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Differential Expressions of ADAM28 and ADAMTSL3 in Gingival Tissue of Patients with Periodontitis

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The modulation of gene expression via DNA methylation modifications is relevant to the pathogenesis of periodontitis. This study aimed at identifying novel biomarkers in gingival tissues from periodontitis by integrally analyzing methylation profiles and gene expression data. Differential gene expressions (DGEs) of dataset GSE106090 were obtained from the Gene Expression Omnibus (GEO) database for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. DNA methylation DGEs (DM-DGEs) were analyzed from dataset GSE173082. After integrating these two datasets, expressions of common genes were validated in gingival tissues from healthy controls and periodontitis patients by real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting. GO analysis of 748 upregulated and 847 downregulated DEGs from the GSE106090 dataset revealed that immune response-regulating signaling pathway, cell-cell junction and signaling receptor activator activity as the top enriched biological process (BP), cellular component (CC) and molecular function (MF), respectively. DEGs were mainly enriched in cytokine-cytokine receptor interaction, Ras signaling pathway, and chemokine signaling pathway. There was one up-regulated mRNA with hypo-methylated gene [ADAM28 (a disintegrin and metalloproteinase 28)] and one down-regulated mRNA with hyper-methylated gene [ADAMTSL3 (a disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like-3)] after integrating GSE106090 and GSE173082 datasets. Increased ADAM28 expression was validated in gingival tissues from periodontitis patients as compared to the healthy controls with decreased ADAMTSL3 expression, which were correlated with disease stage. ADAM28 and ADAMTSL3 may act as novel biomarkers in gingival tissues from periodontitis by a comprehensive analysis of bioinformatics methods and executed validation.

Keywords: ADAM28; ADAMTSL3; DNA methylation; gene expression profiling; periodontitis
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Introduction

As the 6th most prevalent ubiquitous and irreversible chronic inflammatory disease in humans, periodontitis nowadays affects nearly 14% of the world population according to the World Health Organization (WHO) (Alsalleeh et al. 2022; Cardenas et al. 2022). Periodontitis was reported to be strongly associated with age, sex, oral hygiene, smoking status, ethnicity, lower socioeconomic status, and systemic diseases (Tabassum et al. 2022), including obesity, diabetes, nonalcoholic liver disease, and cancers (Mei et al. 2023; Zhang et al. 2023). Periodontitis with a high prevalence in older adults was pathologically caused by dysbiotic changes of the subgingival microbiota attached to the tooth, and finally leading to bone destruction, tooth movement and tooth loss if left untreated (Cardenas et al. 2022; Liaw et al. 2022). Thereafter, patients with periodontitis usually showed to affect their quality of life by the decreased self-confidence and poor esthetics (Elgendy et al. 2023).

DNA methylation is a widely studied epigenetic mechanism due to its role in disease processes (Liaw et al. 2022), which is relevant to the pathogenesis of immunoinflammatory diseases, such as periodontitis (Schulz et al. 2016; Azevedo et al. 2020). DNA methylation modifications could exhibit a more stable nature of gene regulation without changing gene sequence (Wang et al. 2021), provoke alterations in cellular functioning, and favor the risk of

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appearance and/or progression of periodontitis (Cardenas et al. 2022). For example, Toll-like receptor-2 (TLR2) upregulation in association with single CpG-sites' methylation from the TLR2 gene promoter was found in peripheric mononuclear blood cells from apical periodontitis subjects (Bordagaray et al. 2022). In addition, lower levels of DNA methylation of genes expressing pro-inflammatory cytokines have been reported in periodontitis patients resulting in their overexpression in inflamed tissues, such as interleukin-8 (IL-8) (Oliveira et al. 2009; Andia et al. 2010), IL-6 (Ishida et al. 2012) and interferon-gamma (Zhang et al. 2010). Considering many previous studies used computational biology approaches to explore the shared genetic linkages between two pathogenesis-related diseases, we, therefore, investigate the shared genetic links by utilizing bioinformatics analyses.

Differential gene expressions (DGEs) of dataset GSE106090 were obtained from the Gene Expression Omnibus (GEO) database for Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The DNA methylation DGEs (DM-DGEs) were analyzed from dataset GSE173082. Subsequently, after integrating these two sets, there was one up-regulated mRNA with hypo-methylated gene [ADAM28 (a disintegrin and metalloproteinase 28)] and one down-regulated mRNA with hyper-methylated gene [ADAMTSL3 (a disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like-3)]. This finding raised an intriguing hypothesis that ADAM28 and ADAMTSL3 in periodontitis-affected gingival tissues may be potential predictive biomarkers.

