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MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients

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Abstract: Periodontitis is a chronic inflammatory disease caused by specific bacteria and viruses. Local, systemic, and environmental factors affect the rate of disease progression. Immune responses to bacterial products, and the subsequent production of inflammatory cytokines, are crucial in the destruction of periodontal tissue. MicroRNAs (miRNAs) are a class of small RNAs that control various cell processes by negatively regulating protein-coding genes. In this study, we compared miRNA expression in inflamed and noninflamed gingival tissues from Japanese dental patients. Total RNAs were isolated from inflamed and noninflamed gingival tissues. miRNA expression profiles were examined by an miRNA microarray, and the data were analyzed by GeneSpring GX, Ingenuity Pathways Analysis, and the TargetScan databases. Observed miRNA expression levels in inflamed gingiva were confirmed by real-time PCR. The three most overexpressed (by >2.72-fold) miRNAs were hsa-miR-150, hsa-miR-223, and hsa-miR-200b, and the three most underexpressed (by <0.39-fold) miRNAs were hsa-miR-379, hsa-miR-199a-5p, and hsa-miR-214. In IPA analysis, hsa-miR-150, hsa-miR-223, and hsa-miR-200b were associated with inflammatory disease, organismal injury, abnormalities, urological disease, and cancer. The present findings suggest that miRNAs are associated with chronic periodontitis lesions in Japanese. (J Oral Sci 56, 253-260, 2014)

Keywords: gene expression; human; inflammation; microRNA; periodontitis.

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Introduction

Periodontitis is an inflammatory disease of the tissues that support the teeth. It is caused by specific bacteria and viruses and results in progressive destruction of the periodontal ligament and alveolar bone, with pocket formation (1,2). The rate of disease progression may be increased by local, systemic, and environmental factors that influence normal host-bacteria interaction. Immune responses to bacterial products, and the subsequent production of inflammatory cytokines, are of particular importance in the destruction of periodontal tissue (1,3).

MicroRNAs (miRNAs) are single-stranded, small noncoding RNA molecules comprising approximately 22 nucleotides (4). Over 1,000 miRNAs have been identified in the human genome. They bind to the 3'-untranslated region (3'-UTR) of specific target genes and regulate expression of those genes by promoting degradation of transcribed mRNAs or inhibiting their translation (4,5). Several studies indicate that miRNAs are key regulators of cell growth and differentiation, carcinogenesis, rheumatoid arthritis (RA), and inflammation (6-9). Three recent studies compared miRNA profiles in healthy and periodontitis gingival tissues (10-12). Other studies found that comorbidity of periodontitis with obesity was associated with locally upregulated expression of

several miRNAs that share inflammatory and metabolic mRNA targets (13). For example, miRNA-146 inhibits proinflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts (14).

We used miRNA microarray analysis to compare miRNA expression profiles in healthy and periodontitis gingival tissues. In addition, we analyzed key miRNAs to identify candidate miRNA targets in inflamed gingival tissues of Japanese dental patients.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from Wako (Tokyo, Japan). Fetal calf serum (FCS), penicillin, streptomycin, and TrypLE Express were obtained from Invitrogen (Carlsbad, CA, USA). The miRNeasy Mini Kit was purchased from Qiagen (Valencia, CA, USA). The Mir-X miRNA First-Strand Synthesis Kit and SYBR Advantage qPCR Premix were purchased from Clontech (Mountain View, CA, USA). All chemicals used were of analytical grade.

Cell culture

Primary human gingival fibroblasts (HGF) were established from patient gingival connective tissue explants, as previously described (15). Cells were cultured at 37°C in a 5% CO_2/95% air atmosphere in DMEM containing 10% FCS. HGF were grown to confluence in 100-mm tissue culture dishes. The media were then changed to DMEM containing 1% FCS for 12 h, and HGF were incubated in this media with or without IL-1β (1 ng/mL) and TNF-α (10 ng/mL) for 24 h, as previously described (16-18). Total RNA was isolated from triplicate cultures and analyzed for expressions of miRNAs and U6 snRNA by real-time PCR, as described below.

