Construction of potential periodontitis-related miRNA-mRNA regulatory network

Zheng Zhang (✉ zhangzheng@nankai.edu.cn)

Tianjin Stomatological Hospital <https://orcid.org/0000-0002-6954-8790>

Youli Zheng

Tianjin Medical University

Xiaowei Bian

Tianjin Medical University

Mingguang Jin

Jilin University

Research article

Keywords: Periodontitis, microRNA, messenger RNA, biomarker, bioinformatic analysis

Posted Date: November 20th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-109574/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

MicroRNAs (miRNAs) are found to be involved in the pathogenesis of periodontitis, a major cause of tooth loss in adults. However, a comprehensive miRNA-mRNA regulatory network has still not been established.

Methods

One miRNA expression profile and 2 gene expression profiles were downloaded from the GEO database and analyzed using GEO2R. Candidate genes commonly appeared in differentially expressed mRNAs (DE-mRNAs) and target genes of differentially expressed miRNAs (DE-miRNAs) were selected for functional and pathway enrichment analyses using Enrichr database. Multivariate Logistic regression analysis was used to screen independent variables among candidate genes. The diagnostic values of screened genes were determined by the area under the receiver operating characteristic (ROC) curve (AUC).

Results

A total of 5 DE-miRNAs (4 upregulated and 1 downregulated) and 11 candidate genes (3 upregulated and 8 downregulated) were screened. After the construction of miRNA-mRNA regulatory network, 12 miRNA-mRNA pairs were identified. In the network, the upregulated genes were significantly enriched in cellular triglyceride homeostasis and positive regulation of B cell differentiation, whereas the downregulated genes were enriched in vesicle organization, negative regulation of lymphocyte and leukocyte migration. EPCAM and RAB30 were screened as risk factors of periodontitis. The combined AUC of these 2 genes was 0.896 (GSE10334) and 0.916 (GSE16134).

Conclusion

In this study, we established a potential periodontitis-related miRNA-mRNA regulatory network, which brings new insights into the molecular mechanisms and provides key clues in seeking novel therapeutic targets for periodontitis. In the future, more experiments need to be carried out to validate our current findings.

Background

Periodontitis is a common chronic inflammatory condition that significantly affects the integrity of the tooth-supporting tissues, including gingiva, periodontal ligament and alveolar bone[1]. Periodontitis is one of the major causes of tooth loss in adults, and in its severe form it is the sixth most prevalent disease worldwide, affecting 734 million people[2]. It is thought to be caused by a dysbiosis of the commensal oral microbiota, which subsequently leads to dysregulated immune-inflammatory response and the damage of periodontal tissue[3]. Although treatment of periodontitis is successful in the majority of cases, up to 30% of patients with moderate periodontitis respond poorly to treatment[2]. Further understanding of the pathogenesis of periodontitis might assist in the treatment of periodontitis.

MicroRNAs (miRNAs) are a group of small endogenous noncoding RNA molecules that target messenger RNA (mRNA), causing mRNA degradation or the inhibition of protein translation[4]. MiRNAs participate in numerous important biological processes, such as cell proliferation, migration, differentiation, and apoptosis[5]. Because of their biological importance, the dysfunction of specific miRNAs is significantly associated with periodontitis. For example, miRNA-146a are found to be a regulator of inflammatory responses and can contribute to the development of periodontitis[6]. MiRNA-125b regulates osteogenic differentiation of periodontal ligament cells through NKIRAS2/NF- κ B pathway in periodontitis[7]. Together, growing evidence has revealed miRNAs as one of the key players in the onset and progression of periodontitis[8].

During the last decade, advances in microarray and high-throughput sequencing technology have made it possible to quantitatively analyze miRNA expression data sets with clinical profiles, and many miRNAs have been determined in periodontitis[9; 10; 11]. However, there remain questions about how the miRNAs and their target genes interact through molecular pathways in the pathogenesis of

periodontitis. Construction of potential periodontitis-related miRNA-mRNA regulatory network will bring to light a relatively all-round molecular mechanism of miRNAs' impact in periodontitis. In the present study, we analyzed three sizeable and representative microarray profiles, including one miRNA expression profile and two gene expression profiles, and finally established a potential periodontitis-related miRNA-mRNA regulatory network to uncover potential mechanisms of periodontitis occurrence and development.

