

RESEARCH ARTICLE

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# Gene expression changes in *Porphyromonas gingivalis* W83 after inoculation in rat oral cavity

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## Abstract

**Background:** The development of chronic periodontitis was due to not only periodontal pathogens, but also the interaction between periodontal pathogens and host. The aim of this study is to investigate the alterations in gene expression in *Porphyromonas gingivalis* (*P.gingivalis*) W83 after inoculation in rat oral cavity.

**Results:** *P.gingivalis* W83 inoculation in rat oral cavity caused inflammatory responses in gingival tissues and destroyed host alveolar bone. Microarray analysis revealed that 42 genes were upregulated, and 22 genes were downregulated in the detected 1786 genes in the inoculated *P.gingivalis* W83. Real-time quantitative PCR detection confirmed the expression alterations in some selected genes. Products of these upregulated and downregulated genes are mainly related to transposon functions, cell transmembrane transportation, protein and nucleic acid metabolism, energy metabolism, cell division and bacterial pathogenicity.

**Conclusions:** *P.gingivalis* W83 has a pathogenic effect on host oral cavity. Meanwhile, inflammatory oral environment alters *P.gingivalis* W83 gene expression profile. These changes in gene expression may limit the proliferation and weaken the pathogenicity of *P.gingivalis* W83, and favor themselves to adapt local environment for survival.

**Keywords:** Porphyromonas gingivalis, Periodontitis, Microarray, Gene expression

## Background

Periodontitis is a chronic inflammatory disorder mediated by host and bacteria interactions and manifested by damage to the periodontal tissues that may progress to tooth loss. The host inflammatory responses stimulated by periodontal pathogens intend to eliminate the invaded bacteria and attribute to the destruction of tooth supporting tissues and tooth loss [1]. Moreover, the local periodontal environment may change the gene expression profile of periodontal pathogens [2–4]. To a certain extent, the variation of bacterial gene expression may alter the pathogenic ability of bacteria.

*Porphyromonas gingivalis* (*P.gingivalis*) is an opportunistic pathogen of the oral mucosa and a prominent member of the oral biofilms. It is well known that *P.gingivalis* is implicated in the onset and progression of chronic periodontitis. *P.gingivalis* can induce immune cells to secrete cytokines when they invade into hosts. These cytokines are present in inflamed gingiva and aggravate the destruction of oral gingival tissues and alveolar bone [5]. In the

meantime, the expression of *P.gingivalis* genes varies under different conditions, such as iron or hemin [6,7], polyphosphate [8], rhein [9]. *P.gingivalis* may up-regulate or downregulate gene expression to adapt environment and survive [10].

The development of chronic periodontitis was not only due to periodontal pathogens, but also the interaction between periodontal pathogens and host. Most researches focus on periodontal pathogens acting on hosts, but ignore the action of host on *P.gingivalis*. Actually, the changes in *P.gingivalis* gene expression may affect the progression of chronic periodontitis. In the present study, the differential gene expression in *P.gingivalis* W83 inoculated in rat oral cavity and wild strain was analyzed.

## Methods

### Ethical statement

All rats were manipulated in accordance with Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines. The experimental protocols were approved by the ethical committee of China Medical University.

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### Bacteria and animals

This study was carried out with 6-week-old SPF rats (180–220 g) provided by Department of Experimental Animals, China Medical University, and maintained in a temperature-controlled room ( $23 \pm 1$  °C). *P.gingivalis* W83 was obtained from the American Type Culture Collection (ATCC) and grown anaerobically (10 % CO<sub>2</sub>, 10 % H<sub>2</sub>, 80 % N<sub>2</sub>) in enriched brain-heart infusion (BHI) broth containing 5 % fiber-free sheep blood, 1 % vitamin K and hemin, at 37 °C.