RNA preparation and the miRNA microarray

Gingival tissues (containing both epithelial and connective tissues) obtained during periodontal flap surgery (inflamed gingiva from three chronic periodontitis patients) or dental implant surgery (noninflamed gingiva from three edentulous residual ridges) were used in this study. The Institutional Internal Review and Ethics Board of Nihon University School of Dentistry at Matsudo approved the study (EC03-016, EC03-041, EC10-040). Written informed consent was obtained from all participants after all procedures had been fully explained. Periodontal status was assessed using probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP). PPD and CAL were measured with a PCP-11 probe (Hu-Friedy, Chicago, IL, USA). Chronic periodontitis was defined as the presence of at least two sites with a PPD of 6 mm or greater and a CAL of more than 6 mm. The average PPD and CAL (n = 3) among patients with chronic periodontitis was 7.33 ± 1.53 mm and 9.33 ± 1.15 mm, respectively. BOP was detected at all three sites.

Total RNA was isolated using the miRNeasy Mini Kit, and RNA quality was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). miRNA expression profiling of RNA samples was performed using a human miRNA microarray 8 × 15k kit (Agilent Technologies). RNA preparation and hybridization were performed according to the Agilent user manual, and the data were imported into the GeneSpring GX software (Agilent Technologies), to identify induced and repressed genes. IPA software (Ingenuity, CA, USA) was used to analyze the results of the microarray. The identified genes were mapped to genetic networks in the IPA database and ranked by score. A detailed description of IPA can be found at www.ingenuity.com. miRNA targets were identified by IPA and the TargetScan databases.

Real-time PCR

Total RNA was used as a template for cDNA synthesis with a Mir-X miRNA First-Strand Synthesis Kit (Clontech). Quantitative real-time PCR was performed using the following primer sets: hsa-mir-150 forward, 5'-TCTCCCAACCCTTGTACCAGTG-3'; hsa-mir-223 forward, 5'-TGTCAGTTTGTCAAATACCCCA-3'; hsa-mir-200b forward, 5'-TAATACTGCCTGGTAATGATGA-3'; hsa-mir-144* forward, 5'-GGATATCATCATATACTGTAAG-3'; hsa-mir-379 forward, 5'-TGGTAGACTATGGAACGTAGG-3'; hsa-mir-222 forward, 5'-AGCTACATCTGGCTACTGGGT-3'; mRQ 3' reverse primer; U6 forward primer; and U6 reverse primer, including the Mir-X miRNA First-Strand Synthesis Kit using SYBR Advantage qPCR Premix (Clontech) in a TP800 Thermal Cycler Dice Real Time System (TaKaRa, Tokyo, Japan). The amplification reactions were performed in 25 μL of the final reaction mixture containing 2 × SYBR Advantage qPCR Premix (12.5 μL), 10 μM forward and reverse primers (final concentration, 0.2 μM), and 25 ng (2.0 μL) cDNA. To reduce variability between replicates, PCR premixes, which contained all reagents except for cDNA, were prepared and aliquoted into 0.2-mL Hi-8-tubes. The thermal cycling condition was 10 s at 95ºC with 40 cycles of 5 s at 95ºC and 20 s at 60ºC. Post-PCR melting curves confirmed the specificity of single-target amplification. The initial amount