Methods

Microarray data

One miRNA expression profile (GSE54710) and 2 gene expression profiles (GSE10334 and GSE16134) with a sample size greater than 10 were downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>). Dataset GSE54710 was based on the platform of GPL15159 (Agilent-031181 Unrestricted Human miRNA V16.0 Microarray 030840) and contained 98 chronic periodontitis gingival tissue samples, 30 chronic periodontitis control samples, 61 aggressive periodontitis gingival tissue samples and 11 aggressive periodontitis control samples. Both GSE10334 and GSE16134 were based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array). The 2 gene expression profiles included 424 periodontitis tissue samples and 133 normal tissue samples in total.

Data processing

GEO2R online tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) was applied to identify differentially expressed miRNAs (DE-miRNAs) and differentially expressed mRNAs (DE-mRNAs) between periodontitis and normal tissue samples. GEO2R is an interactive web tool based on the R software "LIMMA" package. We set $|\log_2$ fold change (FC)| > 1 and P -value < 0.05 as the thresholds for identifying DE-miRNAs and DE-mRNAs.

Prediction of transcription factors (TF) of DE-miRNAs

The upstream TFs of DE-miRNAs were predicted using TransmiR database. TransmiR database (<http://www.cuilab.cn/transmir>) provides a valuable resource for the study of TF-miRNA regulation and can be used to analyze various processes, such as the evolution of the interactions, expression patterns and associated diseases of miRNAs[12].

Prediction of target genes of DE-miRNAs

The downstream target mRNAs of DE-miRNAs were predicted based on miRNet database (<https://www.mirnet.ca/miRNet/home.xhtml>), which is an easy-to use tool for comprehensive statistical analysis and functional interpretation of data from miRNAs studies.

Construction of miRNA-mRNA regulatory network

Numerous evidences have supported an inverse relationship between miRNA and target gene. Thus the miRNA-mRNA regulatory network was constructed in following steps: (1) Periodontitis specific DE-mRNAs were divided into upregulated and downregulated groups; (2) Candidate target genes were identified by conducting a combined analysis of upregulated DE-mRNAs and target genes of downregulated DE-miRNAs, or downregulated DE-mRNAs and target genes of upregulated DE-miRNAs; (3) The miRNAs that interacted with candidate target genes were selected for construction of the miRNA-mRNA regulatory network.

Functional and pathway enrichment analysis of candidate target genes

To determine the biological processes and pathways of candidate genes in the miRNA-mRNA regulatory networks, Enrichr database (<http://amp.pharm.mssm.edu/Enrichr/>) were used for Gene Ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis. The GO analysis included three categories: biological process, molecular function and cellular component. P -value<0.05 was considered as statistically significant.

Evaluation of risk and diagnostic value of candidate genes

Logistic regression analysis was used to calculate the odds ratio (OR) and 95% confidence interval (CI) for the association between candidate genes expression and the risk of periodontitis. Receiver operating characteristic (ROC) curve was performed, and the area under the curve (AUC) was measured to determine the diagnostic value of these candidate genes.

Results

Identification of DE-miRNAs

We obtained 12 DE-miRNAs (6 upregulated and 6 downregulated) between chronic periodontitis samples and control samples (**Figure 1A**), and 17 DE-miRNAs (14 upregulated and 3 downregulated) between aggressive periodontitis samples and control samples (**Figure 1B**). Only DE-miRNAs commonly appeared in two types of periodontitis were chosen as candidate DE-miRNAs. As shown in **Figure 1C,D**, 5 upregulated DE-miRNAs (hsa-miR-451, hsa-miR-486-5p, hsa-miR-671-5p, hsa-miR-223, hsa-miR-3917) and 1 downregulated DE-miRNA (hsa-miR-1246) were finally identified. The expression levels of candidate DE-miRNAs are shown in **Supplementary Table S1**, and heatmaps of DE-miRNAs are plotted in **Figure 1E,F**.