The human ADAM28 gene is located on chromosome 8p24.2, which was reported to be overexpressed by carcinoma cells, being biomarker for the diagnosis of several cancer types (Hubeau et al. 2020). Moreover, ADAM28 have a potential ability to participate in tooth development (Zhao et al. 2006). ADAMTSL3 (also known as punctin-2 or KIAA1233) as a member of the ADAMTS superfamily of proteins (Adjei et al. 2023) is widely expressed, with highest expression in liver, kidney, heart and skeletal muscle, playing a role in cell-matrix interactions by northern blot analysis of RNA from human adult tissues (Zhang et al. 2023). However, it is not yet fully understood whether ADAM28 and ADAMTSL3 play a significant role in periodontitis. The aims of this study were to validate ADAM28 and ADAMTSL3 expressions by real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting in gingival tissues from periodontitis patients and to find correlations between expressions of ADAM28 and ADAMTSL3 and disease stage.

Materials and Methods

Microarray data

The datasets (GSE106090 and GSE173082) were downloaded from the NCBI's on-line resource Gene Expression Omnibus (GEO), which were summarized in Table 1. GEO2R online analysis tool was used to analyze the differential expression genes (DEGs) (GSE106090) or DNA methylation DEGs (DM-DEGs, GSE173082) in gingival tissues from periodontitis patients and healthy individuals (adjusted $P < 0.05$ and $|\log FC| \geq 1.0$). Subsequently, we carried out the KEGG enrichment and GO function assessment among these DEGs obtained from GSE106090 dataset by the Database for Annotation, Visualization and Integrated Discovery (DAVID) web tool (Sherman et al. 2022) to summarize the expression patterns into biologically relevant categories. The web tool (http://jvenn.toulouse.inra.fr/app/example.html) (Bardou et al. 2014) was used to obtain the Venn diagram to integrate the two sets of data for common genes.

Table 1. Summary of datasets containing differential mRNA transcription (GSE106090) or differential DNA methylation (GSE173082) in gingival tissues from periodontitis patients and healthy individuals.

GEO ID	GSE106090	GSE173082
Platform	GPL21827	GPL21145
Experiment type	Expression profiling by array	Methylation profiling by genome tiling array
Periodontitis		
n	6	12
Sex (female/male)	3/3	6/6
Age (years)	47.3 ± 10.8	56.0 ± 14.9
Healthy individuals		
n	6	12
Sex (female/male)	2/4	10/2
Age (years)	33.2 ± 8.0	48.0 ±15.7

Age was shown as mean ± SD.

Ethical statement

This current study was performed in accordance with the Declaration of Helsinki and obtained ethical board approval by our hospital. The written informed consent was obtained from all participants after explaining about the clinical dental examination, collection of human gingival tissue samples and experimental procedures.

Collection of gingival biopsies

Gingival tissue biopsies were collected from 77 subjects with periodontitis (tooth sites with probing depth ≥ 6 mm, clinical attachment level ≥ 5 mm, and bleeding on probing, periodontitis group) and 77 healthy controls (no signs of periodontal disease, with no gingival/periodontal inflammation, a probing depth ≤ 3.0 mm, a clinical attachment level ≤ 3.5 mm, and no bleeding on probing) (Zhang et al. 2023). Patients with stage II to IV periodontitis was determined by a new periodontitis classification developed by a consensus conference between American Academy of Periodontology (AAP) and European Academy of Periodontology (EFP) (Papapanou et al. 2018), depending on the severity of the disease at presentation and its management complexity (Table 2). Individuals were excluded if they 1) had inflammatory or genetic diseases; 2) received anti-inflammatory treatment, or treatment with antibiotics in the preceding three months; 3) were pregnancy or lactation; and 4) underwent periodontal surgery within the past 6 months. For prosthetic or esthetic reasons, gingival tissues from healthy controls were collected during gingivectomy procedures under asepsis and local anesthesia. The tissue samples from periodontitis group were collected during the Modified Widman flap procedure under local anesthesia and total asepsis as previously described (Ramfjord and Nissle 1974). Gingival tissue biopsies were stored in liquid RNA stabilization reagent (RNA*later*, Applied Biosystems, Waltham, MA, USA) overnight at 4°C and thereafter at −80°C for subsequent RNA isolation.

