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ORIGINAL RESEARCH

AIM2-Driven Inflammation in Periodontitis: Mechanisms and Systemic Implications

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Background and Objective: Periodontitis is a chronic inflammatory condition that can be associated with systemic diseases like diabetes and cardiovascular disease. This study investigates the role of AIM2, a key inflammasome component, in periodontitis, focusing on its involvement in inflammation, DNA repair, and systemic disease links.

Methods: AIM2 expression was analyzed in saliva and gingival crevicular fluid (GCF) from periodontitis patients. A mouse periodontitis model and in vitro gingival fibroblast experiments were used to study AIM2's role. Gene Set Enrichment Analysis (GSEA) and Protein-Protein Interaction (PPI) network analysis explored AIM2's systemic disease associations.

Results: AIM2 was significantly upregulated in periodontitis patients and models, correlating with increased IL-1β, ASC, and Caspase-1. Immunofluorescence revealed AIM2's nuclear localization and co-localization with inflammatory markers. GSEA linked high AIM2 expression to cardiovascular diseases, while its suppression showed protective effects. PPI analysis identified interactions with DNA repair proteins (THOC2, SETX, ATM), suggesting a role in genomic stability and systemic disease.

Conclusion: AIM2 drives local inflammation in periodontitis and may connect periodontitis to systemic diseases via DNA repair and systemic inflammation. This highlights AIM2 as a potential therapeutic target for managing periodontitis and associated systemic risks.

Clinical Significance: Targeting AIM2 could offer a dual therapeutic strategy to control periodontal inflammation and mitigate systemic disease risks, such as cardiovascular disorders.

Keywords: periodontitis, AIM2, inflammasome, systemic diseases, DNA repair

Introduction

Gingival tissue inflammation, alveolar bone resorption elaborated periodontitis is a prevalent chronic inflammatory disease caused by microbial infection in dental plaque and could cause periodontal loss significantly. Moreover, a growing body of evidence suggests that periodontitis transcends the oral cavity and has a great impact on the systemic health increasing the risk for systemic diseases, such as cardiovascular diseases, diabetes, and others.[1–3] The host immune response is critical to the progression of periodontitis and represents a key mechanism during that progression.

The AIM2 (Absent in Melanoma 2) inflammasome is a multiprotein complex assembled largely constituted by the AIM2 receptor, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) and the effector protein caspase-1. AIM2 recruits ASC upon recognition of cytosolic dsDNA (double-stranded dsDNA). ASC then facilitates the assembly of caspase-1. Activated caspase-1 cleaves proIL-1β into IL-1β, and proIL-18 to IL-18. This then triggers the cleaving of pro-IL-1β and pro-IL-18 into their mature versions, IL-1β and IL-18, initiating a robust inflammatory response. The recognition that pathogens by pattern recognition receptors plays a critical role in host defense and the pathogenesis of inflammatory diseases. AIM2 is deeply studied in the different inflammatory diseases such as infectious diseases, autoimmune diseases and cancer via its regulation of inflammation and its effect on disease progression.[4–6]

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AIM2 has now become of interest to us in oral health. They reported that AIM2 expression is highly increased in the patients with different stages of periodontitis, suggesting that AIM2 may be involved in pathogenesis of periodontitis.[7] It appears that such upregulation is associated with the persistence and exacerbation of chronic inflammation in periodontal tissues. Carrillo-Gálvez et al also showed that AIM2 inflammasome activation contributes to alveolar bone resorption in the periodontitis by elevating the release of IL-1β.[8] Although the local inflammatory role of AIM2 appears to be well established, it may also play a role in systemic diseases, such as cardiovascular and metabolic disorders, through systemic inflammatory responses. AIM2 has been linked to DNA repair and aging pathways and has been shown to interact with key genes like ATM and SETX which have a role in maintaining genomic stability as well as regulating the aging of the cellular.[9]

They may explain the link between upregulated AIM2 and age-associated diseases. In addition, tightly linked with AIM2 expression are polymorphisms in inflammation genes, which may render subjects to periodontitis susceptibility or may alter the intensity and duration of the inflammatory response, which then will affect the disease progression. As an example, a study conducted by Li et al found that AIM2 gene polymorphisms play a major role in an individual's periodontitis susceptibility.[10] This indicates that AIM2 may be a significant factor for periodontitis.

The literature goes on to suggest that in addition to having a role in local inflammation, AIM2 also participates in systemic inflammation, DNA repair and aging-related signaling pathways. In this way, this indicates that AIM2 can function as a key link between periodontitis and systemic diseases which may aggravate the pathological processes related to cardiovascular and metabolic disorders.[11–13] As a result, AIM2 is of great significance in investigation of its multifaceted role, particularly in systemic diseases.

Based on previous research, this study will extend to examine the AIM2 expression in periodontitis models and also aims to understand AIM2′s specific roles in periodontal inflammation progression. Therefore, we hypothesize that in addition to exacerbating periodontitis via local inflammatory responses, AIM2 also influences systemic progression of the disease by modulating DNA repair and aging related pathways.

Materials and Methods

Study Subjects and Sample Collection

This study involved 33 patients diagnosed with chronic periodontitis (CP) and 27 healthy individuals serving as the control group. Periodontitis was diagnosed following the 2017 classification system of the World Workshop on the Classification of Periodontal and Peri - Implant Diseases and Conditions.[14] All participants provided informed consent, and the consent process was approved by the Ethics Committee of Hainan Medical University. The study adheres to the ethical principles outlined in the Declaration of Helsinki. No specific approval number was assigned to this project. For patients who meet the criteria, at the initial diagnosis, 500 μL of saliva was collected using the natural flow collection method with an EP tube respectively; gingival crevicular fluid was collected using the filter paper method with a filter paper strip of 1 mm×3 mm.

Inclusion Criteria

- Patients aged 18 to 75 years who had not undergone periodontal treatment in the past six months.[15]
- Periodontitis is diagnosed by probing pocket depth≥4 mm and clinical attachment loss≥2 mm.
- No history of systemic diseases, including cardiovascular disease, diabetes, or immune system disorders.

Exclusion Criteria

- Pregnant or lactating women.
- Individuals who received antibiotic treatment within the last six months.
- Smokers or those with a history of smoking.
- Non-steroidal anti-inflammatory drugs (NSAIDs) or immunosuppressants used within 6 months.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was conducted following the manufacturer's protocol. Each sample was run in triplicate to ensure accuracy. Optical density (OD) readings were taken at 450 nm, and concentrations were calculated based on a standard curve.

ELISA Kits Used

- Human Interleukin-1β (IL-1β) ELISA kit (Jianglaibio, JL13662).
- Human IFI16 ELISA kit (Jianglaibio, JL11400).
- Human Caspase-1 ELISA kit (BYabscience, BYHS100009).
- Human ASC ELISA kit (BYabscience, BY-EH115791).
- Human NLRP3 ELISA kit (Jianglaibio, JL10272).
- Human AIM2 ELISA kit (Jianglaibio, JL49566).
- Human Gasdermin D Protein (GSDMD) ELISA Kit (BYabscience, BYHS501266).

Animal Model Establishment

Eight-week-old C57BL/6 mice were used to establish the periodontitis animal model. All mice were obtained from the institutional animal facility and housed in a pathogen-free environment. The 2017 classification system of the World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions was used to diagnose periodontitis.[14] The Ethics Committee of Hainan Medical University approved the consent process. All participants gave informed consent. The Ethics Committee of Hainan Medical University approved all animal experiments. This project adhered to the ethical principles of Guide for the Care and Use of Laboratory Animals. Periodontitis Model Construction: Orthodontic wire (0.25 mm) was passed between the first and second molars, and a 4–0 surgical suture was tied around the wire on both the buccal and palatal sides. The ligature was placed mesially to the first molar, with the excess wire trimmed to allow penetration into the gingival sulcus. The ligature was checked and retied every two days. After three weeks, one mouse from both the control and experimental groups was euthanized for Micro-CT imaging, which showed significant alveolar bone loss in the model group, confirming successful periodontitis induction. The control group did not undergo ligation but received the same treatment otherwise.