Prediction of upstream TFs of DE-miRNAs

In our study, TransmiR database was used to predict the upstream TFs of DE-miRNAs. The TFs for upregulated and downregulated DE-miRNAs were presented in **Supplementary Table S2**. For upregulated DE-miRNAs, the top 10 TFs were SCML2, PAX4, NOTCH3, POU2F1, RUNX1T1, EIF2C2, NR1H4, MYB, TWIST1 and MED12 (**Figure 2A**). For downregulated DE-miRNA, the TFs were POU5F1, TP73, TP63, TP53, AR and FOXA1 (**Figure 2B**).

Prediction of downstream target genes of DE-miRNAs

The downstream target genes of candidate DE-miRNAs were predicted by using miRNet database. We finally predicted 1858 target genes for upregulated DE-miRNAs and 318 target genes for downregulated DE-miRNA. These predicted target genes were listed in **Supplementary Table S3**. Moreover, upregulated DE-miRNA-target gene network and downregulated DE-miRNA-target gene network were successively established and presented in **Figures 3A,B**, respectively.

Identification of candidate genes

We successfully identified 182 upregulated DE-mRNAs and 75 downregulated DE-mRNAs in GSE10334 datasets (**Figure 3C**), and 250 upregulated DE-mRNAs and 100 downregulated DE-mRNAs in GSE16134 datasets (**Figure 3D**). After conducting a combined analysis of DE-mRNAs and target genes of DE-miRNAs, we further screened 8 candidate target genes for upregulated DE-miRNAs (**Figure 3E**) and 3 candidate target genes for downregulated DE-miRNA (**Figure 3F**).

For the target genes of upregulated DE-miRNAs, the candidate target genes were WASL, RIF1, PLXDC2, HPGD, EEA1, EPCAM, FOXP2 and MAMDC2. For the target genes of downregulated DE-miRNA, the candidate target genes were XBP1, RAB30 and SAMSN1. The expression levels of candidate target genes are shown in **Supplementary Table S4**, and heatmaps of candidate genes are plotted in **Figure 4A,B**.

Construction of miRNA-mRNA regulatory network

According to the predicted miRNA-mRNA pairs, the candidate miRNA-mRNA regulatory network associated with development of periodontitis were finally constructed as presented in **Figure 5**. We found that WASL gene was potentially targeted by 2 miRNAs, including hsa-mir-451 and hsa-mir-223. Hsa-mir-671-5p, one of the most significantly upregulated miRNAs, was predicted to target 3 downregulated genes, including EPCAM, FOXP2 and MAMDC2 genes. Hsa-mir-223 was also upregulated and was found to target WASL, RIF1 and PLXDC2 genes. In addition, hsa-mir-1246, the main downregulated miRNA, potentially targeted XBP1, RAB30 and SAMSN1.

GO enrichment analysis of candidate genes

GO biological process analysis revealed that candidate target genes of upregulated DE-miRNAs were significantly enriched in vesicle organization, negative regulation of lymphocyte migration and negative regulation of leukocyte migration (**Figure 6A**). For molecular function analysis, these genes were significantly enriched in protein homodimerization activity, prostaglandin E receptor activity and NAD⁺ binding (**Figure 6C**). The cellular component analysis for these genes included endocytic patch, actin cortical patch and cortical actin cytoskeleton (**Figure 6E**).

GO biological process analysis demonstrated that candidate target genes of downregulated DE-miRNA were significantly enriched in cellular triglyceride homeostasis, positive regulation of B cell differentiation and regulation of MHC class II biosynthetic process

(**Figure 6B**). For molecular function analysis, these genes were significantly enriched in enhancer binding, phosphotyrosine residue binding, protein phosphorylated amino acid binding, enhancer sequence-specific DNA binding and core promoter binding (**Figure 6D**). At last cellular component analysis for these genes revealed that they were significantly enriched in Golgi stack, cis-Golgi network, Golgi cisterna and trans-Golgi network (**Figure 6F**).

KEGG enrichment analysis of candidate genes

KEGG pathway enrichment analysis showed that candidate target genes of upregulated DE-miRNAs were significantly enriched in endocytosis, pathogenic escherichia coli infection, shigellosis, adherens junction and bacterial invasion of epithelial cells (**Figure 6G**). The enriched pathways for candidate target genes of downregulated DE-miRNAs were non-alcoholic fatty liver disease (NAFLD) and protein processing in endoplasmic reticulum (**Figure 6H**).