RNA isolation and real-time quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted from the tissue specimens using the Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA), and its quality was assessed using the RNA 6000 NanoLabChip kit of the Bioanalyzer system from Agilent Technologies (Santa Clara, CA, USA). SYBR Green was used in the 7500-fast-real-time detection system to detect the mRNA levels of ADAM28 (Forward: 5'-CCTTGCACCAGGCTACACG-3'; Reverse: 5'-CCCGATGTATGGGGCTTAAAG-3') and ADAMTSL3 (Forward: 5'-GGTGGGTGCTGATAGGGATG-3'; Reverse: 5'-AACTCGGGAAGGAAATAGGCT-3'), by specific primers related to the housekeeping gene GAPDH (Forward: 5'-CTGGGCTACACTGAGCACC-3'; Reverse: 5'-AAGTGGTCGTTGAGGGCAATG-3'). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Western blotting

In order to confirm gene expression results, total protein in gingival biopsies was isolated using protein extraction buffer supplemented with a protease inhibitor cocktail for the determination of ADAM28 and ADAMTSL3 protein expressions. After the centrifugation at 12,000 rpm for 10 min, the supernatant was quantitated using the Bradford method. Total protein was then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were washed with Tris-buffered saline containing 0.5% Tween 20 (TBST) and incubated with the primary antibodies, including rabbit polyclonal against human ADAM28 antibody (1:2,000, catalog No. ABIN7150051), rabbit polyclonal against human ADAMTSL3 antibody (1:2,000, catalog No. ABIN758998) and rabbit polyclonal against human GAPDH antibody (1:1,000, catalog No. ABIN7267454) at 4°C overnight, followed by incubation with secondary horseradish peroxidase

Table 2. Periodontal staging according to the new classification of American Academy of Periodontology (AAP) and European Academy of Periodontology (EFP).

Periodontal staging	Definition	Interdental at the sites of greatest bone loss	Radiographic bone loss	Tooth loss	Local
Stage I	Early stages of attachment loss	1-2 mm	Coronal third (<15%)	No	MPD ≤ 4 mm
Stage II	Established periodontitis in which periodontal examinations can identify damages at the tooth support	3-4 mm	Coronal third (15-33%)	No	MPD ≤ 5 mm
Stage III	Periodontitis produces significant damage to the attachment apparatus and tooth loss may occur, in the absence of advanced treatment	≥5 mm	Extending to the mid-third of root and beyond	Tooth loss due to periodontitis≤4	In addition to stage II complexity, MPD≥6 mm, Vertical bone loss≥3 mm, furcation involved class ii/iii, and moderate ridge defect
Stage IV	Significant damage to periodontal support and may cause significant tooth loss, resulting in loss of masticatory function	≥5 mm	Extending to mid- third of root and beyond	Tooth loss due to periodontitis≥5	In addition to stage III complexity

MPD, maximum probing depth.

(HRP)-conjugated secondaries (1:2,000, Catalog No. ABIN3020597) for 1 h. All antibodies were purchased from 4A Biotech Co., Ltd. (Beijing, China). Membranes were developed with a detection reagent. An imaging system were submitted to densitometric analysis using the ImageQuant (Bio-Rad Laboratories, Hercules, CA, USA). Expression of GAPDH was used as a loading control.

Statistical analysis

The minimum sample size for each group was determined n = 64, which was calculated using G*Power with the power of 0.8, the level of significance α of 0.05, and the effect size of 0.5. Considering a dropout of 20%, 77 patients should be recruited in periodontitis patients and healthy controls. After checking normality using the Shapiro Wilk test for continuous data, it was expressed as means ± standard deviation (SD). Nominal variables were expressed as number [proportions (%)]. The statistics of chi-square test (nominal variables) or ANOVA /independent *t* tests (continuous data) were computed in GraphPad prism software (8.0 version, GraphPad Software Inc., San Diego, CA, USA) with a *P* value of less than 0.05 as statistically significant.

Results

Differentially-expressed genes (DEGs) filtered from GSE106090 database

We firstly investigate DEGs from GSE106090 database. We explored 748 upregulated genes and 847 down-regulated genes in gingival tissues from periodontitis as compared to healthy individuals. GO enrichment analysis of the 1,595 genes (Fig. 1A) revealed that immune response-regulating signaling pathway was the top enriched biological process (BP), followed by regulation of cell-cell adhesion, T cell activation, mononuclear cell differentiation, and leukocyte migration. Further analysis showed that the cell-cell junction, external side of plasma membrane, secretory granule membrane, and membrane raft tertiary granule were predominately cell component (CC). Moreover, DEGs in molecular function (MF) mainly included signaling receptor activator activity, receptor ligand activity, cytokine activity, glycosaminoglycan binding, and immune receptor activity. To analyze the involved functional and signaling pathways of DEGs, the results of the KEGG enrichment analysis indicated that the DEGs were mainly enriched in cytokine-cytokine receptor interaction, Ras signaling pathway, and chemokine signaling pathway, as demonstrated in Fig. 1B.