Micro-CT Analysis

After 4 weeks of standard housing, the experimental mice were euthanized via cervical dislocation under general anesthesia. Subsequently, the left mandibular bone samples containing the first, second and third mandibular molars were collected. The mandibles are then scanned with a high-resolution micro-CT (SkyScan1176, Bruker). The scanning parameters were 50 kV and 200 μA with a 9 μm resolution. The scanned images were reconstructed, and the bone mineral density (BMD) and bone volume (BV) were calculated.

Immunohistochemistry (IHC) Staining

Tissue Preparation

Mouse gingival tissues were fixed in 4% paraformaldehyde for 24 hours, embedded in paraffin, and sectioned into 4-μm thickness for immunohistochemical (IHC) analysis.

IHC Staining

After deparaffinization and antigen retrieval at 4°C, tissue sections were incubated overnight with primary antibodies to AIM2, IL-1β and Caspase-1 (1: 200 dilution). Detection was performed using 3,3'-diaminobenzidine (DAB) for chromogenic visualization, and nuclei were counterstained with hematoxylin. Images were acquired using an Olympus BX51 microscope. Under microscopic examination, hematoxylin-stained nuclei displayed a blue coloration, while DAB-positive expression regions exhibited a brown-yellow signal. Quantitative image analysis was conducted using ImageJ software.

Cell Culture and Transfection

Gingival fibroblasts(GFs) were isolated from healthy gingival tissues of volunteers and cultured in Dulbecco's Modified Eagle Medium supplemented by 10% fetal bovine serum (FBS). Cells were maintained at 37°C in a 5% CO_2 atmosphere. For experiments, cells were seeded in 6-well plates and transfected at 80–90% confluency. Overexpression of AIM2: Gingival fibroblasts were transfected with pcDNA3.4-AIM2-3xFlag plasmid using Lipofectamine 6000 (Invitrogen), with empty vector (Vec) as the control. Cells were collected 48 hours after transfection for subsequent mRNA and protein

analysis. Knockdown of AIM2: Small interfering RNA (siRNA) was used to specifically knock down AIM2 expression, with Lipofectamine 6000 (Invitrogen) used for transfection. Non-targeting siRNA (NC) was used as a control. Cells were collected for analysis 48 hours after transfection.

Quantitative Real-Time PCR (qRT-PCR)

RNA was extracted using the EastepR Super Total RNA Extraction Kit from Promega (Beijing), followed by cDNA synthesis using the HifairR III First Strand cDNA Synthesis Kit from Yeasen Biotech (Shanghai). SYBR Green Master Mix (Yeasen, Shanghai) was used for quantitative PCR on a Roche LightCyclerR 96 platform. The 2-DDCt technique was used to determine the gene expression levels of AIM2, IL-1β and Caspase-1 using the GAPDH internal control.

Western Blot Analysis

BCA Protein Quantification Kits (Beyotime Biotech Inc) were used to quantify the proteins extracted with RIPA buffer. SDS-PAGE was used to separate samples (40 ug), which were then transferred onto PVDF membranes from Millipore. After blocking with 5% milk fat for 2 h the membranes were incubated at 4degC overnight with primary antibodies. These included AIM2 (Bioss bs5986R 1:1000), ASC (Bioss, bs-6741R, 1:1000), IL-1β(Bioss, bs-6319R, 1:1000), Cleaved Caspase-1 (Affinity Biosciences, AF4005, 1:1000), Caspase-1 (Bioss, bs-10442R, 1:1000), and β-actin (Proteintech 20536-1AP, 1:5000). Secondary antibodies conjugated with HRP were applied to anti-rabbit or anti-mouse secondary antibodies (Jackson 111-035-013, 1:10,000). Thermo Scientific's ECL detection system was used to visualize the protein bands. ImageJ software was then used to analyze them.

Immunofluorescence Staining

The cells were fixed with 4% paraformaldehyde and permeabilized by 0.5% Triton X - 100 over 20 minutes. QuickBlock™ solution (Beyotime Biotech Inc) was applied for 1 hour to block nonspecific binding. Primary antibodies targeting AIM2, IL-1β, ASC, and Caspase-1 (1:200 dilution) were incubated at 4°C overnight. Images were captured with a confocal microscopy using Alexa Fluor 594 or 488 conjugated secondary antibodies (1:500). Nuclei were counterstained in DAPI. Quantification was performed using ImageJ software.

Bioinformatics Analysis

RNA sequencing was performed on AIM2 overexpression and knockdown groups to identify differentially expressed genes (DEGs). GSEA and PPI network analysis were conducted using the STRING database and visualized with Cytoscape software to identify AIM2-regulated key proteins and pathways.

Statistical Analysis

Data were analyzed using GraphPad Prism 9.0 software. All quantitative data were expressed as mean ± SEM. Statistical differences between groups were analyzed using Student's *t*-test or Mann-Whitney *U*-test, with $P < 0.05$ deemed significant.

Results

Expression of AIM2, IFI16, NLRP3, IL-1β, ASC, and GSDMD in Saliva and Gingival Crevicular Fluid of Periodontitis Patients

In saliva and gingival Crevicular Fluid (GCF), 33 chronic periodontitis patients (CP) and 27 healthy controls were compared to determine the expression levels of AIM2, IFI16 and GSDMD. All molecules were significantly more abundant in the CP group compared with controls (Figure 1).

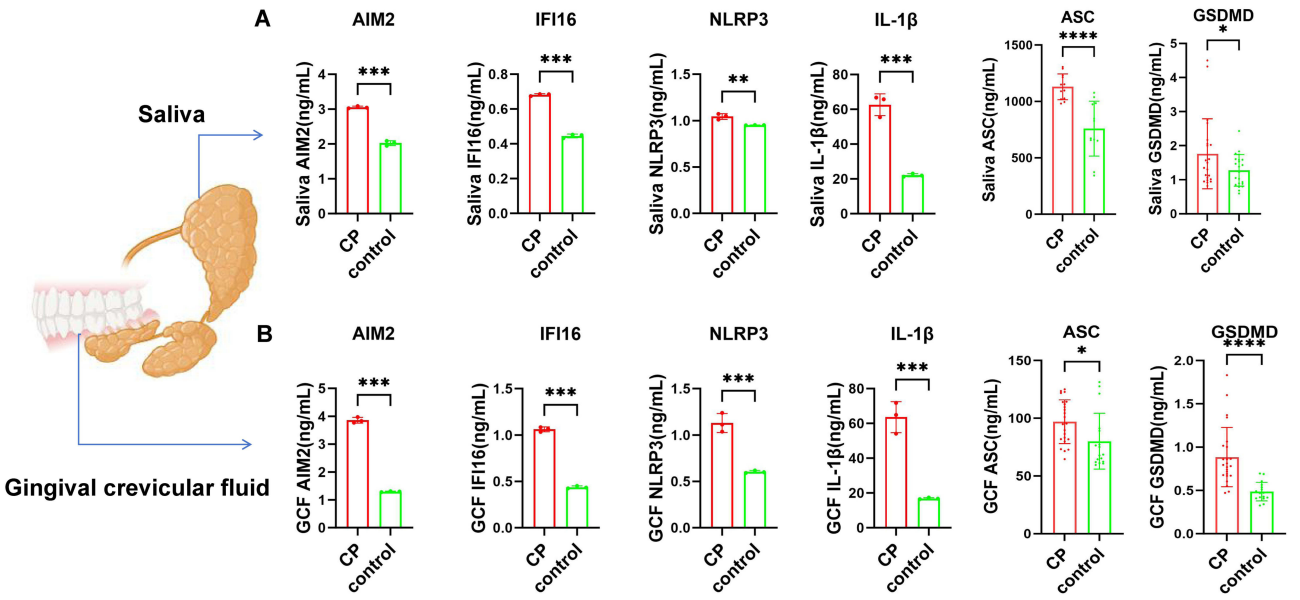

Figure 1 Expression of AIM2, IFI16, NLRP3, IL-1β, ASC, and GSDMD in Saliva and Gingival Crevicular Fluid (GCF) of Periodontitis Patients. This figure compares the concentrations of inflammatory and pyroptosis-related molecules (AIM2, IFI16, NLRP3, and IL-1β) in the saliva (**A**) and GCF (**B**) of periodontitis patients (CP) and healthy controls (Control). Results show significantly higher expression levels of these molecules in periodontitis patients, with statistical significance denoted by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). The findings suggest that these molecules play a key role in the inflammatory response associated with periodontitis.