The risk and diagnostic value of candidate genes

To determinate the association between candidate gene expression and the risk of periodontitis, we conducted univariate Logistic regression analysis. As shown in **Table 1**, candidate target genes of upregulated DE-miRNAs were associated with decreased risk of periodontitis, whereas candidate target genes of downregulated DE-miRNA were associated with increased risk of periodontitis. Multivariate Logistic regression analysis was used to screen independent variables among candidate genes, and EPCAM and RAB30 were finally screened in both GSE10334 and GSE16134 datasets. The expression levels of EPCAM and RAB30 are shown in **Figure 7A-D**.

Then ROC curves were next established to explore the diagnostic value of EPCAM and RAB30 for periodontitis. The AUCs for EPCAM were 0.826 (GSE10334) and 0.819 (GSE16134) (**Figure 7E,H**), and for RAB30 were 0.888 (GSE10334) and 0.908 (GSE16134) (**Figure 7F,I**). When EPCAM and RAB30 were combined, the AUCs increased to 0.896 (GSE10334) and 0.916 (GSE16134) (**Figure 7G,J**).

Discussion

MiRNAs are critical regulators of immune-inflammatory response and play important roles in the pathogenesis of periodontitis[8]. MiRNAs usually function by regulating target genes within the miRNA-mRNA regulatory network[13]. The dysregulation of miRNA-mRNA regulatory network has been reported to lead to a variety of human diseases, such as pancreatitis[14], chronic obstructive pulmonary disease[4] and cancer[15]. During the past few years, many studies have intensively demonstrated that alteration of miRNAs and their downstream target genes expression levels is closely associated with the development of periodontitis[8]. However, to our knowledge, up to now, a comprehensive miRNA-mRNA regulatory network in periodontitis has still not been created. In the present study, we conducted a potential periodontitis-related miRNA-mRNA regulatory network using miRNA and mRNA data from GEO database.

Based on this regulatory network, 4 upregulated DE-miRNAs and 1 downregulated DE-miRNA were finally identified. Although hsa-miR-3917 was initially identified as one of the most significantly upregulated miRNAs, it was excluded from the miRNA-mRNA regulatory network, because none of target genes of hsa-miR-3917 was screened as the candidate target genes in this regulatory network. In addition, most of DE-miRNAs that we screened was identical with previous studies. For example, hsa-miR-223 and hsa-miR-671-5p were found to be significantly upregulated in periodontitis tissue[9; 11]. Hsa-miR-1246 expression is lower in periodontitis than that in normal tissues[11]. Previous studies have showed that several DE-miRNAs, such as hsa-miR-223, could modulate the host immune response[8], and the expression of proinflammatory cytokines[16]. Thus, we speculated that these DE-miRNAs could play a potential regulatory role in the immune-inflammatory response in periodontal disease.

As reported in previous studies, the expression of miRNA can be modulated by TFs[17; 18]. We therefore predicted the TFs in relation to these DE-miRNAs. Pax4, a TF located on chromosome 7q32, was predicted as the TF that could potentially regulate expression of a majority of upregulated DE-miRNAs. Pax4 has been demonstrated to serve as a vital player in modulating miRNA expression and function. For instance, PAX4 was reported to promote migration and invasion in human epithelial cancers by decreasing miR-144 and miR-451 expression levels. NOTCH3, a signaling receptor involved in cell differentiation, was also predicted as one of the TFs in relation to upregulated DE-miRNAs. A recent investigation has demonstrated a critical role for NOTCH3 signaling in the modulation of tissue damage during inflammation[19]. In the future, the roles of these predicted TFs in periodontitis need to be further investigated.