Integrated two sets of data to screen for differentially expressed genes with differential methylation level

We then explore the primary data from the DNA methylation profiling dataset GSE173082, and the result showed 2 hypomethylated DEGs (ACSS2 and ADAM28) and 4 hypermethylated DEGs (ADAMTSL3, ADAMTS14, ADRA1A, ADH4). The heatmap of these differently methylated DEGs (DM-DEGs) was displayed in Fig. 2A. Subsequently, we integrated the two sets of data (GSE106090 and GSE173082) to screen for differentially expressed genes with differential methylation level, and there was one up-regulated mRNA with hypo-methylated gene (ADAM28, Fig. 2B) and one down-regulated mRNA with hyper-methylated gene (ADAMTSL3, Fig. 2C), indicating ADAM28 and ADAMTSL3 in periodontitis-affected

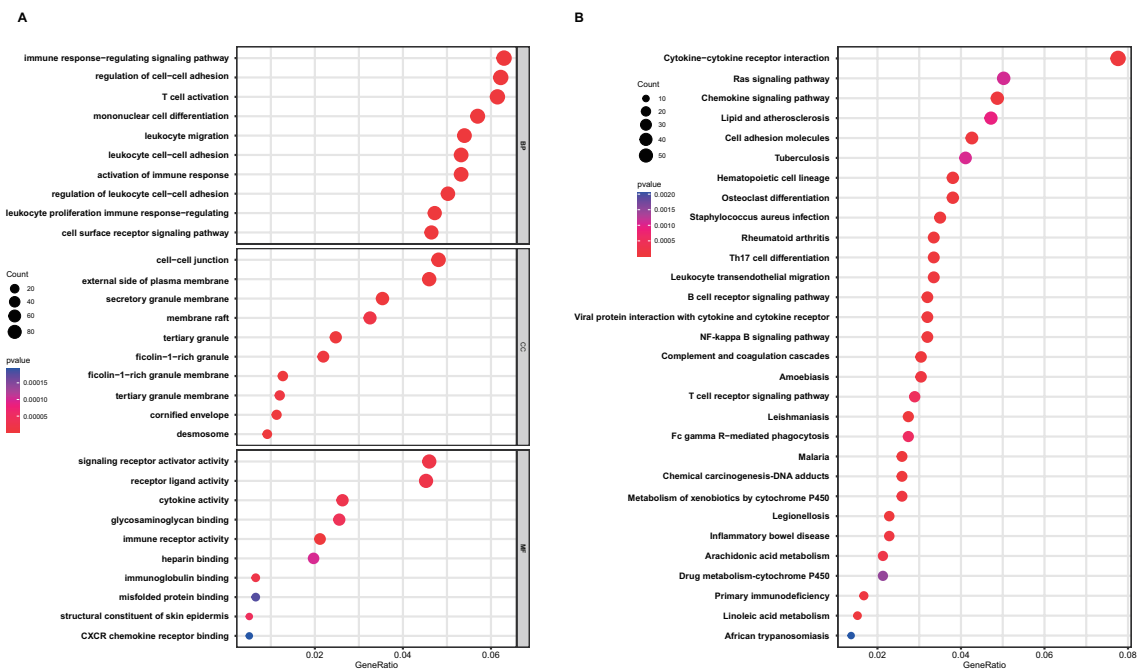

Fig. 1. Gene Ontology (GO) analysis (A) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (B) of differential expression genes (DEGs) in gingival tissues from periodontitis as compared to healthy individuals.