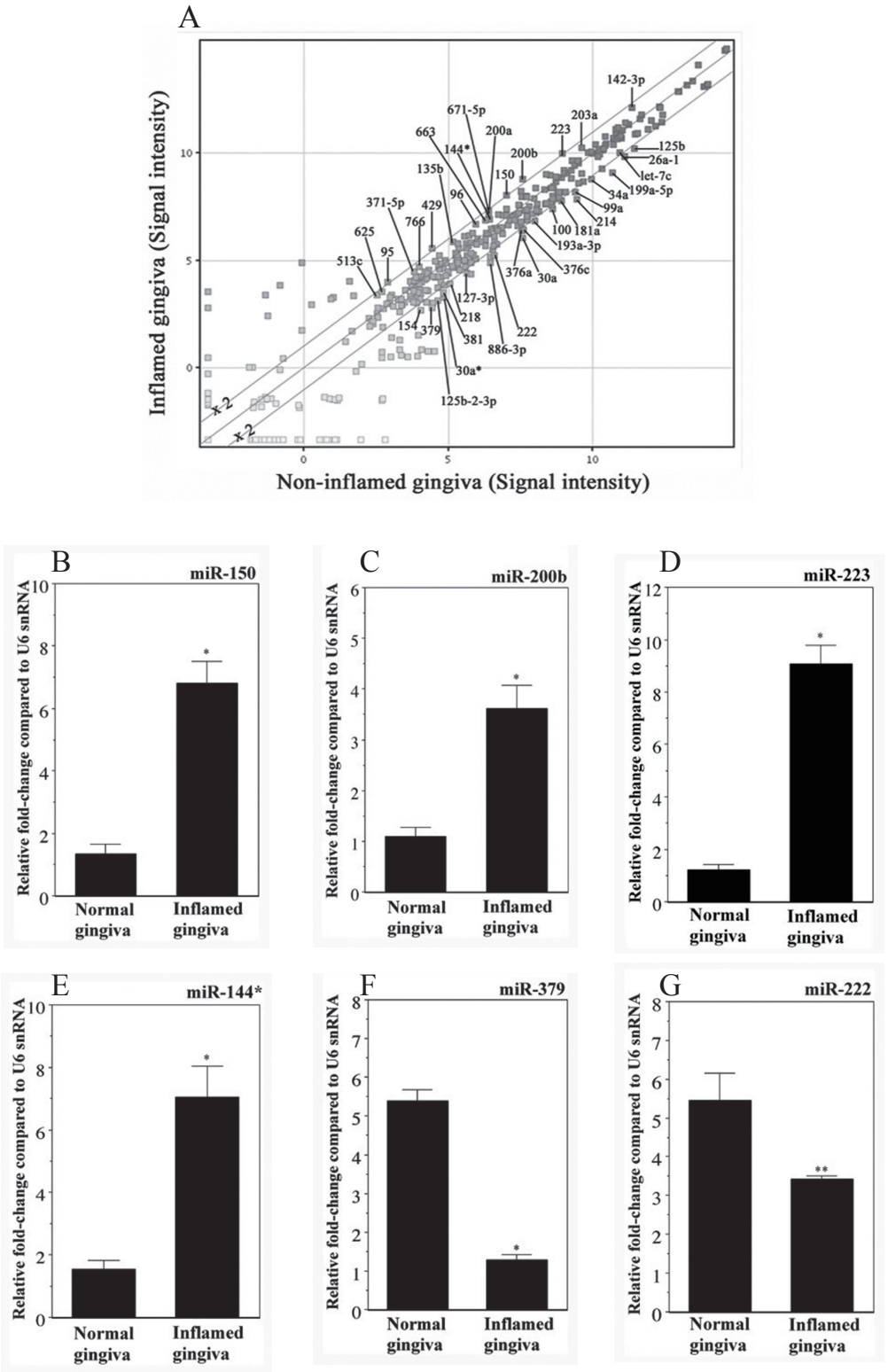

Fig. 1 **A**: Scatterplot of the results of miRNA microarray analysis of inflamed and noninflamed gingiva shows the hybridization signal pattern of miRNAs. In inflamed gingiva, 17 miRNAs were overexpressed and 22 were underexpressed. **B-G**: Expressions of miR-150 (B), miR-200b (C), miR-223 (D), miR-144* (E), miR-379 (F), and miR-222 (G) and U6 snRNA in three samples each of inflamed and noninflamed gingiva were measured by real-time PCR. Quantitative analyses of the datasets are shown with standard errors. *$P < 0.01$, significant difference versus control