### *P.gingivalis* W83 inoculation

12 Rats were given azithromycin (10 mg/500 ml) *ad libitum* for 4 days to reduce the original oral flora. This was followed by a 7-day antibiotic-free period. 6 Rats were then orally challenged with *P.gingivalis* W83 ( $1 \times 10^9$  CFU) by gavage into the esophagus and oral cavity five times every other day [11]. The other 6 rats (control group) were only challenged with BHI broth. All 12 rats received steel wire ligature in cervical part in two sides of first molars and an 8-week high sugar feeding.

### Alveolar bone loss analysis

Horizontal bone loss was assessed morphometrically by measuring the distance between the cement–enamel junction and the alveolar bone crest of the first, the second and the third molar. The alveolar bone destruction was detected by morphological and macroscopic observation, radiographic (PLANMECA, Finland) and stereomicroscope (SZX12, Olympus, Japan) fitted with a DIGIMED Viewer imaging measurement system evaluation at 6 sites per molars. Alveolar bone loss of every molar was presented in the figures as mean  $\pm$  SD. Independent samples *t*-test was used to calculate the significance among the groups (SPSS Inc., Chicago, IL, USA). *P*-value < 0.05 was considered statistically significant.

### Isolating culture and acquiring plaque

After *P.gingivalis* W83 inoculation in rat oral cavity for 8 weeks, plaques were acquired from periodontal pockets of first molar using toothpicks and put into 0.5 ml transfer tube. The plaques were dispersed by oscillator. 100  $\mu$ l ten-fold serial dilutions were inoculated on BHI culture medium anaerobically at 37 °C for 5–7 days. The morphology of colonies was observed in primary cultures. *P.gingivalis*W83 colonies were identified by their black pigmentation, gram staining and PCR. The single clone was purified in BHI medium for subcultures in order to detect the differences in the gene of *P.gingivalis*W83.

### Microarray hybridization

3 samples were picked up from wild strain *P.gingivalis* W83 and inoculated *P.gingivalis* W83, respectively. The

total RNA was extracted and labeled with Klenow, and then hybridism with *P.gingivalis* W83 chip. The commercial GeneChip *P.gingivalis* W83 Genome Array used here was provided by CapitalBio Corporation (<http://www.capitalbio.com/>, Beijing, China), a service provider authorized by Roche NimbleGen (Wisconsin, USA). Array hybridization, washing, scanning and data analysis were performed at the CapitalBio Corporation, Beijing, China and carried out according to the NimbleGen's Expression user's guide.

### Real-time quantitative PCR

To independently confirm the expression data generated by the microarray experiments, we performed real-time quantitative PCR analyses for 14 genes differentially regulated. Total RNA was extracted. Quality and concentration of the RNA were determined by measuring its absorbance at 260 and 280 nm using a microplate reader (M-200, Tecan, Switzerland). Total bacterial RNA was subsequently reverse-transcribed using the M-MLV RTase cDNA Synthesis Kit (Takara, China) following the manufacturer's protocol. Real-time quantitative PCR analysis was conducted in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in combination with the SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II PCR Master Mix Reagents Kit (Takara), as recommended by the manufacturer of the Wall Clear PCR Strip Tubes (Axygen, USA). The primers for the real-time quantitative PCR analysis were designed using Primer3 (<http://bioinfo.ut.ee/primer3/>) (Table 1). *P.gingivalis* W83 16 s DNA was used as the internal reference. Real-time quantitative PCR was performed three times for each sample. The data were analyzed according to relative gene expression by the  $2^{-\Delta\Delta Ct}$  method.

### Statistics

Significantly differentially expressed genes between the inoculated periodontitis and wild strains were identified using two class unpaired method in the Significant Analysis of Microarray software (SAM, version 3.02). Genes were determined to be significantly differentially expressed with a selection threshold of false discovery rate, FDR < 5 % and fold change > 2.0 in the SAM output result.