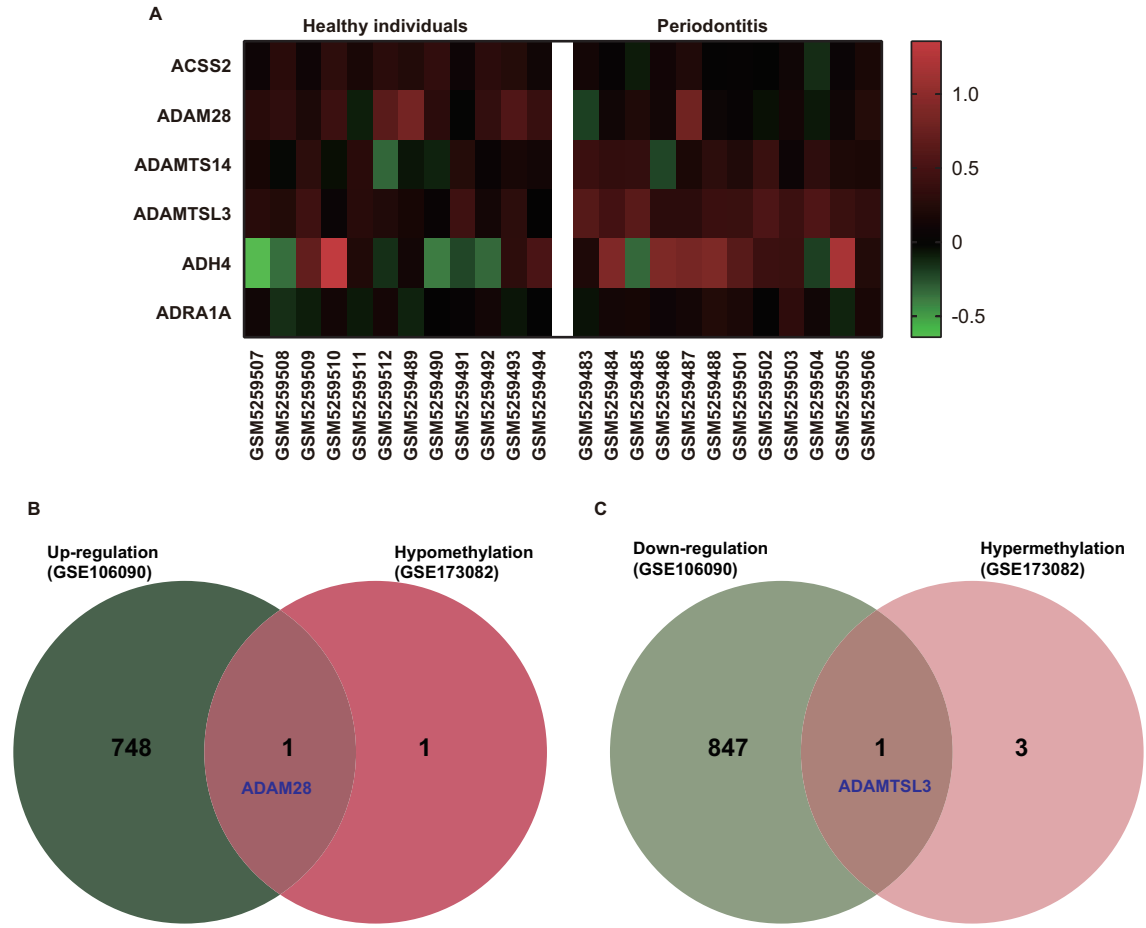

Fig. 2. Integrated two sets of data (GSE106090 and GSE173082) to screen for differentially expressed genes with differential methylation level.
(A) HapMap showing differential methylation level from dataset GSE173082. (B) One up-regulated mRNA with hypomethylated gene [ADAM28 (a disintegrin and metalloproteinase 28)]. (C) One down-regulated mRNA with hypermethylated gene [ADAMTSL3 (a disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like-3)].

gingival tissues may be potential predictive biomarkers.

Validation of the ADAM28 and ADAMTSL3 expressions in gingival tissues from periodontitis

The two genes in connection with periodontitis, ADAM28 and ADAMTSL3, were investigated further in patients with periodontitis and healthy controls. Demographic characteristics of periodontitis and healthy controls groups (Table 3), and the result showed that the mean age of participants was not significantly different in the periodontal disease group in comparison with the healthy controls (P = 0.116). Also, sex distribution (P = 0.479) and body mass index (BMI, P = 0.193) were not statistically different within and between the groups. Relative mRNA expression of ADAM28 and ADAMTSL3 in gingival tissues from healthy controls and periodontitis patients were analyzed by qRT-PCR and normalized to GAPDH. The results showed that the increased ADAM28 expression (P = 3.72E-71, Fig. 3A) was found in gingival tissues from periodontitis patients as compared to the healthy controls with decreased ADAMTSL3 expression (P = 1.75E-51, Fig. 3B). Western blotting analysis of gingival tissue from periodontitis patients and healthy controls showed higher levels of ADAM28 (P = 5.35E-61) and lower levels of ADAMTSL3 (P = 6.51E-57) in periodontitis (Fig. 3C-E).