Table 1 List of the top differentially expressed miRNAs in inflamed and noninflamed gingival tissues

miRNAs overexpressed in inflamed gingiva	Fold change	miRNAs underexpressed in inflamed gingiva	Fold change
hsa-miR-150	3.20	hsa-miR-379	0.36
hsa-miR-223	2.73	hsa-miR-199a-5p	0.38
hsa-miR-200b	2.72	hsa-miR-214	0.39
hsa-miR-429	2.68	hsa-miR-30a	0.41
hsa-miR-95	2.48	hsa-miR-886-3p	0.43
hsa-miR-200a	2.36	hsa-miR-125b-2*	0.43
hsa-miR-142-3p	2.33	hsa-miR-222	0.44
hsa-miR-625	2.17	hsa-miR-26a	0.45
hsa-miR-96	2.09	hsa-miR-193a-3p	0.47
hsa-miR-144*	2.08	hsa-miR-127-3p	0.47
hsa-miR-513c	2.04	hsa-miR-381	0.47
hsa-miR-203	2.02	hsa-miR-154	0.48
hsa-miR-671-5p	2.00	hsa-miR-30a*	0.48
hsa-miR-135b	1.90	hsa-miR-181a	0.50
hsa-miR-371-5p	1.76	hsa-miR-34a	0.51
hsa-miR-766	1.70	hsa-miR-376c	0.52
hsa-miR-663	1.63	hsa-miR-376a	0.53
		hsa-miR-125b	0.55
		hsa-miR-100	0.57
		hsa-miR-218	0.57
		hsa-miR-99a	0.60
		hsa-let-7c	0.65

of RNA was quantified using a standard curve, and fold expressions of miRNA relative to U6 were determined in quadruplicate (Fig. 1) or triplicate (Fig. 2).

Statistical analysis

Significant differences between noninflamed and inflamed gingival tissues were analyzed using one-way ANOVA.

Results

Differences in miRNA expression in inflamed and noninflamed gingiva

A scatterplot of the miRNA microarray results for inflamed and noninflamed gingiva is shown in Fig. 1A. In inflamed gingiva, we identified 17 overexpressed (>3.20- to 1.63-fold) miRNAs and 22 underexpressed (<0.36- to 0.65-fold) miRNAs (Table 1). The three most overexpressed miRNAs were hsa-miR-150, hsa-miR-223, and hsa-miR-200b, and the three most underexpressed miRNAs were hsa-miR-379, hsa-miR-199a-5p, and hsa-miR-214 in inflamed gingiva. Next, we used TargetScanHuman (6.2) and IPA of these miRNAs to analyze the number of predicted miRNA targets (Table 2).

Real-time PCR analysis

To confirm the results of miRNA microarray analysis, we selected 6 miRNAs for real-time PCR analysis. The results showed that expressions of hsa-miR-150, hsa-miR-200b, hsa-miR-223, and hsa-miR-144* were significantly increased in inflamed gingiva (Fig. 1B, C, D, E). In contrast, expressions of hsa-miR-379 and hsa-miR-222 were significantly decreased in inflamed gingiva (Fig. 1F, G).

Next, we used a cell culture system using HGF to determine which miRNA expressions were increased in inflamed gingiva. When we stimulated HGF by inflammatory cytokines (IL-1β and TNF-α) for 24 h, hsa-miR-150 expression was significantly increased in HGF (Fig. 2).

Network, disease, and biofunctional analyses

IPA identified two networks associated with overexpression of hsa-miR-150, hsa-miR-223, and hsa-miR-200b (Table 3A). One network is involved in Argonaute 2 (AGO2), miR-150-5p, and miR-223-3p gene expression and is related to cancer, endocrine system disorders, and gastrointestinal disease. Another network is involved in the B-cell receptor (BCR complex), cell division cycle 25C (CDC25C), cyclin-dependent kinase inhibitor 1B (CDKN1B), formin homology 2 domain containing 1 (FHOD1), jagged 1 (JAG1), myristoylated alanine-rich C-kinase substrate (MARCKS), c-Myc (MYC), polycystin-1 (PKD1), phospholipase C-gamma 1 (PLCG1), peroxisome proliferator-activated receptor alpha (PPAR-alpha, PPARA), protein phosphatase 1F (PPM1F), tyrosine-protein phosphatase non-receptor type 13