Candidate genes were next screened through integrating DE-mRNAs and target genes of DE-miRNAs. A total of 11 genes were identified, including 3 up-regulated genes and 8 down-regulated genes. The majority of these genes were firstly identified to act as potential modulators in periodontitis. Among them, XBP1 has been reported to play a critical role in inflammatory diseases[20]. The role of XBP1 in mammalian host defenses and the innate immune response has also been uncovered by previous studies[21]. Subsequent functional enrichment analysis revealed that the downregulated genes were enriched in 56 biological processes, such as “negative regulation of lymphocyte migration” and “negative regulation of leukocyte migration”, whereas the upregulated genes were enriched in 106 biological processes, such as “positive regulation of B cell differentiation” and “regulation of immunoglobulin secretion”. Therefore, we speculate that these candidate genes may be of great importance in the immune-inflammatory response in periodontitis.

To evaluate the diagnostic value of candidate genes, multivariate Logistic regression analysis was performed on 11 DE-mRNAs, and EPCAM and RAB30 were finally screened. EPCAM is a type I transmembrane protein that regulates cell cycle progression and differentiation[22]. The extracellular domain of EPCAM can significantly enhance osteogenesis of mesenchymal stem cells under differentiation conditions[23]. RAB30 is a novel anti-bacterial autophagic regulator which plays an important role in the autophagy regulated immune response[24]. Using ROC analyses, we found that the aforementioned two genes could be considered biomarkers for the diagnosis of periodontitis. More interestingly, combining these two genes resulted in an extremely high diagnostic value, indicating that the two-gene diagnostic model had a good performance for the clinical detection of periodontitis.

Although a potential periodontitis-related miRNA-mRNA regulatory network has been constructed in the present study, some limitations should be recognized. First, the sample size is still small for miRNA expression profile; Second, the direct relationship of the miRNA-mRNA pairs in the constructed network lacks of experimental validation. Third, miRNA and mRNA expression profiles are from the GEO database but different tissue origination, which may affect the accuracy of results. Finally, EPCAM and RAB30 were found to be gene biomarkers of periodontitis in this study, but the evaluation of periodontitis activity using gingival tissue specimens seems to be infeasible. In the future, the biomarkers associated with the two genes in the saliva and gingival crevicular fluid need to be explored in periodontitis.

Conclusion

In summary, we identified a number of DE-miRNAs and DE-mRNAs between periodontitis tissues and normal tissues. Based on these DE-RNAs, we constructed a potential periodontitis-related miRNA-mRNA regulatory network and revealed important miRNA-mRNA regulatory axes, which may contribute to the finding of molecular mechanisms underlying the initiation and development of periodontitis.

Abbreviations

AUC, area under the curve; CI, confidence interval; DE-miRNAs, differentially expressed miRNAs; DE-mRNAs, differentially expressed mRNAs; FC, fold change; GEO, Gene Expression Omnibus; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNAs, MicroRNAs; mRNA, messenger RNA; OR, odds ratio; ROC, Receiver operating characteristic; TF; transcription factors.

Declarations

Ethics approval and consent to participation

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The detailed data supporting the present study can be obtained upon reasonable request.

Competing interests

The authors declared that there is no conflict of interest in this work.

Funding support

The work was supported by the Science and Technology Foundation of Tianjin Stomatological Hospital (# 2019BSZD02) and the Natural Science Foundation of Tianjin, China (# 20JCQNJC00200).

Authors' contributions

Z.Z and M.J conceived and designed the study, Y.Z and X.B downloaded the GEO datasets and statistically analyzed and summarized the data, Z.Z and Y.Z drafted and wrote the manuscript. M.J developed the search strategy and reviewed drafts of the paper. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors would like to thank the anonymous reviewers for their valuable comments and the constructive criticism that helped in the improvement of the manuscript.