ADAM28 and ADAMTSL3 expressions in gingival tissues were correlated with the severity of periodontitis

To better understand the distribution of ADAM28 and ADAMTSL3 expressions in patients with periodontitis at different severity, we analyzed their expression levels based on disease stages. In detail, the mRNA expression levels of ADAM28 showed a fold change of 1.07- and 1.17-fold in patients with moderate (P = 0.012) and severe (P = 5.24E-09) periodontal disease compared to mild periodontitis, respectively (Fig. 4A). Furthermore, in our patients, ADAMTSL3 mRNA expression was significantly increased in mild periodontitis patients also in comparison with moderate (P = 0.005) and severe (P = 5.11E-09) periodontitis patients (Fig. 4B). Moreover, as compared to moderate

Table 3. Demographic characteristics of periodontitis and healthy control (HC) groups.

Variable	Periodontitis (n=77)	HC (n=77)	*P*
Sex, n (%)			
Male	52 (67.53)	57 (74.02)	
Female	25 (32.47)	20 (25.98)	0.479
Age (years), mean±SD	53.77 ± 5.82	55.32 ± 6.40	0.116
Body mass index (BMI, kg/m^2), mean±SD	22.47 ± 1.84	22.06 ± 2.04	0.193

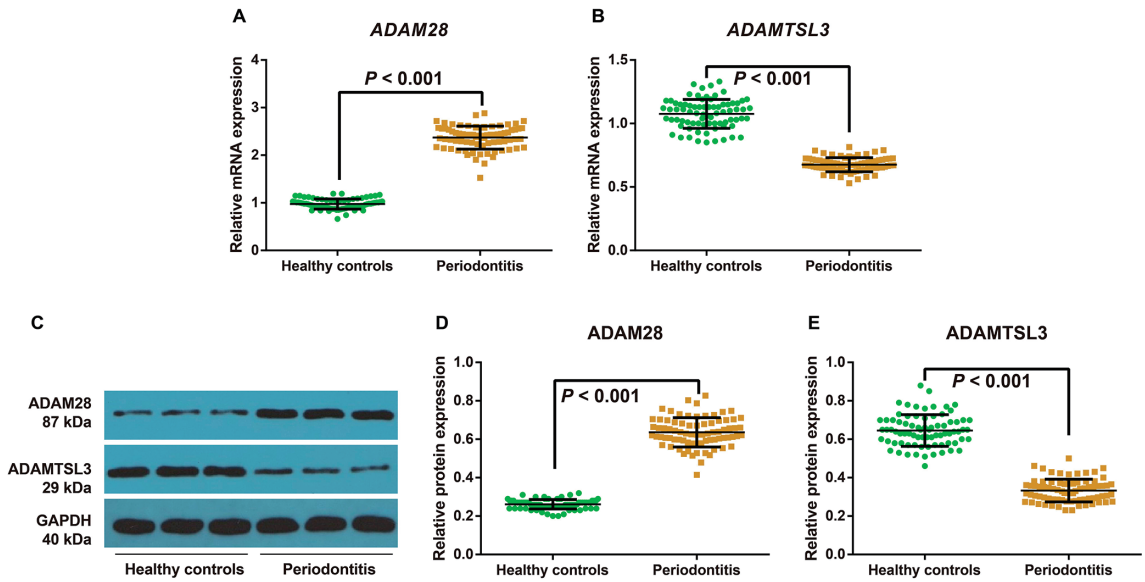

Fig. 3. Validation of the ADAM28 and ADAMTSL3 expressions in gingival tissues from periodontitis.
(A, B) Relative mRNA expression of ADAM28 (A) and ADAMTSL3 (B) in gingival tissues from healthy controls (n = 77) and periodontitis patients (n = 77) analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) and normalized to GAPDH. (C-E) Western blotting analysis of gingival tissue from periodontitis patients and healthy controls showed higher levels of ADAM28 (C, D) and lower levels of ADAMTSL3 (C, E) in periodontitis. The continuous data were presented as mean ± standard deviation (SD) and calculated by independent *t* test.

periodontitis, higher ADAM28 gene expression (*P* = 1.22E-04) and lower ADAMTSL3 gene expression (*P* = 5.73E-05) were found in severe periodontitis patients. In addition, significant increase of ADAM28 protein expression (moderate vs. mild: *P* = 0.016; severe vs. mild: *P* = 5.11E-09; severe vs. moderate: *P* = 1.30E-05, Fig. 4C) and decrease of ADAMTSL3 protein expression (moderate vs. mild: *P* = 0.006; severe vs. mild: *P* = 5.10E-09; severe vs. moderate: *P* = 1.56E-05, Fig. 4D) were observed in relation to the disease stage.