### Results

#### Pathogenic effects of *P.gingivalis* W83 on rat oral cavity

After *P.gingivalis* W83 inoculation in rat oral cavity for 8 weeks, the gingival tissues were inflammatory and bleeding (Fig. 1A). Severe alveolar bone losses were found in rats with *P.gingivalis* W83 inoculation (Fig. 1B). The distance between cemento-enamel junction and alveolar bone crest (CEJ: ABC) was measured at proximal, middle and distal sites of buccal and palatal per molar, respectively. In first molars, second

**Table 1** Sequences for real-time PCR

| Gene   | Sequence(5'-3')                                   | PCR product (bp) |
|--------|---|------------------|
| PG1005 | F: CGGTGAGGTTTACAGAAGAA<br>R: AGGGAGGTGTAAGTCACG  | 79               |
| PG1006 | F: GGAATGGAGCGAAAGACC<br>R: CCAACAAGCAGAACCGAC    | 169              |
| PG1007 | F: TCTGTTTGTTCCTCCATT<br>R: TATGGCTCCTCAAAGTAGAG  | 62               |
| PG1008 | F: TTACAACAGCGGCTACCA<br>R: TATCCACTGCCACAGCCT    | 103              |
| PG1009 | F: AAGCGTGCTACCATTGCG<br>R: TCAGGCTATACCCGTTCT    | 78               |
| PG1010 | F: TCTGTCCTGCGATACTT<br>R: CACTCATCTCCTATCTTTT    | 99               |
| PG0874 | F: AGGGTGTCTGAGCAAGTA<br>R: TGGAGGAATCGAAGATAGAA  | 73               |
| PG1513 | F: GAAACGGCTCAAGTCATA<br>R: TCCCTCCTCCATTCCAC     | 114              |
| PG0684 | F: GAATACGGAGGTCAATCGC<br>R: GAACGCTGAGAAGGAGGC   | 90               |
| PG0682 | F: CGGTGAGTTCTATTATTGCG<br>R: CAGCACCAGGCATGACCA  | 123              |
| PG1975 | F: CGTGACGGGCATAAGACA<br>R: AGTGAGTCGTGGGTTTAC    | 134              |
| PG1982 | F: GTAATACCGAGGAAACTGAA<br>R: GTGTTTCAGGGATAAGTCC | 60               |
| PG2008 | F: CTGCGGTTTCAACCAAGT<br>R: ATACCGAACCTCGTCTAC    | 115              |
| PG0001 | F: AGGTGGTCATGTTCTCTCC<br>R: TGACTACCCTCTGCATTGG  | 78               |

molars, third molars, the distances were significantly increased, which were  $1216.00 \pm 305.98 \mu\text{m}$ ,  $987.28 \pm 238.14 \mu\text{m}$ ,  $725.11 \pm 202.71 \mu\text{m}$ , compared with normal rats, which were  $414.89 \pm 209.67 \mu\text{m}$ ,  $300.44 \pm 127.92 \mu\text{m}$ ,  $357.56 \pm 281.06 \mu\text{m}$ .

**Identification of the inoculated *P.gingivalis* W83 in rat with periodontitis**

Suspicious gram-negative bacilli were taken from plaque culture. After pure subculture, gram staining and polymerase chain reaction (PCR) proved that the bacteria were *P.gingivalis* W83. PCR fragment length of the product was 857 bp, as shown in Fig. 2.

**Genes upregulated in the inoculated *P.gingivalis* W83**

We determined the expression of 1786 genes by microarray analysis in *P.gingivalis* wild strain and *P.gingivalis*

inoculated in oral cavity. The complete list of gene expression values has been deposited in NCBI's Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67608>). The detection showed that 42 genes were up-regulated in the inoculated *P.gingivalis* W83 compared with wild strain (Table 2) (Fig. 3). In these upregulated genes, 30 expressed hypothetical proteins. Among other 12 genes, *PG0874*, *PG0009*, *PG0427*, *PG0942* and *PG0590* function as transposons. *PG0009*, *PG0427*, *PG0942* and *PG0590* encode ISPg5 transposases; and *PG0874* encodes an int protein as a mobilizable transposon.

