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Periodontitis may modulate long-non coding RNA expression

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ABSTRACT

Periodontitis is a chronic inflammatory disease that compromises the integrity of the periodontium. Despite extensive research involving periodontitis, the detailed mechanisms underlying periodontal inflammation remain unclear. However, new important expression regulators have been emerging, such as non-coding RNAs, which are important determinants in the molecular control of the inflammatory process. Taking into consideration the vital role of non-coding RNAs, we determined for the first time the expression profiles of different long non-coding RNAs (lncRNAs) that are implicated in inflammation. In this study, we take periodontal samples of healthy subjects, patients with gingivitis and with periodontitis. In both disease groups, the lncRNA OIP5-AS1 expression levels were lower than levels in healthy subjects ($P < 0.05$). This study reveals new insights into the relative levels of OIP5-AS1 lncRNA in healthy, gingivitis and periodontal tissue, which may have important applications as a potential biomarker with protagonist activity in the development and manifestation of destructive periodontitis.

1. Introduction

Severe periodontal disease is the most common oral disease among adults. Approximately 11.2% of the population suffers from periodontitis worldwide (Kassebaum et al., 2014). Periodontitis is a chronic inflammatory disease that compromises the integrity of the tissues supporting the teeth, including the gingiva, periodontal ligament, dental cementum and alveolar bone, and are collectively known as the periodontium (Pihlstrom, Michalowicz, & Johnson, 2005). According to information from the Department of Health of Mexico, the percentage of periodontitis increases with age (Salud, 2015).

Despite extensive research involving periodontitis, the detailed mechanisms of the pathogenesis behind the periodontal inflammation remains unclear (Yucel-Lindberg & Båge, 2013). To attempt to explain the molecular mechanisms of periodontitis, many scientific articles have attempted to determine the expression profiles of genes in healthy periodontal tissues and contrast them with results in diseased tissue. This has helped to identify genes involved in the homeostasis of these tissues and also find transcendent biological markers that could help to determine why certain patients are susceptible to periodontal disease (Beikler, Peters, Prior, Eisenacher, & Flemmig, 2008).

Importantly, non-coding RNAs (ncRNAs) have been found to have a relevant role in the new regulation mechanisms of the inflammatory response. Moreover, micro RNAs (miRNAs) are considered to participate in the progression and management of the inflammatory response because several miRNAs (e.g., miR-126, miR-132, miR-146, miR-155 and miR-221) have emerged as important regulators of inflammation gene expression (Marques-Rocha et al., 2015), suggesting the significant need to investigate the role of lncRNAs in periodontal disease.

Recently, the expression of lncRNA was analysed by microarrays in

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chronic periodontitis tissues (Zou et al., 2015) and periodontal mesenchymal stem cells of periodontitis patients (Wang et al., 2016), and lncRNA has been investigated as a genetic risk factor for this inflammatory illness (Bochenek et al., 2013). However, further studies regarding the whole-genome properties by microarrays are required to determine a more precise mechanism underlying the role of lncRNAs in periodontal disease. To deeply characterise the status and action of lncRNA dysregulation by periodontitis, we conducted a pilot study using tissues from healthy, gingivitis and periodontitis subjects.

2. Material and methods

2.1. Ethics statement

The ethical approval was obtained from the Ethics Committee of Facultad de Enfermería Mexicali, Universidad Autónoma de Baja California (No. POSG/017-2-076). Written informed consent of all participating subjects was obtained prior to inclusion in the study.

2.2. Subjects

The characteristics of the patients are shown in Table 1. A total of nine patients were recruited in this study after being diagnosed by a dental surgeon with a specialty in periodontics. The subjects had an average age of 34 years old (67% were female and 33% were male patients). All the groups (control, gingivitis and chronic periodontitis) had three patients, and all the samples came from the maxilla specifically, the buccal side of incisors and mesial side of molars.

2.3. Periodontal tissue specimens

Periodontal tissues were obtained from three patients with chronic periodontitis, three patients with gingivitis and three subjects with clinically healthy gingiva. These specimens were collected from patients referred to the Periodontal Clinic, Facultad de Odontología Mexicali, Universidad Autónoma de Baja California. All participants were clinically examined for periodontal disease, and those with a tooth site demonstrating bleeding on probing, clinical attachment level ≥ 5 mm and radiographic assessment of bone loss (Supplementary Fig. 1 in Supplementary material) were included in the periodontitis-affected group; gingival biopsies were obtained at the time of periodontal surgery or extraction of severely involved teeth.