Expression of AIM2, IL-1β, ASC, and Caspase-1 in a Periodontitis Animal Model

In our periodontitis mouse model, micro-CT analysis (Figure 2A) revealed significant periodontal bone loss in the periodontitis group compared to controls, confirming the successful model establishment. Quantitative results in Figures 2B and C indicated a significant reduction in bone density and bone volume in the periodontitis group ($P < 0.001$).

Immunohistochemical analysis (Figure 2D) demonstrated a marked upregulation of AIM2 in the periodontal tissues of periodontitis mice, especially in areas of active inflammation (indicated by the red arrow). Similarly, IL-1β, ASC, and Caspase-1 expression levels were significantly higher in periodontitis mice, showing a strong co-localization with AIM2 in inflamed regions.

Histological and quantitative analyses further verified that compared with the control group, the expression of AIM2, IL-1β, ASC, and Caspase-1 was significantly upregulated in the periodontitis model.

Effects of AIM2 Overexpression and Knockdown on IL-1β, ASC, and Caspase-1 Expression in Gingival Fibroblasts

To further investigate the role of AIM2 in gingival fibroblasts (GFs), we analyzed the effects of AIM2 overexpression (OE-AIM2) and knockdown (siAIM2) on the expression of inflammatory factors IL-1β, ASC, and Caspase-1.

Figure 3 A shows the mRNA expression levels of IL-1β, ASC, and Caspase-1 in AIM2 overexpression (OE-AIM2) and control (Vec) cells. The results indicate that AIM2 overexpression significantly upregulated the mRNA levels of IL-1β, ASC, and Caspase-1, demonstrating AIM2's active role in promoting the expression of these inflammatory factors.

At the protein level, Western blot analysis in Figures 3B and C further validated this phenomenon. AIM2 overexpression not only increased the protein expression of AIM2 itself but also significantly elevated the levels of IL-1β, ASC, and Caspase-1, particularly the active form of Caspase-1 (Cleaved-Caspase-1). In its precursor form (pro-Caspase-1), Caspase-1 is inactive, but AIM2 overexpression promoted its cleavage into the active form, which is crucial for amplifying the inflammatory response. The increase in Cleaved-Caspase-1, along with the elevated ratio of Cleaved-Caspase-1 to pro-Caspase-1, indicates a significant increase in inflammasome activation, potentially enhancing the intensity of the inflammatory response.

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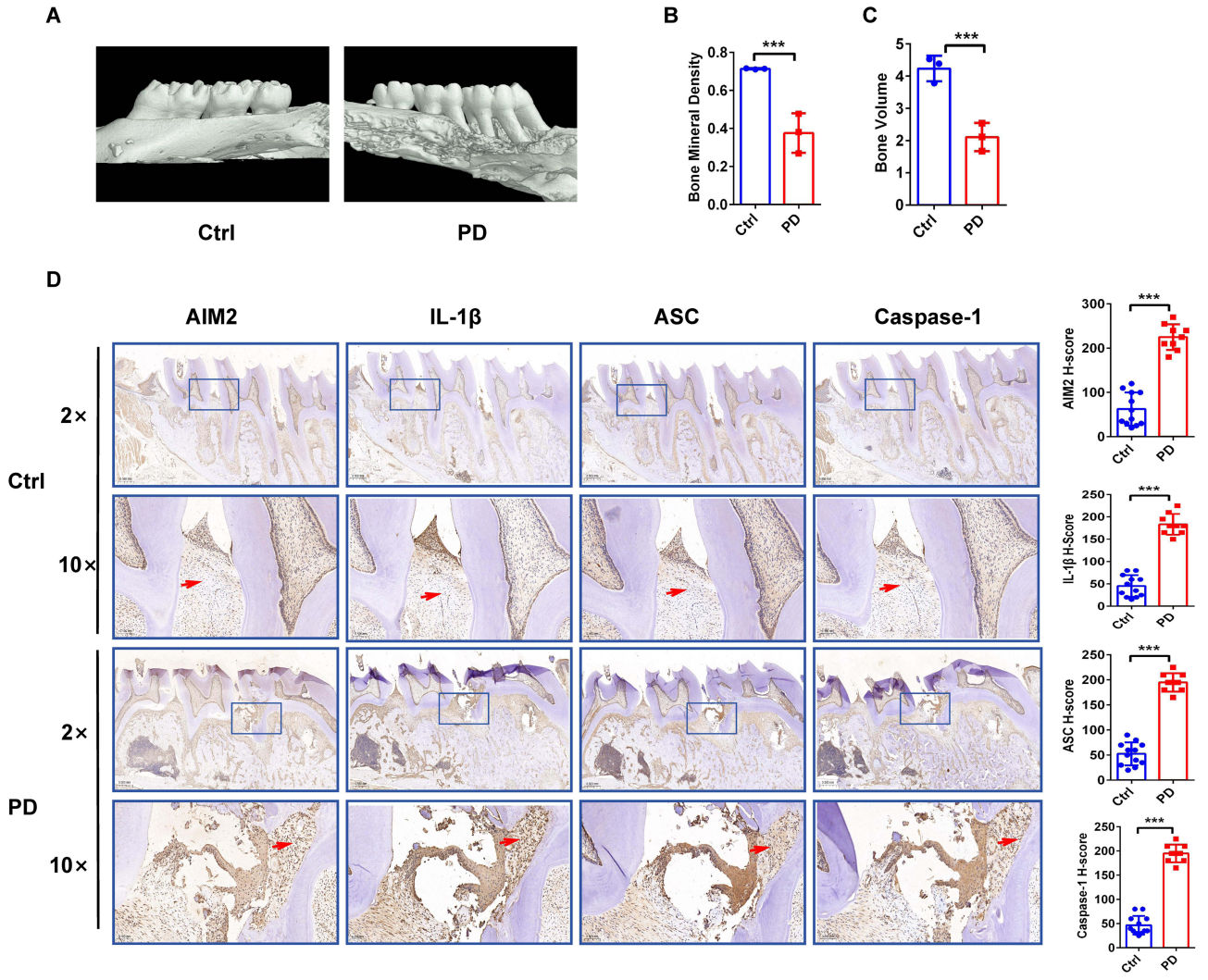

Figure 2 Expression of AIM2, IL-1β, ASC, and Caspase-1 in a Periodontitis Animal Model (**A**) Micro-CT images compare the periodontal bone structure in normal control mice (Ctrl) and periodontitis model mice (PD), showing significant bone loss in the PD group. (**B**) Quantitative analysis reveals significantly lower bone mineral density (BMD) in the periodontitis group compared to controls (***$P < 0.001$). (**C**) Bone volume (BV) is also significantly reduced in the periodontitis group (***$P < 0.001$). (**D**) Immunohistochemistry demonstrates significantly higher expression of AIM2, IL-1β, ASC, and Caspase-1 in periodontal tissues of periodontitis mice, with representative images at low (2×) and high (10×) magnification. The blue box indicates the enlarged area, and red arrows highlight regions of high expression in inflamed tissues. Quantitative data are presented as mean ± SEM (***$P < 0.001$). These results underscore the structural and molecular changes associated with periodontitis, emphasizing the role of inflammatory and pyroptosis-related markers in disease progression.

