that the knockdown or overexpression of SNHG5 respectively upregulated or down-regulated NLRP3 inflammasome expression in hPDLCs. Moreover, applying the NF-κB p65-specific inhibitor, JSH-23, significantly reduced the expression levels of NLRP3 and pro-IL-1β. Collectively, these data imply that the NLRP3 inflammasome is associated with the inflammatory effect of SNHG5, which is mediated by the NF-κB signalling pathway.

As regulatory non-coding RNA molecules, lncRNA performs various functions depending on subcellular location. For instance, nucleolar lncRNA mainly participates in transcription and chromatin remodelling processes, whereas cytoplasmic lncRNA is mainly involved in gene regulatory mechanisms by forming complexes with specific proteins (Martianov et al., 2007; Tsai et al., 2010). SNHG5 can regulate gene expression in numerous ways by serving as a competing endogenous RNA against microRNA (Chi et al., 2019) or stabilizing target transcripts (Damas et al., 2016). In the present study, we demonstrated that SNHG5 directly binds to the NF-κB p65 subunit, thereby inhibiting the nuclear translocation of p65, further regulating the transcription of inflammation-associated genes.

5 | CONCLUSION

The current study demonstrates that SNHG5 is down-regulated in patients with periodontitis. Reduced SNHG5 exacerbates periodontitis, possibly through increased NF-κB pathway signalling (Figure 7). Thus, targeting the SNHG5/NF-κb/NLRP3 axis may be a promising strategy for treating periodontitis.

AUTHOR CONTRIBUTIONS

Yineng Han contributed to the conception, design, data acquisition, analysis, and interpretation and drafted and critically revised the manuscript; Yiping Huang and Qiaolin Yang contributed to data analysis and interpretation and critically revised the manuscript; Lingfei Jia contributed to data analysis and critically revised the manuscript; Weiran Li and Yunfei Zheng contributed to the conception, design, data acquisition, analysis, and interpretation and critically revised the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of the work.

ACKNOWLEDGEMENTS

This study was financially supported by grants from the National Natural Science Foundation of China (Nos. 82071119, 82071142).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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How to cite this article: Han, Y., Huang, Y., Yang, Q., Jia, L., Zheng, Y., & Li, W. (2022). Long non-coding RNA SNHG5 mediates periodontal inflammation through the NF-κB signalling pathway. *Journal of Clinical Periodontology*, *49*(10), 1038–1051. https://doi.org/10.1111/jcpe.13684