Discussion

One of the main findings in the current study identified 748 upregulated genes and 847 downregulated genes obtained from GSE106090 dataset in gingival tissues from periodontitis as compared to healthy individuals. In our study, GO enrichment analysis indicated that DEGs were mainly involved in immune response-regulating signaling pathway, cell-cell junction and signaling receptor activator activity as the top enriched BP, CC and MF, respectively. The KEGG pathway showed a strong correlation with the cytokine-cytokine receptor interaction. Altogether, the GO and KEGG enrichment analysis results provide additional evidence for the potential role of DEGs co-expressed in the pathological process of periodontitis.

Another finding of the current study from the DNA methylation profiling dataset GSE173082 showed 2 hypomethylated DEGs (ACSS2 and ADAM28) and 4 hypermethylated DEGs (ADAMTSL3, ADAMTS14, ADRA1A, ADH4). A previous study using the microarray dataset and bioinformatics tools under a high-throughput analysis showed an inverse correlation between variations in methylation and the mRNA levels of the immune genes during periodontitis (De Souza et al. 2014). There was one upregulated mRNA with hypo-methylated gene (ADAM28) and one down-regulated mRNA with hyper-methylated gene (ADAMTSL3) after integrating GSE106090 and GSE173082 datasets. Members of ADAM family belong to the superfamily of zinc-dependent metalloproteinases, known as metzincins, which also comprises MMPs (Matrix Metalloproteinases) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) (Hubeau et al.