Conversely, Figure 3D displays the mRNA expression levels of IL-1β, ASC, and Caspase-1 in AIM2 knockdown (siAIM2) and control (NC) cells. After AIM2 knockdown, the mRNA levels of these inflammatory factors were significantly reduced, further supporting AIM2's critical role in regulating the expression of these factors.

Western blot results in Figures 3E and F further illustrate the impact of AIM2 knockdown on protein expression. Knockdown of AIM2 led to a significant reduction in the protein levels of IL-1β, ASC, Caspase-1, and Cleaved-Caspase-1, indicating that AIM2 downregulation inhibited Caspase-1 activation, reduced the generation of active Caspase-1, and potentially weakened the inflammatory response.

Immunofluorescence Double Staining Reveals AIM2 Localization and Its Relationship with Inflammatory Factors

Immunofluorescence double staining was performed to explore AIM2 localization in gingival fibroblasts and its relationship with the inflammatory factors IL-1β, ASC, and Caspase-1. Confocal microscopy allowed the visualization of the distribution and co-localization of these molecules within the cells.

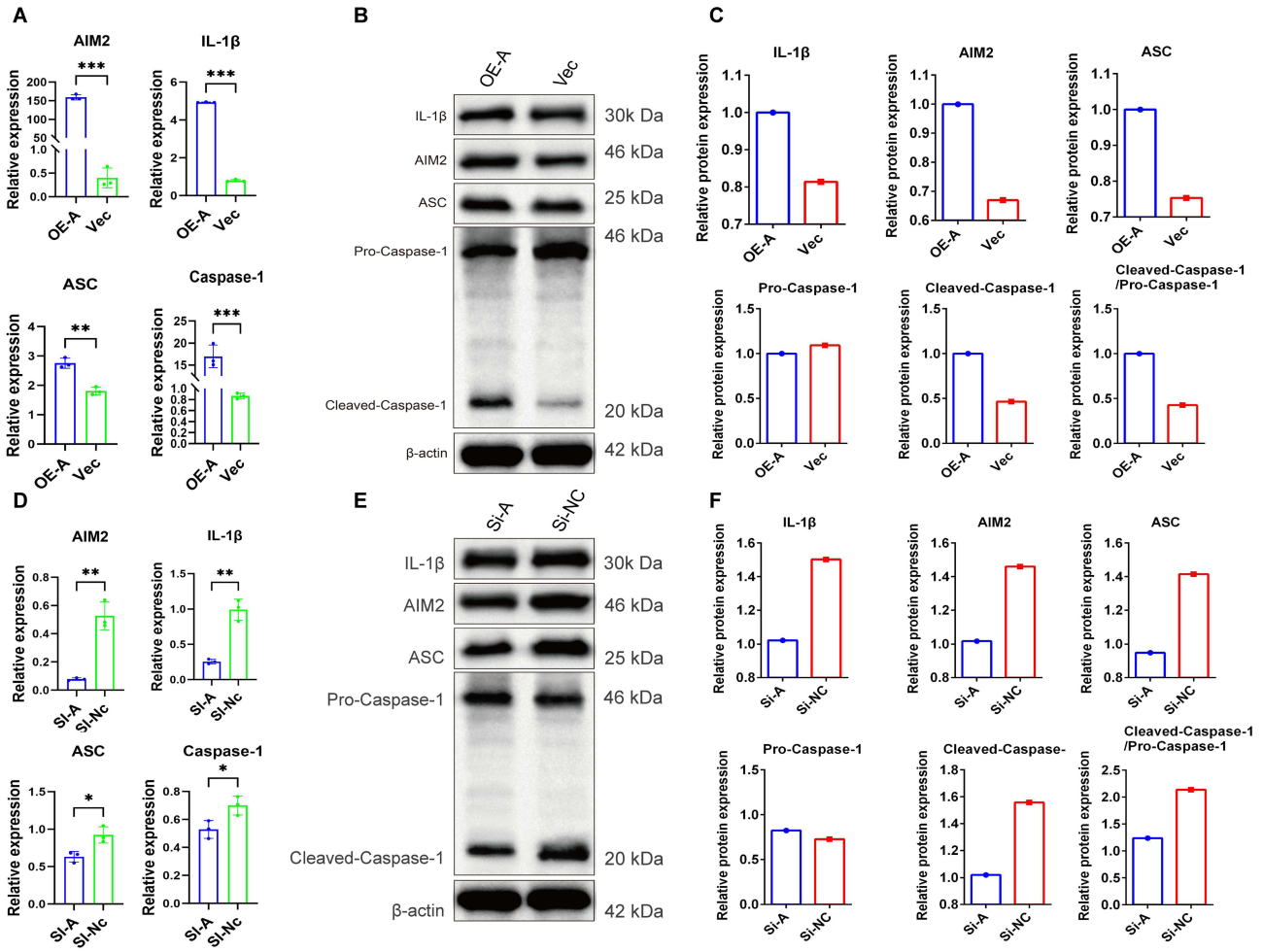

Figure 3 Effects of AIM2 Overexpression and Knockdown on IL-1β, ASC, and Caspase-1 Expression in Gingival Fibroblasts This figure demonstrates the regulatory role of AIM2 in the expression of key inflammatory mediators. (**A**) qRT-PCR results show that AIM2 overexpression (OE-A) significantly upregulates mRNA levels of IL-1β, ASC, and Caspase-1, while the control group (Vec) shows no effect. (***P* < 0.01 vs the Vec group, ****P* < 0.001 vs the Vec group) (**B**) Western blot analysis confirms increased protein levels of AIM2, IL-1β, ASC, and Cleaved-Caspase-1 upon AIM2 overexpression. (**C**) Quantitative analysis reveals a higher Cleaved-Caspase-1/pro-Caspase-1 ratio, indicating enhanced inflammation. (**D**) Conversely, AIM2 knockdown (siAIM2) significantly reduces mRNA levels of IL-1β, ASC, and Caspase-1 compared to the control (SI-NC) (**P* < 0.05 vs the SI-NC group, ***P* < 0.01 vs the SI-NC group). (**E**) Western blot shows decreased protein levels of these mediators, particularly Cleaved-Caspase-1, upon AIM2 knockdown. (**F**) Quantitative analysis confirms a reduced Cleaved-Caspase-1/pro-Caspase-1 ratio, suggesting diminished inflammation. Together, these findings highlight AIM2 as a key regulator of inflammatory responses in gingival fibroblasts.

AIM2 was primarily localized in the nuclear region, especially in AIM2 overexpression (OE-A) cells where the nuclear signal was significantly enhanced. Conversely, AIM2 knockdown (siA) cells exhibited a marked reduction in AIM2 expression. DAPI staining highlighted the cell nuclei, and merged images (Figure 4A) demonstrated AIM2's co-localization with the nuclei. Quantitative analysis confirmed a significant increase in AIM2's nuclear localization following overexpression.

The analysis of IL-1β levels indicated a substantial increase in IL-1β expression in the overexpression group, predominantly localized within the nucleus. In contrast, knockdown of AIM2 led to a significant reduction in IL-1β expression, which was primarily found in the cytoplasm (Figure 4B). Both control groups exhibited a similar cytoplasmic distribution, suggesting that AIM2 may regulate IL-1β expression, influencing the inflammatory response.