Table 2 Number of predicted miRNA targets of the top differentially expressed miRNAs in inflamed and noninflamed gingival tissues

miRNAs overexpressed in inflamed gingiva	Number of predicted miRNA targets	miRNAs underexpressed in inflamed gingiva	Number of predicted miRNA targets
hsa-miR-150	275	hsa-miR-379	37
hsa-miR-223	311	hsa-miR-199a-5p	408
hsa-miR-200b	789	hsa-miR-214	301
hsa-miR-429	236	hsa-miR-30a	536
hsa-miR-95	6	hsa-miR-886-3p	0
hsa-miR-200a	84	hsa-miR-125b-2*	0
hsa-miR-142-3p	331	hsa-miR-222	210
hsa-miR-625	119	hsa-miR-26a	184
hsa-miR-96	434	hsa-miR-193a-3p	207
hsa-miR-144*	878	hsa-miR-127-3p	13
hsa-miR-513c	256	hsa-miR-381	233
hsa-miR-203	868	hsa-miR-154	129
hsa-miR-671-5p	339	hsa-miR-30a*	536
hsa-miR-135b	67	hsa-miR-181a	626
hsa-miR-371-5p	350	hsa-miR-34a	387
hsa-miR-766	356	hsa-miR-376c	254
hsa-miR-663	32	hsa-miR-376a	199
		hsa-miR-125b	229
		hsa-miR-100	28
		hsa-miR-218	931
		hsa-miR-99a	19
		hsa-let-7c	12

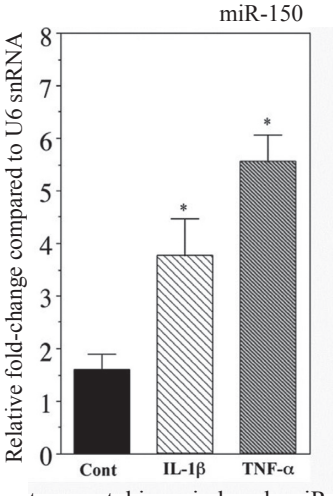

Fig. 2 Inflammatory cytokines induced miR-150 expression in HGF. Expressions of miR-150 and U6 snRNA in HGF after stimulation by IL-1β (1 ng/mL) and TNF-α (10 ng/mL) for 24 h were measured by real-time PCR. Quantitative analyses of the datasets are shown with standard errors. *$P < 0.01$, significant difference versus control

(PTPN13), retinoblastoma 1 (RB1), retinoblastoma-like 1 (RBL1), retinoblastoma-like 2 (RBL2), vimentin (VIM), zinc finger E-box-binding homeobox 1 (ZEB1), and miR-200b-3p gene expressions and is related to the cell cycle, connective tissue development and function, and cellular movement.

Next, we identified the diseases and biofunctions associated with overexpression of hsa-miR-150, hsa-miR-223, and hsa-miR-200b (Table 3B). miR-223-3p is involved in the inflammatory response (interstitial pneumonia, granulocyte activation, and leukocyte hyperactivation) and connective tissue disorders (interstitial pneumonia); miR-150-5p, miR-200b-3p, and miR-223-3p are related to another inflammatory response (lupus nephritis). miR-150-5p is involved in infectious disease (sepsis), immunological disease (nasal natural killer cell lymphoma), and metabolic disease (pituitary ACTH hypersecretion); miR-150-5p, miR-200b-3p, and miR-223-3p are related to another metabolic disease (diabetes mellitus). miR-200b-3p is involved in cellular function and maintenance (formation of actin stress fibers).

Discussion

In this study, we compared miRNA expression profiles in inflamed and noninflamed gingiva from Japanese and found 17 overexpressed and 22 underexpressed miRNAs. In inflamed gingiva, the three most overexpressed miRNAs were hsa-miR-150, hsa-miR-223, and hsa-miR-200b (>2.72-fold), and the three most underexpressed miRNAs were hsa-miR-379, hsa-miR-199a-5p, and hsa-miR-214 (<0.39-fold).