References

- [1] G. Hajishengallis, Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 15 (2015) 30-44.
- [2] J. Marchesan, M.S. Ginary, L. Jing, M.Z. Miao, S. Zhang, L. Sun, T. Morelli, M.H. Schoenfish, N. Inohara, S. Offenbacher, and Y. Jiao, An experimental murine model to study periodontitis. *Nat Protoc* 13 (2018) 2247-2267.
- [3] D.F. Kinane, P.G. Stathopoulou, and P.N. Papapanou, Periodontal diseases. *Nat Rev Dis Primers* 3 (2017) 17038.
- [4] H. Tasena, A. Faiz, W. Timens, J. Noordhoek, M.N. Hylkema, R. Gosens, P.S. Hiemstra, A. Spira, D.S. Postma, G.W. Tew, M.A. Grimbaldeston, M. van den Berge, I.H. Heijink, and C.A. Brandsma, microRNA-mRNA regulatory networks underlying chronic mucus hypersecretion in COPD. *Eur Respir J* 52 (2018).
- [5] W. Lou, B. Ding, L. Xu, and W. Fan, Construction of Potential Glioblastoma Multiforme-Related miRNA-mRNA Regulatory Network. *Front Mol Neurosci* 12 (2019) 66.
- [6] S. Zhao, Y. Cheng, and J.G. Kim, microRNA-146a downregulates IL-17 and IL-35 and inhibits proliferation of human periodontal ligament stem cells. *J Cell Biochem* 120 (2019) 13861-13866.
- [7] N. Xue, L. Qi, G. Zhang, and Y. Zhang, miRNA-125b Regulates Osteogenic Differentiation of Periodontal Ligament Cells Through NKIRAS2/NF-kappaB Pathway. *Cell Physiol Biochem* 48 (2018) 1771-1781.
- [8] X. Luan, X. Zhou, A. Naqvi, M. Francis, D. Foyle, S. Nares, and T.G.H. Diekwisch, MicroRNAs and immunity in periodontal health and disease. *Int J Oral Sci* 10 (2018) 24.
- [9] Y. Ogata, S. Matsui, A. Kato, L. Zhou, Y. Nakayama, and H. Takai, MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. *J Oral Sci* 56 (2014) 253-60.
- [10] Y.H. Lee, H.S. Na, S.Y. Jeong, S.H. Jeong, H.R. Park, and J. Chung, Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. *Biocell* 35 (2011) 43-9.
- [11] C. Stoecklin-Wasmer, P. Guarnieri, R. Celenti, R.T. Demmer, M. Kebschull, and P.N. Papapanou, MicroRNAs and their target genes in gingival tissues. *J Dent Res* 91 (2012) 934-40.
- [12] J. Wang, M. Lu, C. Qiu, and Q. Cui, TransmiR: a transcription factor-microRNA regulation database. *Nucleic Acids Res* 38 (2010) D119-22.
- [13] B. Zhang, MicroRNA: a new target for improving plant tolerance to abiotic stress. *J Exp Bot* 66 (2015) 1749-61.

[14] D. Wang, L. Xin, J.H. Lin, Z. Liao, J.T. Ji, T.T. Du, F. Jiang, Z.S. Li, and L.H. Hu, Identifying miRNA-mRNA regulation network of chronic pancreatitis based on the significant functional expression. *Medicine (Baltimore)* 96 (2017) e6668.

[15] M.N. Uddin, M. Li, and X. Wang, Identification of Transcriptional Markers and microRNA-mRNA Regulatory Networks in Colon Cancer by Integrative Analysis of mRNA and microRNA Expression Profiles in Colon Tumor Stroma. *Cells* 8 (2019).

[16] S. Matsui, and Y. Ogata, Effects of miR-223 on expression of IL-1beta and IL-6 in human gingival fibroblasts. *J Oral Sci* 58 (2016) 101-8.

[17] L.E. Mullany, J.S. Herrick, R.K. Wolff, J.R. Stevens, W. Samowitz, and M.L. Slattery, MicroRNA-transcription factor interactions and their combined effect on target gene expression in colon cancer cases. *Genes Chromosomes Cancer* 57 (2018) 192-202.

[18] W. Si, J. Shen, C. Du, D. Chen, X. Gu, C. Li, M. Yao, J. Pan, J. Cheng, D. Jiang, L. Xu, C. Bao, P. Fu, and W. Fan, A miR-20a/MAPK1/c-Myc regulatory feedback loop regulates breast carcinogenesis and chemoresistance. *Cell Death Differ* 25 (2018) 406-420.