ASC exhibited a punctate distribution within the cells, indicative of potential inflammasome formation. AIM2 overexpression markedly increased ASC expression and enhanced its aggregation around the nucleus, while AIM2 knockdown significantly reduced these aggregates. In control cells, ASC was primarily expressed in the cytoplasm (Figure 4C), supporting AIM2's role in promoting inflammasome assembly.

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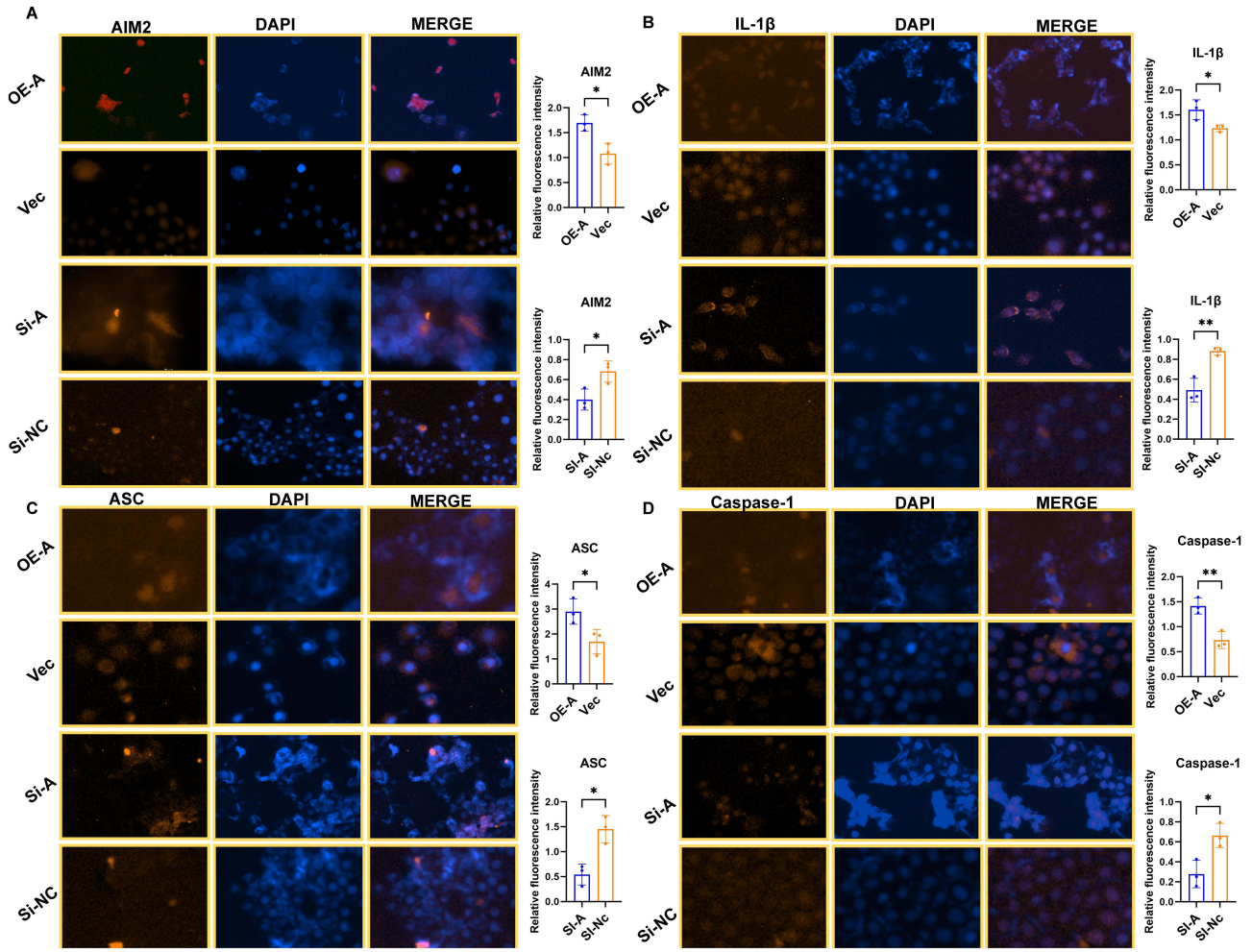

Figure 4 Immunofluorescence Double Staining Reveals AIM2 Localization and Its Relationship with Inflammatory Factors This figure illustrates the localization of AIM2 and its regulatory effects on key inflammatory factors in gingival fibroblasts. (**A**) Immunofluorescence results (400x magnification) show the distribution of AIM2 in gingival fibroblasts. AIM2 is primarily localized in the nucleus in the overexpression group (OE-A), with significantly enhanced signals (*P < 0.05 vs the VEC group), while its expression is notably reduced in the knockdown group (siA) (*P < 0.05 vs the SI-NC group). DAPI stains the nuclei, and the merged images show co-localization of AIM2 with the nucleus. Quantitative analysis indicates that AIM2 overexpression significantly increases its nuclear localization. (**B**) Immunofluorescence results for IL-1β show that IL-1β expression is significantly increased in the overexpression group and mainly localized in the nucleus (*P < 0.05 vs the VEC group). AIM2 knockdown significantly reduces IL-1β expression, which is primarily localized in the cytoplasm (**P < 0.01 vs the SI-NC group). In the control groups (Vec and si-NC), IL-1β is also primarily expressed in the cytoplasm, suggesting that AIM2 may regulate IL-1β expression to influence the inflammatory response. (**C**) Immunofluorescence results for ASC show a punctate distribution within the cells, suggesting the formation of inflammasome aggregates. AIM2 overexpression significantly increases ASC expression, particularly around the nucleus (*P < 0.05 vs the VEC group), while AIM2 knockdown reduces the formation of these aggregates (*:P < 0.05 vs the SI-NC group). In the control groups, ASC is also primarily expressed in the cytoplasm. (**D**) Immunofluorescence results for Caspase-1 show a punctate aggregate distribution within the cells, and the number of aggregates is significantly increased in the AIM2 overexpression group, concentrating near the nucleus (**P < 0.01 vs the VEC group). AIM2 knockdown significantly reduces Caspase-1 expression and the formation of aggregates (*P < 0.05 vs the SI-NC group). This suggests that AIM2 not only promotes Caspase-1 expression but may also regulate its activation, further amplifying the inflammatory cascade.

Caspase-1 showed a similar punctate distribution pattern, with the number of aggregates increasing significantly in AIM2 overexpression cells, particularly near the nucleus (Figure 4D). This finding suggests that AIM2 not only elevates Caspase-1 expression but also regulates its activation, further amplifying the inflammatory response. In contrast, AIM2 knockdown significantly decreased both Caspase-1 expression and the formation of aggregates.