**Genes downregulated in the inoculated *P.gingivalis* W83**

Compared with wild strain, 22 genes were down-regulated in the inoculated strain (Table 3) (Fig. 3). Among these genes, *PG0682*, *PG0683*, *PG0684*, *PG0282* and *PG0946* encode ABC transporters. Products of *PG0682*, *PG0683* and *PG0684* are putative permease proteins; and products of *PG0282* and *PG0946* are ATP-binding proteins. In addition, *PG2008* and *PG0283* also encode transport and binding proteins. All these proteins are involved in cell transmembrane transportation. Some downregulated genes encode proteins related to protein and nucleic acid metabolism, including *PG1129*, *PG1993*, *PG0001* and *PG0522*. Products of other genes are related to energy metabolism (*PG1042*), cell division (*PG0141*), bacterial pathogenicity (*PG1975*), and so on.

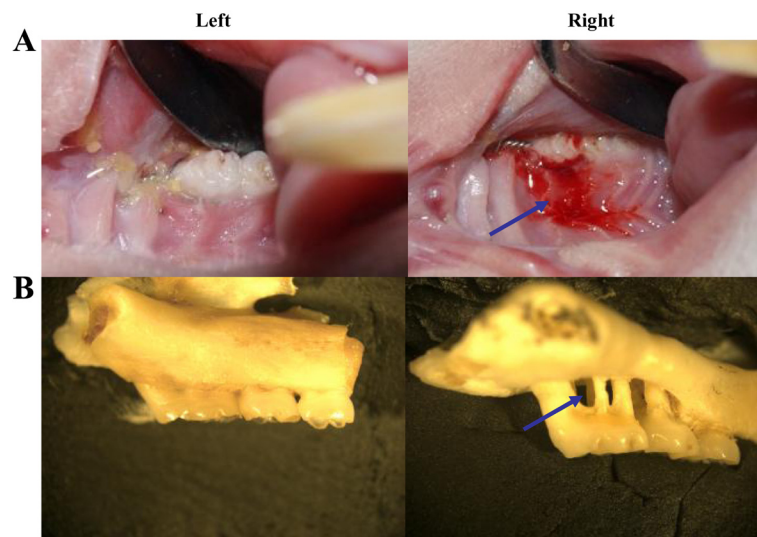
**Microarray result confirmation by real-time quantitative PCR**

Among the upregulated and downregulated genes, we picked up 14 genes to detect the expression by real-time quantitative PCR. Consistent with microarray hybridization, real-time quantitative PCR detection showed similar expression trends in these genes (Table 4).