Three previous studies compared miRNA expression in periodontitis and healthy gingival tissues (10-12). A

Table 3A Main networks identified by Ingenuity Pathway Analysis

ID	Molecules in network	Score [a]	Focus molecular [b]	Main diseases and functions
1	AGO2, ⬆ miR-150-5p, ⬆ miR-223-3p	7	2	Cancer, endocrine system disorders, gastrointestinal disease
2	BCR (complex), CDC25C, CDKN1B, FHOD1, JAG1, MARCKS, ⬆ miR-200b-3p, MYC, PKD1, PLCG1, PPARA, PPM1F, PTPN13, RB1, RBL1, RBL2, VIM, ZEB1	2	1	Cell cycle, connective tissue development and function, cellular movement

[a] Score is a measure of how accurately the focus genes are matched. Assessment is based on the number of focus genes and network size.
[b] Number of focus genes in network

Table 3B Diseases and biofunctions identified by Ingenuity Pathway Analysis

Category	Disease or function annotation	*P* value	Molecules
Inflammatory response	Interstitial pneumonia	2.46E-02	⬆ miR-223-3p
	Activation of granulocytes	1.29E-02	⬆ miR-223-3p
	Hyperactivation of leukocytes	2.42E-02	⬆ miR-223-3p
	Lupus nephritis	6.06E-07	⬆ miR-150-5p, miR-200b-3p, miR-223-3p
Cellular function and maintenance	Formation of actin stress fibers	4.50E-02	⬆ miR-200b-3p
Infectious disease	Sepsis	2.96E-02	⬆ miR-150-5p
Connective tissue disorders	Interstitial pneumonia	2.46E-02	⬆ miR-223-3p
Immunological disease	Nasal natural killer cell lymphoma	8.77E-03	⬆ miR-150-5p
Metabolic disease	Pituitary ACTH hypersecretion	3.74E-03	⬆ miR-150-5p
	Diabetes mellitus	8.67E-04	⬆ miR-150-5p, miR-200b-3p, miR-223-3p

study of Chinese found that five miRNAs (hsa-miR-126*, hsa-miR-190, hsa-miR-20a, hsa-miR-32, and hsa-miR-362-3p) were greatly upregulated (>5-fold) in inflamed gingival tissues, as compared with healthy gingival tissues; 86 miRNAs were slightly upregulated (2- to 5-fold) (10). A study of Koreans noted that six miRNAs (hsa-let-7a, hsa-let-7c, hsa-miR-130a, hsa-miR-301a, hsa-miR-520d, and hsa-miR-548a) were upregulated by more than 8-fold in periodontitis tissue as compared with healthy gingiva, and the expression of 22 miRNAs was upregulated by more than 4-fold (11). A study of Americans found 25 miRNAs that were upregulated (>2.63- to 1.48-fold) in inflamed gingiva. The three most overexpressed miRNAs were hsa-miR-451, hsa-miR-223, and hsa-miR-486-5p (12). Surprisingly, these three reports yielded completely different results, probably because of differences in ethnicity and experimental conditions. However, hsa-miR-223 was overexpressed in inflamed gingiva in both this study and the American study (12). miR-223 is involved in many types of cancer, inflammatory diseases, autoimmune diseases, and in pathological processes such as leukemia, RA, and cardiovascular disease (19-23). miR-223 is overexpressed in the T-lymphocytes of persons with RA, is involved in obesity-associated adipose tissue inflammation, and is a key factor in osteoclast differentiation (19,21,23). Platelet-secreted miR-223 promotes endothelial cell apoptosis induced by advanced glycation end products via targeting of the insulin-like growth factor 1 receptor (24). Activated platelets can deliver mRNA regulatory AGO2-miR223 complexes to endothelial cells via microparticles (25).

We identified two other miRNAs (hsa-miR-150 and hsa-miR-200b) that were overexpressed in inflamed gingiva. miR-150 overexpression in hematopoietic progenitor cells significantly reduced the number of mature B cells in circulation, the spleen, and lymph nodes, with little or no change in population levels of T cells and myeloid cells (26). Diminished serum miR-150 levels are associated with unfavorable outcomes in patients with critical illnesses, independent of the presence of sepsis (27). miR-150 promotes development of natural killer (NK) cells. However, miR-150 overexpression reduces the number of invariant NK T (iNKT) cells in the thymus and peripheral lymphoid organs (28). miR-150 differentially controls development of NK and iNKT cell lineages by targeting c-Myb transcription factor (28). In this study, miR-150 expression was significantly increased in HGF after stimulation by the inflammatory cytokines IL-1β and TNF-α for 24 h (Fig. 2). Diminished numbers of iNKT cells in peripheral blood lymphocytes were found in persons with autoimmune diseases such as RA, systemic lupus erythematosus, systemic sclerosis, and Sjogren's syndrome (29). Therefore, miR-150 might

induce autoimmune disease.

miR-200b stimulates tumor growth in TGFBR2-null colorectal cancer by negatively regulating CDKN1B (p27/kip1) (30). Tamoxifen inhibits miR-200 expression and promotes epithelial-to-mesenchymal transition by upregulating c-Myc in endometrial cancer cells (31).