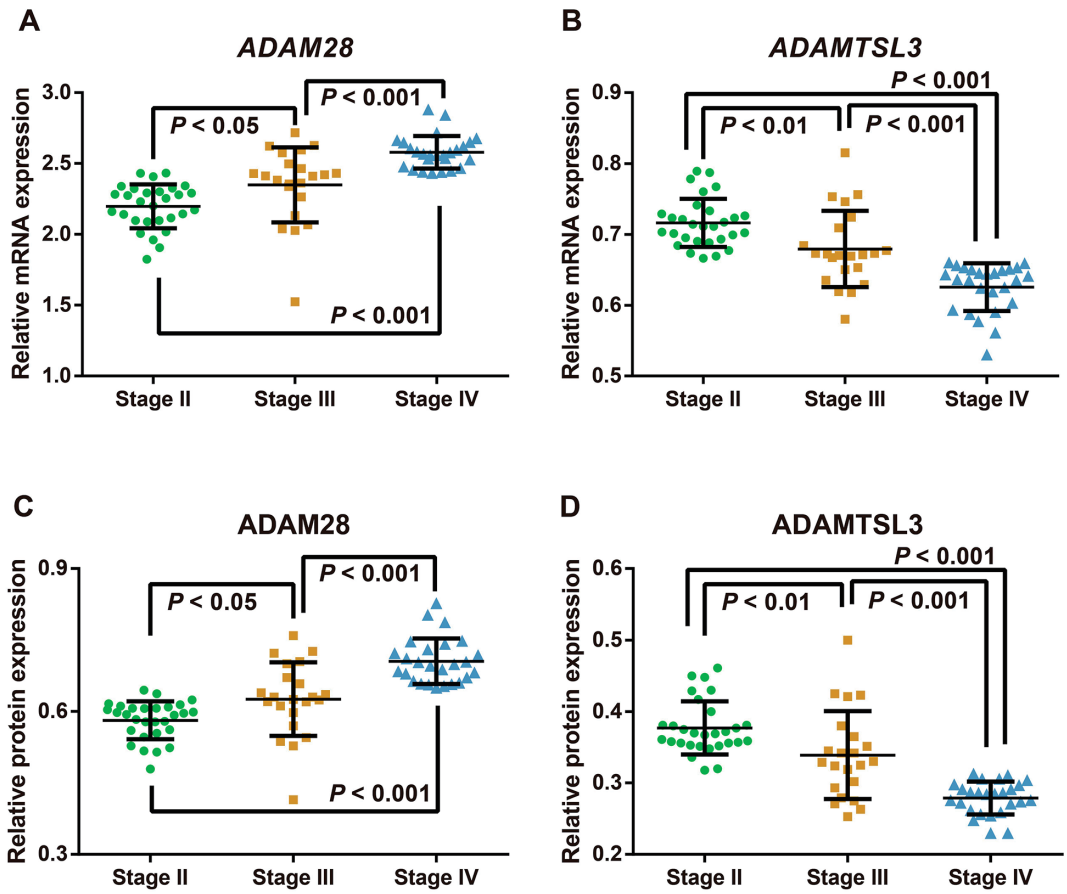

Fig. 4. ADAM28 and ADAMTSL3 expressions in gingival tissues were correlated with the severity of periodontitis. Relative mRNA (A, B) and protein (C, D) expressions of ADAM28 (A, C) and ADAMTSL3 (B, D) in gingival tissues from periodontitis at different stage. The continuous data were presented as mean ± standard deviation (SD) and calculated by ANOVA test.

2020). As reported by several previous studies, members of the ADAMTS family of metalloproteinases are associated with pathogenesis and progression of periodontal disease, such as increased ADAMTS-1, ADAMTS-4 and ADAMTS-5 mRNA expression in periodontitis (Tayman et al. 2020; Tayman and Koyuncu 2023). Members of the ADAMTS-like subfamily lack a metalloprotease domain and hence, lack proteolytic activity, but they otherwise have a similar structural organization including the ADAMTS ancillary domain, which has an essential substrate binding function in the extracellular (Hall et al. 2003).

A previous study by Zhao et al. (2006) found that ADAM28 with autocatalytic activity could regulate odontogenic mesenchymal cells through progressive reciprocal inductive interactions between the epithelium and the mesenchyme, thus participating in the regulation of tooth development. In current study, we validated that the increased gene and protein expressions of ADAM28 were found in gingival tissues from periodontitis patients as compared to the healthy controls, which were closely correlated with the severity of periodontitis. As one of the most prominent pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) was overexpressed in periodontitis patients and correlate with disease progression (Plemmenos et al. 2021). As reported, ADAM28 might activate TNF-α to release mature TNF-α in the supernatant of HEK-293 cells transfected with ADAM28 overexpression plasmid, and inhibition of ADAM28 by siRNA in macrophages resulted in a reduced mature TNF-α release (Jowett et al. 2012). We therefore speculating that ADAM28 might activate pathways leading to or supporting periodontitis-related inflammation by modulating the biological activity of mediators as TNF-α.

Additionally, ADAMTSL3 was dysregulated in several diseases. Findings of an *in-vitro* and *in-vivo* research by Rypdal et al. (2022) revealed upregulated ADAMTSL3 in failing hearts of patients, and also demonstrated a cardioprotective role for ADAMTSL3 to inhibits pathological TGF-β signaling and cardiac fibroblast phenotype, showing beneficial effects of ADAMTSL3 in heart failure. Moreover, downregulation of ADAMTSL3 displayed poor prognosis in hepatocellular carcinoma, supporting its tumor-suppressive role (Zhou et al. 2020). ADAMTSL3 was reported to be a contribution of genetic factors in type 2 diabetes patients (Minohara et al. 2021). With positive evidence of periodontal disease being implicated in diabetes, the present study was undertaken to investigate

ADAMTSL3 in periodontitis (Shetty et al. 2022). It is noteworthy to mention the chronically inflamed periodontal tissue exhibits extracellular matrix (ECM) degradation (Duplancic et al. 2022), and ADAMTSL3 as a component of the ECM (Hall et al. 2003) may be reduced in periodontitis. Our study actually brought a new point of view regarding the gene and protein expression of ADAMTSL3 in relation to the disease stage in gingival tissues from periodontitis patients.

Some limitations apply to the present investigation, including the low number of patients involving more demographic information due to the epidemiological situation with COVID-19 at the time of sampling. Moreover, further experiments such as immunohistochemistry would be performed to validate the expression of ADAM28 and ADAMTSL3 in gingival tissues of periodontitis.

In conclusion, there was one up-regulated mRNA with hypo-methylated gene (ADAM28) and one down-regulated mRNA with hyper-methylated gene (ADAMTSL3) in gingival tissues from periodontitis patients and healthy individuals after integrating GSE106090 and GSE173082 datasets. The increased ADAM28 expression was validated in gingival tissues from periodontitis patients as compared to the healthy controls with decreased ADAMTSL3 expression, both showing closely relation to the disease stage.

Author Contributions

Jin-Juan Zhu and Zhi-Hong Zhong contributed equally to this work, regarding to the design of manuscript, the collection of data, the data analysis, the preformation of figures, and the revision of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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