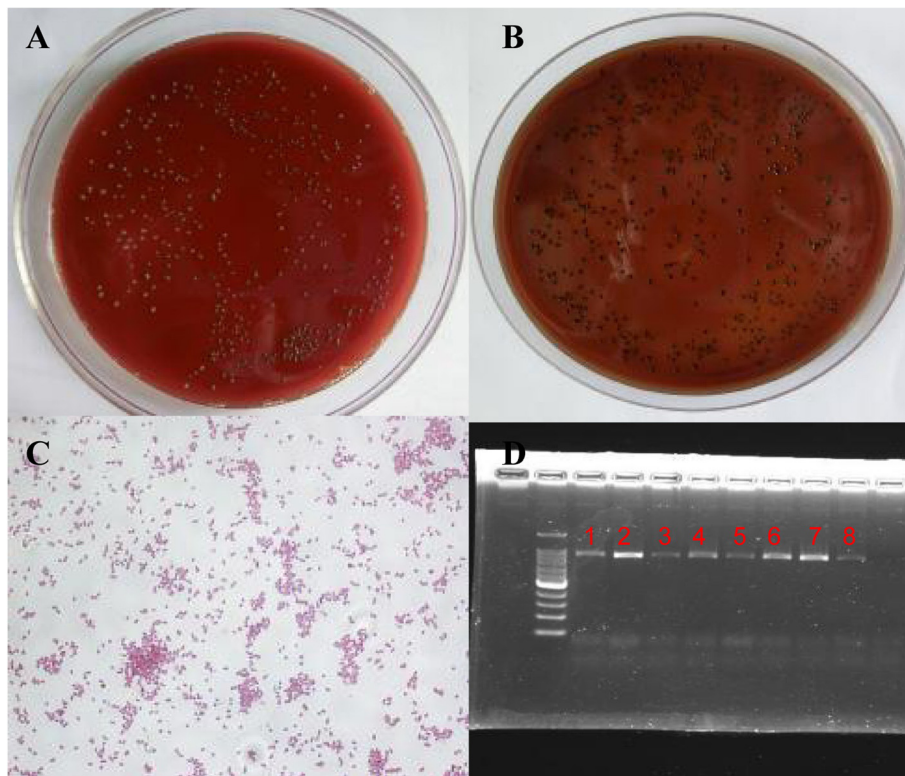
**Discussion**

Chronic periodontitis is initiated by periodontal pathogens, including *P.gingivalis*. Our study showed that *P.gingivalis* W83 induced rat gingival tissue inflammation, and alveolar bone loss, which is the key feature of periodontitis. Therefore, our study demonstrates that *P.gingivalis* W83 has pathogenic effects on rat oral cavity. After inoculation in rat oral cavity for 8 weeks, *P.gingivalis* W83 were isolated, and analyzed by microarray. In the detected 1786 genes, 42 genes were upregulated, whereas 22 genes were downregulated, indicating that the local periodontal environment can change the gene expression profile of *P.gingivalis* W83.

In the 42 upregulated genes, 30 expressed hypothetical proteins. Among other 12 genes, *PG0874*, *PG0009*, *PG0427*, *PG0942* and *PG0590* are in the same class in JCVI cell function classification. They all function as mobile extrachromosomal factor: transposon. Transposon



**Fig. 1** Pathogenic effects of *P.gingivalis* W83 on rat oral cavity. **(A)** The gingival tissues were inflammatory and bleeding after *P.gingivalis* W83 inoculation in rat oral cavity for 8 weeks. **(B)** Severe alveolar bone losses were found in rats with *P.gingivalis* W83 inoculation. Left: control groups. Right: rats inoculated with *P.gingivalis* W83 intraorally for 8 weeks



**Fig. 2** Isolation and identification of inoculated *P.gingivalis* W83. **(A)** Some suspicious black colonies were found from plaque mixture. **(B)** Suspicious black colonies were pure cultured. **(C)** Gram staining proved the pure culture as gram-negative brevibacterium (x400). **(D)** Agarose gel electrophoresis proved that PCR fragment length was 857 bp. 1 and 2: Wild type *P.gingivalis* W83; 3–8: three inoculated *P.gingivalis* W83 samples for microarray analysis (two columns for each sample)