RNA Seq Uencing Reveals Key Pathways and Molecular Mechanisms Regulated by AIM2

RNA-seq analysis of AIM2-overexpressing (OE-AIM2) and AIM2-knockdown (siAIM2) gingival fibroblasts, followed by GO and KEGG enrichment analyses, revealed distinct functional roles for AIM2. Overexpression of AIM2 primarily influenced biological processes and pathways related to immune response, inflammation, and gene regulation, including "antimicrobial peptide production", "regulation of inflammatory response", "inflammasome assembly", and "NF-κB

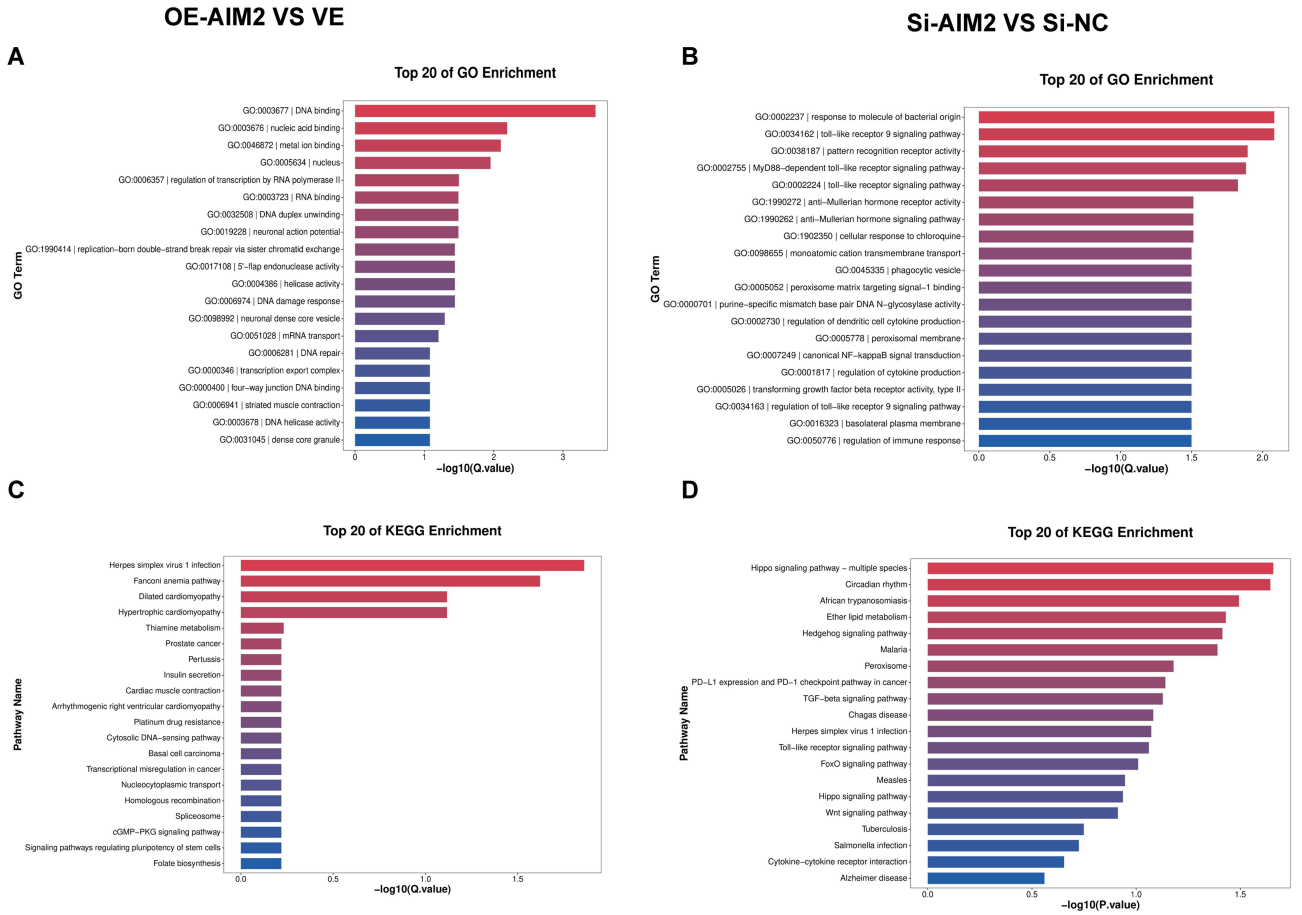

Figure 5 RNA Sequencing Reveals Key Biological Pathways and Molecular Mechanisms Regulated by AIM2 (**A**) GO enrichment analysis of gingival fibroblasts after AIM2 overexpression (OE-AIM2). Results show that AIM2 overexpression significantly influences biological processes related to immune response, inflammation, and gene regulation, with "antimicrobial peptide production", "regulation of inflammatory response", and "DNA repair" being the top enriched GO terms. (**B**) GO enrichment analysis of gingival fibroblasts after AIM2 knockdown (siAIM2). The significantly enriched biological processes include "cell cycle regulation", "apoptotic process", and "maintenance of genome stability", suggesting AIM2's role in these key cellular functions. (**C**) KEGG pathway enrichment analysis of gingival fibroblasts after AIM2 overexpression. Cells with AIM2 overexpression are significantly enriched in pathways such as "inflammasome assembly", "NF-κB signaling pathway", and "apoptosis", suggesting that AIM2 may promote inflammation and cell death through these pathways. (**D**) KEGG pathway enrichment analysis of gingival fibroblasts after AIM2 knockdown. Cells with AIM2 knockdown are significantly enriched in pathways such as "cell cycle regulation", "DNA repair", and "autophagy", indicating that AIM2 may suppress these pathways.

signaling (Figure 5A and C)". In contrast, AIM2 knockdown significantly enriched processes and pathways associated with "cell cycle regulation", "apoptosis", "genome stability maintenance", "DNA repair", and "autophagy". These findings highlight AIM2's critical involvement in immune-inflammatory responses and cellular homeostasis, suggesting its potential role in diseases linked to immune dysregulation and genomic instability (Figure 5B–D).

GSEA Enrichment and Protein-Protein Interaction Network Reveal Potential Mechanisms Regulated by AIM2

The Disease Ontology (DO) enrichment analysis revealed that AIM2 overexpression was significantly associated with disease categories such as "multiple endocrine neoplasia type 1", "polycystic ovary syndrome", "infertility", and "autoimmune diseases (Figure 6A)", while AIM2 knockdown enriched categories included "hereditary tumor syndromes", "mitochondrial dysfunction", and "neurodegenerative diseases (Figure 6B)". Gene set enrichment analysis (GSEA) further demonstrated that AIM2 overexpression was linked to periodontitis and systemic diseases, including cardiovascular (eg, acute myocardial infarction, hypertension), metabolic (eg, diabetes), neurological (eg, Alzheimer's disease), and respiratory diseases, whereas AIM2 knockdown showed reduced enrichment for these conditions (Figure 6A and B). PPI network analysis identified key molecular interactions: under AIM2 overexpression, networks involving THOC2-PNN/SETX, SETX-ATM, PNN-RBM25,