IPA analysis of the main networks, diseases, and biofunctions related to miR-150, miR-223, and miR-200b showed associations with cancer, endocrine system disorders, gastrointestinal disease, the cell cycle, connective tissue development and function, and cellular movement (Table 3A), and with inflammation, infectious disease, immunological disease, metabolic disease, connective tissue disorders, and cellular function and maintenance (Table 3B). The results suggest that these networks, diseases, and biofunctions regulate the cell cycle and cellular movement of inflammatory and immune cells in gingival tissues. The expressions of specific miRNAs in inflamed gingival tissue and obesity may share inflammatory metabolic mRNA targets (13). These findings offer insight regarding the mechanisms of periodontal inflammation.

In this study, we used miRNA microarray profiling and real-time PCR analysis to compare miRNA expression profiles in inflamed and noninflamed gingival tissues from Japanese dental patients. In addition, we used IPA to identify important relations to networks, diseases, and biofunctions. The findings suggest a relationship between miRNAs and periodontitis in periodontal tissue destruction.

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References

1. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366, 1809-1820.
2. Kato A, Imai K, Ochiai K, Ogata Y (2013) Higher prevalence of Epstein–Barr virus DNA in deeper periodontal pockets of chronic periodontitis in Japanese patients. PLoS One 8, e71990.
3. Seymour GJ, Powell RN, Cole KL, Aitken JF, Brooks D, Beckman I et al. (1983) Experimental gingivitis in humans. A histochemical and immunological characterization of the lymphoid cell subpopulations. J Periodontal Res 18, 375-385.
4. Maqbool R, Hussain MU (2014) MicroRNAs and human diseases: diagnostic and therapeutic potential. Cell Tissue Res DOI 10.1007/s00441-013-1787-3.
5. Kozomara A, Griffiths-Jones S (2011) miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 39, D152-157.
6. Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M et al. (2008) Expression of microRNA-146 in rheumatoid arthritis synovial tissue. Arthritis Rheum 58, 1284-1292.
7. Hung PS, Chen FC, Kuang SH, Kao SY, Lin SC, Chang KW (2010) miR-146a induces differentiation of periodontal ligament cells. J Dent Res 89, 252-257.
8. Nahid MA, Rivera M, Lucas A, Chan EKL, Kesavalu L (2011) Polymicrobial infection with periodontal pathogens specifically enhances microRNA miR-146a in ApoE-/- mice during experimental periodontal disease. Infect Immun 79, 1597-1605.
9. Park MG, Kim JS, Park SY, Lee SA, Kim HJ, Kim CS et al. (2014) MicroRNA-27 promotes the differentiation of odontoblastic cell by targeting APC and activating Wnt/β-catenin signaling. Gene 538, 266-272.
10. Xie YF, Shu R, Jiang SY, Liu DL, Zhang XL (2011) Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. Int J Oral Sci 3, 125-134.
11. Lee YH, Hee SN, Jeong SY, Jeong SH, Park HR, Chung J (2011) Comparison of inflammatory microRNA expression in healthy and periodontitis tissues. Biocell 35, 42-49.
12. Stoecklin-Wasmer C, Guarnieri P, Celenti R, Demmer RT, Kebschull M, Papapanou PN (2012) MicroRNAs and their target genes in gingival tissues. J Dent Res 91, 934-940.
13. Perri R, Nares S, Zhang S, Barros SP, Offenbacher A (2012) MicroRNA modulation in obesity and periodontitis. J Dent Res 91, 33-38.
14. Xie Y, Shu R, Jiang S, Liu D, Ni J, Zhang X (2013) MicroRNA-146 inhibits pro-inflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts. J Inflammation 10, 20.
15. Ogata Y, Niisato N, Sakurai T, Furuyama S, Sugiya H (1995) Comparison of the characteristics of human gingival fibroblasts and periodontal ligament cells. J Periodontol 66, 1025-1031.
16. Nakao S, Ogata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H (2002) Tumor necrosis factor α (TNF-α)-induced prostaglandin E_2 release is mediated by the activation of cyclooxygenase-2 (COX-2) transcription via NFκB in human gingival fibroblasts. Mol Cell Biochem 238, 11-18.
17. Nakao S, Ogata Y, Yamamoto Y, Furuyama S, Sugiya H (2004) Platelet-derived growth factor-induced arachidonic acid release for enhancement of prostaglandin E_2 synthesis in human gingival fibroblasts pretreated with interleukin-1β. J Cell Biochem 92, 579-590.
18. Sato K, Yoshimura A, Kaneko T, Ukai T, Ozaki Y, Nakamura H et al. (2012) A single nucleotide polymorphism in 3'-untranslated region contributes to the regulation of Toll-like receptor 4 translation. J Biol Chem 287, 25163-25172.
19. Sugatani T, Hruska KA (2007) MicroRNA-223 is a key factor