[19] K. Wei, I. Korsunsky, J.L. Marshall, A. Gao, G.F.M. Watts, T. Major, A.P. Croft, J. Watts, P.E. Blazar, J.K. Lange, T.S. Thornhill, A. Filer, K. Raza, L.T. Donlin, A. Accelerating Medicines Partnership Rheumatoid, C. Systemic Lupus Erythematosus, C.W. Siebel, C.D. Buckley, S. Raychaudhuri, and M.B. Brenner, Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* 582 (2020) 259-264.

[20] A. Kaser, A.H. Lee, A. Franke, J.N. Glickman, S. Zeissig, H. Tilg, E.E. Nieuwenhuis, D.E. Higgins, S. Schreiber, L.H. Glimcher, and R.S. Blumberg, XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 134 (2008) 743-56.

[21] M. Duarte, P. Vende, A. Charpilienne, M. Gratia, C. Laroche, and D. Poncet, Rotavirus Infection Alters Splicing of the Stress-Related Transcription Factor XBP1. *J Virol* 93 (2019).

[22] J.Y. Tretter, K. Schorpp, E. Luxenburger, J. Trambauer, H. Steiner, K. Hadian, O. Gires, and D. Niessing, A high-content screen for small-molecule regulators of epithelial cell-adhesion molecule (EpCAM) cleavage yields a robust inhibitor. *J Biol Chem* 293 (2018) 8994-9005.

[23] Kuan, Il, C.C. Lee, C.H. Chen, J. Lu, Y.S. Kuo, and H.C. Wu, The extracellular domain of epithelial cell adhesion molecule (EpCAM) enhances multipotency of mesenchymal stem cells through EGFR-LIN28-LET7 signaling. *J Biol Chem* 294 (2019) 7769-7786.

[24] K. Nakajima, T. Nozawa, A. Minowa-Nozawa, H. Toh, S. Yamada, C. Aikawa, and I. Nakagawa, RAB30 regulates PI4KB (phosphatidylinositol 4-kinase beta)-dependent autophagy against group A Streptococcus. *Autophagy* 15 (2019) 466-477.

Tables

TABLE 1 The association between candidate gene expression and the risk of periodontitis.

Gene symbol	Target genes of upregulated DE-miRNAs								Target genes of downregulated DE-miRNA		
	WASL	RIF1	PLXDC2	HPGD	EEA1	EPCAM	FOXP2	MAMDC2	XBP1	RAB30	SAMSN1
GSE10334											
OR	0.21	0.16	0.14	0.23	0.14	0.21	0.25	0.38	5.38	14.30	3.16
95%CI	0.12-0.32	0.09-0.27	0.08-0.24	0.15-0.36	0.08-0.23	0.13-0.33	0.17-0.37	0.28-0.51	3.46-8.30	6.94-29.47	2.21-4.52
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GSE16134											
OR	0.26	0.20	0.16	0.23	0.12	0.21	0.23	0.36	6.27	17.03	3.82
95%CI	0.18-0.38	0.13-0.31	0.10-0.26	0.15-0.34	0.08-0.21	0.14-0.32	0.16-0.34	0.27-0.49	4.08-9.64	8.45-34.34	2.69-5.44
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CI, confidence interval; DE-miRNAs, differentially expressed microRNAs; OR, odds ratio.

Figures

Figure 1

Identification of differentially expressed miRNAs (DE-miRNAs). (A) Volcano plot of DE-miRNAs in chronic periodontitis (CP) and normal gingival tissue samples. (B) Volcano plot of DE-miRNAs in aggressive periodontitis (AgP) and normal gingival tissue samples. (C) Venn diagrams of overlapping upregulated DE-miRNAs between CP and AgP data. (D) Venn diagrams of downregulated upregulated DE-miRNAs between CP and AgP data. (E) Heatmap of DE-miRNAs in CP and normal gingival tissue samples. (F) Heatmap of DE-miRNAs in AgP and normal gingival tissue samples.

Figure 1

Identification of differentially expressed miRNAs (DE-miRNAs). (A) Volcano plot of DE-miRNAs in chronic periodontitis (CP) and normal gingival tissue samples. (B) Volcano plot of DE-miRNAs in aggressive periodontitis (AgP) and normal gingival tissue samples. (C) Venn diagrams of overlapping upregulated DE-miRNAs between CP and AgP data. (D) Venn diagrams of downregulated upregulated DE-miRNAs between CP and AgP data. (E) Heatmap of DE-miRNAs in CP and normal gingival tissue samples. (F) Heatmap of DE-miRNAs in AgP and normal gingival tissue samples.