Healthy gingival tissues were collected during crown-lengthening procedure showing no bleeding on probing and a probing depth ≤ 3 mm. Gingivitis tissue, collected during the crown-lengthening procedure, were obtained from clinically inflamed sites showing redness, probing depth ≤ 3 mm and bleeding upon probing. Bone loss was not observed in either group. The excised tissues were washed with phosphate-buffered saline (PBS) and immediately placed in an Eppendorf tube with RNAlater solution (Thermo Fisher Scientific, Waltham, MA), at room temperature. Then, the tissue was transported at 4 °C and placed in −70 °C refrigeration until the tissues were used.

2.4. Total RNA isolation and cDNA synthesis

Fifty milligrams of periodontal tissue was disrupted using a tissue homogeniser (Polytron Kinematica) in 1 mL of TriPure reagent (ROCHE, Sciences, Maryland, USA). The RNA integrity evaluation, was performed by visualising ribosomal RNA 18S and 28S on agarose 1.5% gels stained with ethidium bromide, whereas, concentration and purity were assessed by spectrophotometry on a NanoDrop 1000 (Thermo Fisher Scientific Lafayette, CO, USA).

The synthesis of cDNA was performed using 1 μg of total RNA with a RT^2 First Strand Kit (QIAGEN). The cDNA manufacturing protocol includes a treatment to avoid DNA carryover (DNase I for 30 min at 42 °C), and it was performed according to the manufacturer instructions.

2.5. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis

The qRT-PCR was performed using RT2 SYBR® Green qPCR MasterMix (QIAGEN, Germany). The reaction (25 μl) was deposited into the wells of the QIAGEN Inflammatory Responses RT2 lncRNA PCR array kit (QIAGEN, Germany), which contains the predesigned and laboratory verified pairs of specific oligonucleotides. The mRNA relative quantification of the target genes was conducted using the reference genes included in the array and the $2-\Delta Ct$ method. For quality control of qPCR assays, we determined the linearity and reproducibility (VC $<$ 10%). Data are presented as means and standard errors. Groups were analysed by two-way analysis of variance (ANOVA), and we used the Benjamini-Hochberg Procedure (FDR method to avoid statistical error type I) ($p < 0.05$).

3. Results

The Human Inflammatory Response and Autoimmunity RT2 lncRNA PCR Array profiles the expression of 84 lncRNAs validated or predicted to be involved in pro-inflammatory and anti-inflammatory processes. We found that six lncRNA expression levels presented statistical differences when using typical ANOVA; these lncRNAs include AC000120.7, MZF1-AS1, FGD5-AS1, HOTAIR, OIP5-AS1 and RP11-29G8.3 (Fig. 1). In this study, we detected the decreased expression of AC000120.7, MZF1-AS1, FGD5AS1 and OIP5-AS1 lncRNAs in tissue samples of patients with gingivitis and periodontitis as compared with healthy gingival samples (Fig. 1a, b, c, and e). In contrast, the expression level of RP11-29G8.3 lncRNA was significantly higher in periodontitis-affected gingival tissue samples as opposed to gingivitis and healthy gingival tissues (Fig. 1f). Interestingly, gingivitis samples overexpressed HOTAIR lncRNA as compared with periodontitis samples while expression of HOTAIR lncRNA was not detected in healthy tissues (Fig. 1d). Notably, when the Benjamini-Hochberg Procedure was used,

Table 1
Patient characteristics.

Group	Patient	Gender	Age	Bleeding on probing	Sample site		
Control	1	M	51	Absent	Central incisor	3 mm	Probing depth
	2	F	23	Absent	Central incisor	3 mm	
	3	F	25	Absent	Central incisor	3 mm	
Gingivitis	4	F	38	Present	Central incisor	3 mm	
	5	M	18	Present	Central incisor	3 mm	
	6	F	30	Present	Central incisor	3mm	
Chronic Periodontitis	7	M	41	Present	left molar	7 mm	Clinical attachment
	8	F	22	Present	Central incisor	5 mm	level
	9	F	55	Present	right molar	7 mm	

F, female. **M**, male.