**Table 2** Genes upregulated in the inoculated *P.gingivalis* W83

| Locus no. | Putative identification <sup>a</sup>             | Cellular role <sup>a</sup>  | Fold   |
|-----------|--|---|--------|
| PG0102    | hypothetical protein                             |   | 3.7076 |
| PG2116    | hypothetical protein                             |   | 3.6850 |
| PG1007    | GntR family transcriptional regulator            | Regulatory functions: DNA interactions  | 3.6786 |
| PG0265    | hypothetical protein                             |   | 3.3714 |
| PG1008    | hypothetical protein                             |   | 3.3338 |
| PG1510    | hypothetical protein                             |   | 3.0890 |
| PG1009    | hypothetical protein                             |   | 3.0258 |
| PG1655    | hypothetical protein                             |   | 2.8935 |
| PG0132    | hypothetical protein                             |   | 2.7298 |
| PG2114    | hypothetical protein                             |   | 2.6986 |
| PG2064    | hypothetical protein                             |   | 2.5952 |
| PG1010    | ABC transporter, ATP-binding protein             | Transport and binding proteins: Unknown substrate   | 2.4885 |
| PG0542    | hypothetical protein                             |   | 2.4794 |
| PG1005    | putative lipoprotein                             | Cell envelope   | 2.4686 |
| PG1514    | glycerol dehydrogenase-related protein           | Unknown function: General   | 2.4537 |
| PG0844    | hypothetical protein                             |   | 2.4324 |
| PG0874    | mobilizable transposon, int protein              | Mobile and extrachromosomal element functions:<br>Transposon functions                    | 2.4307 |
| PG0507    | hypothetical protein                             |   | 2.4290 |
| PG1357    | hypothetical protein                             |   | 2.3151 |
| PG1410    | hypothetical protein                             |   | 2.3094 |
| PG0617    | hypothetical protein                             |   | 2.2814 |
| PG0855    | hypothetical protein                             |   | 2.2153 |
| PG0009    | ISPg5, transposase Orf1                          | Mobile and extrachromosomal element functions:<br>Transposon functions                    | 2.1609 |
| PG0427    | ISPg5, transposase Orf1                          | Mobile and extrachromosomal element functions:<br>Transposon functions                    | 2.1431 |
| PG0942    | ISPg5, transposase Orf1                          | Mobile and extrachromosomal element functions:<br>Transposon functions                    | 2.1424 |
| PG0541    | hypothetical protein                             |   | 2.1291 |
| PG1398    | hypothetical protein                             |   | 2.1216 |
| PG1027    | hypothetical protein                             |   | 2.1204 |
| PG0590    | ISPg5, transposase Orf1                          | Mobile and extrachromosomal element functions:<br>Transposon functions                    | 2.1151 |
| PG1006    | hypothetical protein                             |   | 2.1122 |
| PG0340    | hypothetical protein                             |   | 2.1119 |
| PG0256    | CvpA family protein                              | Unknown function: General   | 2.1090 |
| PG0749    | hypothetical protein                             |   | 2.0834 |
| PG1662    | hypothetical protein                             |   | 2.0788 |
| PG2220    | hypothetical protein                             |   | 2.0771 |
| PG1871    | hypothetical protein                             |   | 2.0589 |
| PG2187    | 1,4-dihydroxy-2-naphthoate octaprenyltransferase | Biosynthesis of cofactors, prosthetic groups, and carriers:<br>Menaquinone and ubiquinone | 2.0471 |
| PG0410    | hypothetical protein                             |   | 2.0409 |
| PG1233    | hypothetical protein                             |   | 2.0367 |
| PG0325    | hypothetical protein                             |   | 2.0349 |

**Table 2** Genes upregulated in the inoculated *P.gingivalis* W83 (Continued)

|        |  |                          |        |
|--------|--|--------------------------|--------|
| PG0409 | hypothetical protein   |                          | 2.0259 |
| PG1513 | phosphoribosyltransferase, putative/phosphoglycerate mutase family protein | Energy metabolism: Other | 2.0032 |