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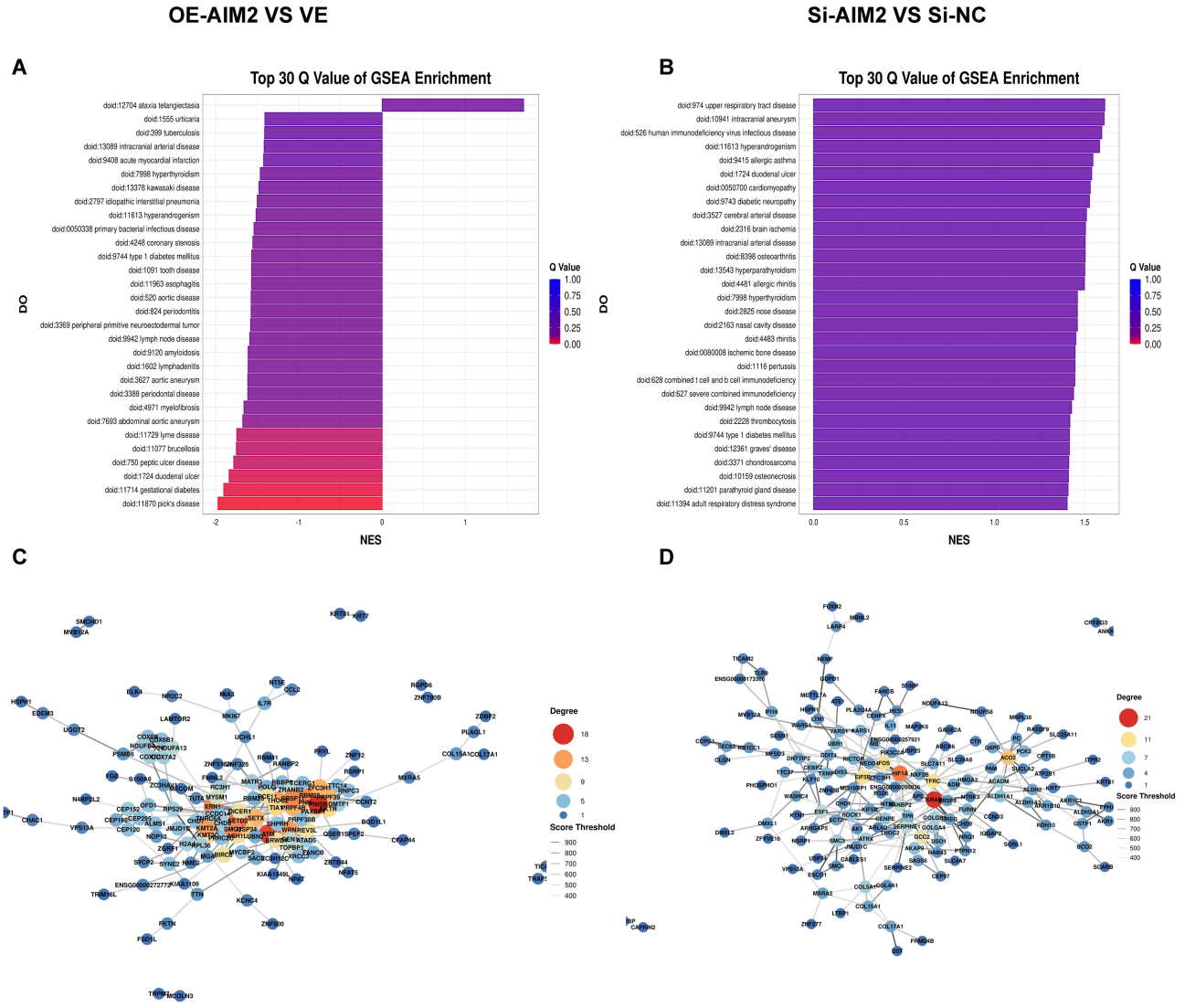

Figure 6 GSEA Enrichment and Protein-Protein Interaction Network Reveal Potential Mechanisms Regulated by AIM2 This figure shows the molecular mechanisms regulated by AIM2 based on GSEA enrichment analysis and PPI network analysis. (**A** and **B**) display the top 30 significantly enriched Disease Ontology (DO) terms and their corresponding Normalized Enrichment Score (NES) and Q values after AIM2 overexpression and knockdown. AIM2 overexpression is associated with various systemic diseases, including periodontitis and cardiovascular diseases, suggesting AIM2 may serve as a key link between periodontitis and systemic conditions. In contrast, AIM2 knockdown significantly reduces the enrichment of disease-related terms, indicating potential protective effects. (**C** and **D**) show the PPI networks for AIM2 overexpression (**C**) and knockdown (**D**), identifying key protein interactions altered by changes in AIM2 expression. In the overexpression network (**C**), significant interactions include: THOC2-PNN/THOC2-SETX: involved in RNA metabolism and transcription regulation. SETX-ATM: related to DNA damage response and genome stability. PNN-RBM25: involved in RNA splicing and post-transcriptional regulation. ATM-WRN/GEN1: involved in DNA repair pathways. In the knockdown network (**D**), core interactions are significantly reduced, highlighting AIM2's crucial role in gene regulatory networks. Key interactions include:ACO2-PCK2/SLC25A11: associated with mitochondrial function and metabolic pathways. KRAS-APC/ALDH1A1: involved in tumor suppression, cell proliferation, and metabolism. CENPE-RANBP2/SASS6: primarily involved in regulating cell division and mitosis. THOC2-SRSF6/EIF5B: related to RNA post-transcriptional processing and protein synthesis.

and ATM-WRN/GEN1 were prominent, highlighting roles in RNA metabolism, DNA repair, and transcriptional regulation (Figure 6C). In contrast, AIM2 knockdown weakened interactions, particularly in mitochondrial function (ACO2-PCK2 /SLC25A11), cell proliferation (KRAS-APC/ALDH1A1), cell division (CENPE-RANBP2/SASS6), and RNA processing (THOC2-SRSF6/EIF5B) (Figure 6D). These findings underscore AIM2's broad influence on disease-related pathways and molecular networks, connecting it to immune, metabolic, and neurological disorders.

Discussion

This study analyzed AIM2 expression in the saliva and gingival crevicular fluid of periodontitis patients, using a mouse model and functional experiments in gingival fibroblasts to reveal AIM2's role in periodontitis and systemic diseases. The results

indicate that AIM2 expression is significantly increased in both periodontitis patients and mouse model. In addition, AIM2 facilitates expression of inflammatory media, such as IL-1β, ASC and Caspase-1 to amplify inflammatory response.[16,17] AIM2 clearly plays a central role in periodontitis, and hinted at a possible crucial link between periodontal disease and systemic disorders,[18] while the function and the mechanism of AIM2 in periodontal inflammation is less validated than that of NLRP3.[19] They signal through common pathway[s], although whether the mechanisms are the same remain[s] to be verified. Moreover, IFI16, another member of this family acting as an IFN-inducible protein family, has identical function to AIM2 in sensing intracellular pathogenic DNA to trigger inflammasomes.[20] Because of AIM2's specific role in periodontitis remains to be established, we investigated its expression and its association with the IL-1β, ASC and Caspase-1 inflammatory mediators important to the disease.

IL-1β, ASC, and Caspase-1 are essential to the inflammatory response.[21] The maturation and secretion of IL-1β (a potent pro-inflammatory cytokine) is a process that largely requires inflammasome activation and, on the whole, is mediated by Caspase-1.[22] As an adaptor protein, ASC bridges inflammasome sensors AIM2 or NLRP3 to Caspase-1 to form the active inflammasome complex and initiates the inflammatory cascade.[23] Caspase-1 is the effector protein which cleaves pro-IL-1β and other precursors to generate the active cytokine.[24] To better understand AIM2's role in inflammatory mechanisms of periodontitis, a study of co-expression patterns of AIM2, IL-1β, ASC and Caspase-1 was conducted. In our study, we have confirmed through histological and quantitative analysis, the co-expression of AIM2 with IL-1β, ASC and Caspase-1. This finding suggests that AIM2 is of central importance in modulating inflammatory response and may participate in periodontal tissue pathological damage through regulating the expression and activation of the above key inflammatory factors. These results are in agreement with recent studies and provide further confirmation of the role of AIM2 in regulating the inflammatory response in periodontitis.[25] The AIM2 (Figure 4) mainly localizes in the nuclear region by immunofluorescence double staining. Further, in over expression conditions, AIM2 localizes with inflammatory proteins such as ASC, Caspase 1 and IL 1β around the nucleus confirming its role in the inflammatory response. By the virtue of this localization, AIM2 could regulate the expression and the activation of inflammatory factors by its nuclear or perinuclear signaling, thereby influencing the pathology of periodontal disease. Finally, upon aiming to purify AIM2 during our research of its role in periodontitis, the high expression of AIM2 mRNA levels along with its interaction with the other key factors of inflammation point to a direct part of AIM2 in the initiation and amplification of the inflammatory response. Our results further cement AIM2 as a major regulator of inflammatory responses in periodontitis, and offer theoretical basis for AIM2 as a potential drug target. Further studies must be performed in other populations of patients to better define the role of AIM2 as well as examine its possible role as a therapeutic target.