Figure 2

Predicted transcription factors of differentially expressed miRNAs (DE-miRNAs). (A) Transcription factors of upregulated DE-miRNAs. (B) Transcription factors of downregulated DE-miRNAs.

Figure 2

Predicted transcription factors of differentially expressed miRNAs (DE-miRNAs). (A) Transcription factors of upregulated DE-miRNAs. (B) Transcription factors of downregulated DE-miRNAs.

Figure 3

Screen of candidate genes. (A) Upregulated DE-miRNAs-target genes network constructed using miRNet. (B) Downregulated DE-miRNAs-target genes network constructed using miRNet. (C) Volcano plot of differentially expressed mRNAs (DE-mRNAs) in GSE10334. (D) Volcano plot of DE-mRNAs in GSE16134. (E) Venn diagrams of overlapping genes from downregulated DE-mRNAs and the target genes of upregulated DE-miRNAs. (F) Venn diagrams of overlapping genes from upregulated DE-mRNAs and the target genes of downregulated DE-miRNAs.

Figure 3

Screen of candidate genes. (A) Upregulated DE-miRNAs-target genes network constructed using miRNet. (B) Downregulated DE-miRNAs-target genes network constructed using miRNet. (C) Volcano plot of differentially expressed mRNAs (DE-mRNAs) in GSE10334. (D) Volcano plot of DE-mRNAs in GSE16134. (E) Venn diagrams of overlapping genes from downregulated DE-mRNAs and the target genes of upregulated DE-miRNAs. (F) Venn diagrams of overlapping genes from upregulated DE-mRNAs and the target genes of downregulated DE-miRNAs.

Figure 4

Heatmap of candidate genes. (A) Heatmap of candidate genes in GSE10334. (B) Heatmap of candidate genes in GSE16134.

Figure 4

Heatmap of candidate genes. (A) Heatmap of candidate genes in GSE10334. (B) Heatmap of candidate genes in GSE16134.

Figure 5

The construction of miRNA-mRNA regulatory network in periodontitis.

Figure 5

The construction of miRNA-mRNA regulatory network in periodontitis.

Figure 6

Functional and pathway enrichment analysis of candidate genes in the miRNA-mRNA regulatory network. (A,C,E,G) Functional and pathway enrichment analysis of downregulated candidate genes. (B,D,F,H) Functional and pathway enrichment analysis of upregulated candidate genes.

Figure 6

Functional and pathway enrichment analysis of candidate genes in the miRNA-mRNA regulatory network. (A,C,E,G) Functional and pathway enrichment analysis of downregulated candidate genes. (B,D,F,H) Functional and pathway enrichment analysis of upregulated candidate genes.

Figure 7

The expression and receiver operating characteristic (ROC) curve of EPCAM and RAB30. (A-B) The expression of EPCAM and RAB30 in GSE10334. (C-D) The expression of EPCAM and RAB30 in GSE16134. (E-G) The ROC curve of EPCAM and RAB30 in GSE10334. (H-J) The ROC curve of EPCAM and RAB30 in GSE16134.

Figure 7

The expression and receiver operating characteristic (ROC) curve of EPCAM and RAB30. (A-B) The expression of EPCAM and RAB30 in GSE10334. (C-D) The expression of EPCAM and RAB30 in GSE16134. (E-G) The ROC curve of EPCAM and RAB30 in GSE10334. (H-J) The ROC curve of EPCAM and RAB30 in GSE16134.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1TheexpressionlevelsofDEmiRNAs.pdf](#)
- [SupplementaryTableS1TheexpressionlevelsofDEmiRNAs.pdf](#)
- [SupplementaryTableS2.pdf](#)
- [SupplementaryTableS2.pdf](#)
- [SupplementaryTableS3.pdf](#)
- [SupplementaryTableS3.pdf](#)
- [SupplementaryTableS4.pdf](#)

- [SupplementaryTableS4.pdf](#)