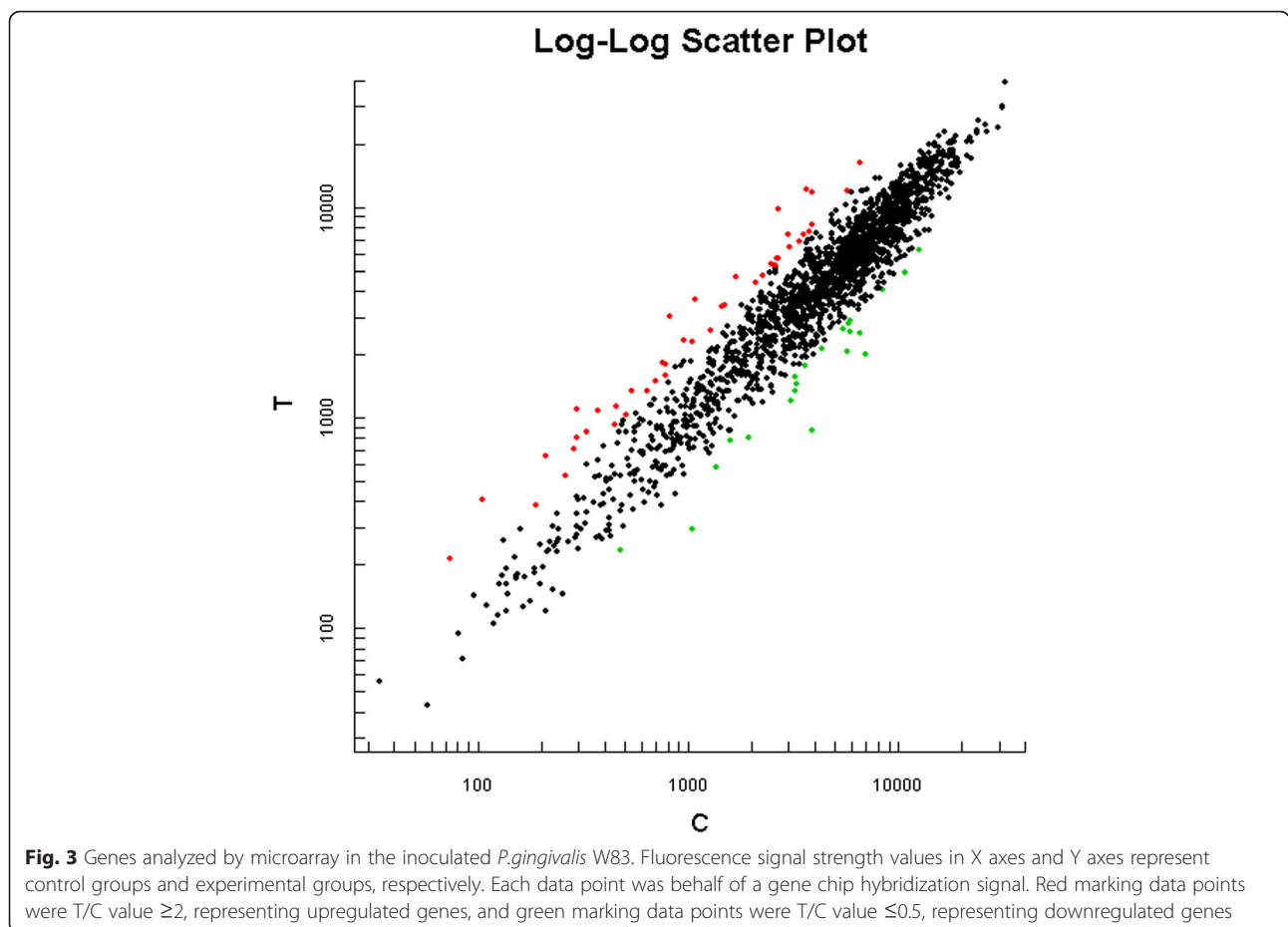
<sup>a</sup>Locus number, identification and functional classification according to JCVI *P.gingivalis* genome database

is a removable genome DNA sequence, which can “jump” in genome from one location to another through the process of cutting and integration. Transposition is generally known to be triggered by cellular stress [12–14], therefore upregulation of these transposons suggests that *P.gingivalis* W83 inoculated in rat oral cavity may adapt local environment for its own survival, which is consistent with some other studies [15,16].