Furthermore, by using GSEA enrichment analysis, we extended our association analysis between AIM2 and systemic diseases within periodontitis. It was found that the overexpression of AIM2 is linked to cardiovascular diseases (acute myocardial infarction, hypertension and cerebral artery diseases) and metabolic diseases (such as diabetes), a well as neurological diseases (such as Alzheimer's disease and cerebral ischemia).[26–28] This raises the possibility that AIM2 functions as a correlation between periodontal disease and cardiovascular disease through enhancing systemic inflammatory response, which makes contribution to the pathological correlation between these two conditions. Previous studies have shown a bidirectional relationship between cardiovascular disease and periodontitis. AIM2 could be of importance in this regard. On the other hand, the depletion of AIM2 substantially decreased enrichment to related gene sets, especially to gene sets in pathways for cardiovascular diseases. This indicates that AIM2 knockdown will be protective, slowing down disease progression. Furthermore, this finding confirms the bidirectional regulatory role of AIM2 in systemic diseases, and overexpression may exacerbate pathological processes, but knockdown may be protective.[29,30] Along with its function in the inflammatory response, AIM2 is associated with DNA damage repair. Secondly, PPI network analysis identified significant interactions between AIM2 and several proteins associated with RNA metabolism, transcriptional regulation and DNA repair (ie THOC2, SETX, ATM and WRN) upon over expression of AIM2. This suggests that AIM2 may participate in the progression of inflammation related diseases by affecting the stability of genome, possibly by these key pathways. SETX and ATM are mainly reported to be involved in DNA repair and genome stability, and these processes can affect the development of chronic diseases such as heart disease, Alzheimer's and diabetes, indirectly. In many long lived bat species, telomere length did not significantly shorten with age and there are indications that DNA repair genes like ATM and SETX are important for telomere dynamics. Accordingly, healthy aging may be maintained by ATM and SETX to preserve genome stability and

ultimately, age related diseases such as Alzheimer's disease and cardiovascular disease.[9] The work demonstrates that, through the ATM/p53 signaling pathway, WRN plays an important role in inhibiting oxidative stress induced apoptosis in human lens epithelial cells. DNA methylation has been found to downregulate WRN expression. This suggests that WRN and ATM not only have the function of DNA repair but are involved in other reactions to oxidative stress and are associated with age related disease including Alzheimer and cardiovascular disease.[31] The roles of the MRN complex and WRN protein in the ATM pathway are critical for DNA double stranded break repair by their activation. This may lead to a new understanding of other diseases, since DNA damage and repair are closely associated with the onset of aging, cancer, and neurodegenerative diseases (ie, Alzheimer's).[32] Experiments using AIM2 knockdown revealed significant changes in gene axes expression of metabolic functions, cell proliferation and cell division. Firstly, the association of the ACO2-PCK2/SLC25A11 axis with mitochondrial function and metabolic regulation is close. The key genes that are involved in tricarboxylic acid cycle and energy metabolism include ACO2 (acetyl-CoA synthase 2) and SLC25A11 (mitochondrial malate transporter). Mitochondrial dysfunction usually leads to more severe neurodegenerative diseases like Alzheimer's as studies show.[33] Gluconeogenesis is a vastly important metabolic reaction and PCK2 (phosphoenolpyruvate carboxykinase 2) is a key enzyme in that reaction and regulates cellular response to glucose metabolism.[34] Secondly, KRAS is necessary for cell proliferation and suppresses tumor progression in part through the control of the APCCDH1 E3 ligase and the deacetylase ALDH1A1. While under the PubMed and Google Scholar databases, KRAS is a known proto-oncogene that had been mutated in several types of cancer including pancreatic cancer and lung cancer.[35] APC (adenomatous polyposis coli) is a negative regulator of such Wnt signaling pathway and APC loss or mutation is a common event in the pathogenesis of colorectal cancer. ALDH1A1 (aldehyde dehydrogenase 1 family member A1) is involved in vitamin A metabolism and involved also in stem cell function and oxidative stress response.[36] In the end, the CENPE-RANBP2/SASS6 axis has an important role in cell division and mitosis. RANBP2 (RAN binding protein 2) is involved in nuclear pore complex function, SASS6 (sperm associated antigen 6), functioning in centriole duplication,[37,38] and CENPE (centromere protein E) is essential for microtubule attachment to the chromosomes during mitosis.

In summary, AIM2's regulatory role extends beyond inflammation, affecting metabolic regulation, cell proliferation, and division. These findings further emphasize AIM2's bidirectional regulatory role in systemic diseases: it may exacerbate pathological processes through overexpression, but knockdown may provide protective effects. AIM2's role in metabolism, proliferation, and cell cycle regulation, especially in the context of chronic diseases like cardiovascular disease and Alzheimer's disease, is crucial and warrants further investigation.

Limitations

Future research should address several limitations, despite the fact that this study revealed AIM2's potential role in periodontitis. Firstly, this study primarily used gingival fibroblasts as a model. Although these cells play a crucial role in periodontitis, periodontal tissue also includes other cell types, such as osteoblasts and immune cells, whose roles in the inflammatory response were not thoroughly investigated. Future studies should extend to other cell types to comprehensively uncover AIM2's mechanisms of action.

Additionally, we did not conduct detailed functional experiments in this study to validate the direct effects of AIM2 on cell senescence or inflammation. By supplementing functional experiments, especially in vivo validation using AIM2 knockout or overexpression animal models, a better understanding of AIM2's specific role in periodontitis and systemic diseases could be achieved.

In terms of bioinformatics analysis, we primarily relied on data from this study and did not integrate data from public databases for deeper exploration. Future research could enhance the generalizability and robustness of the findings by integrating data from public databases. Such supplementary studies would provide stronger evidence for AIM2 as a potential therapeutic target.

Conclusion and Outlook

As an important component of the inflammasome, AIM2 recognizes intracellular double-stranded DNA and activates an inflammatory response, playing a key role in the pathogenesis of periodontitis. Our study demonstrates that AIM2 is significantly elevated in the saliva and gingival crevicular fluid of patients with periodontitis, suggesting that it may

exacerbate periodontal tissue damage by triggering the production of inflammatory factors. Additionally, the high expression of AIM2 may connect periodontitis with systemic diseases, such as cardiovascular disease, through systemic inflammation.

Moreover, AIM2 is closely related to DNA repair and aging. When recognizing DNA damage, AIM2 activates the inflammatory response to eliminate damaged cells and maintain tissue homeostasis. However, prolonged inflammation may lead to genome instability, thereby accelerating the progression of aging-related diseases. Notably, AIM2's interactions with DNA repair proteins such as ATM and SETX suggest its potential influence on DNA repair and aging.

In conclusion, AIM2's role in periodontitis extends beyond inflammation regulation and may also influence systemic diseases by impacting DNA repair and aging processes. This study provides new research directions to develop AIM2 into a potential therapeutic target for the future.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. However, some data cannot be shared due to ethical and privacy concerns.

Ethical Approval

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and its subsequent amendments. This study was reviewed and approved by the Medical Ethics Committee of Hainan General Hospital. The animal samples used in this study were obtained from a previous experiment approved by the Ethics Committee of Hainan Medical University (Approval No: HYLL-2024-145). The samples were shared with permission from the original investigators and were used in compliance with ethical guidelines for secondary use of biological materials. All procedures involving animals were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH, USA) and the ARRIVE guidelines for reporting animal research.

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Author Contributions

Zhen Fan and Rui Chen contributed equally to this work and are considered co-first authors. Zhen Fan was responsible for conducting the in vitro experiments, while Rui Chen oversaw the human experiments. Xiaomei Xie established the periodontitis model. Zhifeng Chen contributed to data analysis and interpretation. Dan Yang assisted with bioinformatics analysis and manuscript revision. Chunbo Hao and Shan Wang, as corresponding authors, supervised the project and provided critical feedback throughout the research process.

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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

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