in osteoclast differentiation. J Cell Biochem 101, 996-999.
20. Eyholzer M, Schmid S, Schardt JA, Haefliger S, Mueller BU, Pabst T (2010) Complexity of miR-223 regulation by CEBPA in human AML. Leuk Res 34, 672-676.
21. Fulci V, Scappucci G, Sebastiani GD, Giannitti C, Franceschini D, Meloni F et al. (2010) miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. Hum Immunol 71, 206-211.
22. Rangrez AY, M'Baya-Moutoula E, Metzinger-Le Meuth V, Henaut L, Djelouat MS, Benchitrit J et al. (2012) Inorganic phosphate accelerates the migration of vascular smooth muscle cells: evidence for the involvement of miR-223. PLoS One 7, e47807.
23. Zhuang G, Meng C, Guo X, Cheruku PS, Shi L, Xu H et al. (2012) A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. Circulation 125, 2892-2903.
24. Pan Y, Liang H, Liu H, Li D, Chen X, Li L et al. (2014) Platelet-secreted microRNA-223 promotes endothelial cell apoptosis induced by advanced glycation end products via targeting the insulin-like growth factor 1 receptor. J Immunol 192, 437-446.
25. Laffont B, Corduan A, Plé H, Duchez AC, Cloutier N, Boilard E et al. (2013) Activated platelets can deliver mRNA regulatory Ago2•microRNA complexes to endothelial cells via microparticles. Blood 122, 253-261.
26. Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF (2007) miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc Natl Acad Sci U S A 104, 7080-7085.
27. Roderburg C, Luedde M, Cardenas DV, Vucur M, Scholten D, Frey N et al. (2013) Circulating microRNA-150 serum levels predict survival in patients with critical illness and sepsis. PLoS one 8, e54612.
28. Bezman NA, Chakraborty T, Bender T, Lanier LL (2011) miR-150 regulates the development of NK and iNKT cells. J Exp Med 208, 2717-2731.
29. Kojo S, Adachi Y, Keino H, Taniguchi M, Sumida T (2001) Dysfunction of T cell receptor AV24AJ18+, BV11+ double-negative regulatory natural killer T cells in autoimmune diseases. Arthritis Rheum 44, 1127-1138.
30. Fu Y, Liu X, Zhou N, Du L, Sun Y, Zhang X et al. (2014) MicroRNA-200b stimulates tumour growth in TGFBR2-null colorectal cancers by negatively regulating p27/kip1. J Cell Physiol 229, 772-782.
31. Bai JX, Yan B, Zhao ZN, Xiao X, Qin W, Zhang R et al. (2013) Tamoxifen represses miR-200 microRNAs and promotes epithelial-to-mesenchymal transition by up-regulating c-Myc in endometrial carcinoma cell lines. Endocrinol 154, 635-645.