In the 22 downregulated genes, 7 genes encode transport and binding proteins. All these proteins are involved in cell transmembrane transportation. They can transport many substrates, such as metabolites, ion, sugar, amino acids, lipids, cholesterol and drugs [17]. *PG2008* encodes a TonB dependent receptor protein, responsible for iron transmembrane transportation [18]. As iron ion is necessary for the breeding and spreading

of *P.gingivalis* W83, downregulation of *PG2008* suggests the subdued iron transferring and proliferation of *P.gingivalis* W83. There are 4 downregulated genes encoding proteins related to nucleic acid and protein metabolism. *PG1129* encodes a nucleotide reductase, which is related to purine, pyrimidine, nucleotide and DNA metabolism, and plays a regulating role in cell proliferation. Products of *PG1993* and *PG0001* are related to the metabolism of DNA, such as copy, restructuring and repair. Therefore, downregulation of these genes means that the proliferation of inoculated *P.gingivalis* W83 is in certain obstacles.

*PG1042* encodes a putative glycogen synthase, involved in biosynthesis and degradation of polysaccharides. Downregulation of *PG1042* suggests a disturbed energy metabolism. *PG0141* encodes a spoOJ protein related to



**Table 3** Genes downregulated in the inoculated *P.gingivalis* W83

| Locus no. | Putative identification <sup>a</sup>               | Cellular role <sup>a</sup>  | Fold   |
|-----------|--|---|--------|
| PG2008    | TonB-dependent receptor, putative                  | Transport and binding proteins: Cations and iron carrying compounds                   | 0.2234 |
| PG0929    | hypothetical protein                               |   | 0.2806 |
| PG1129    | ribonucleotide reductase                           | Purines, pyrimidines, nucleosides, and nucleotides: 2'-Deoxyribonucleotide metabolism | 0.2862 |
| PG0684    | ABC transporter, permease protein, putative        | Transport and binding proteins: Unknown substrate                                     | 0.3594 |
| PG0683    | ABC transporter, permease protein, putative        | Transport and binding proteins: Unknown substrate                                     | 0.3848 |
| PG0682    | ABC transporter, permease protein, putative        | Transport and binding proteins: Unknown substrate                                     | 0.3938 |
| PG0522    | tRNA delta(2)-isopentenylpyrophosphate transferase | Protein synthesis: tRNA and rRNA base modification                                    | 0.4190 |
| PG1648    | RelA/SpoT family protein                           | Cellular processes: Adaptations to atypical conditions                                | 0.4192 |
| PG0282    | ABC transporter, ATP-binding protein               | Transport and binding proteins: Unknown substrate                                     | 0.4303 |
| PG0946    | ABC transporter, ATP-binding protein               | Transport and binding proteins: Unknown substrate                                     | 0.4347 |
| PG1042    | glycogen synthase, putative                        | Energy metabolism: Biosynthesis and degradation of polysaccharides                    | 0.4485 |
| PG0890    | alkaline phosphatase, putative                     | Central intermediary metabolism: Other  | 0.4552 |
| PG1100    | hypothetical protein                               |   | 0.4813 |
| PG1993    | excinuclease ABC subunit C                         | DNA metabolism: DNA replication, recombination, and repair                            | 0.4814 |
| PG0226    | transglutaminase-related protein                   | Unknown function: General   | 0.4823 |
| PG0141    | spoOJ protein                                      | Cellular processes: Cell division   | 0.4865 |
| PG0144    | hypothetical protein                               |   | 0.4888 |
| PG1975    | hemagglutinin protein HagC                         | Cellular processes: Pathogenesis  | 0.4941 |
| PG1982    | CRISPR-associated Cas1 family protein              | Mobile and extrachromosomal element functions: Other                                  | 0.4950 |
| PG1718    | hypothetical protein                               |   | 0.4955 |
| PG0283    | RND family efflux transporter MFP subunit          | Transport and binding proteins: Unknown substrate                                     | 0.4965 |
| PG0001    | chromosomal replication initiation protein         | DNA metabolism: DNA replication, recombination, and repair                            | 0.4984 |

<sup>a</sup>Locus number, identification and functional classification according to JCVI *P.gingivalis* genome database

**Table 4** Microarray result confirmation by real-time PCR

| Gene   | Fold increase measured by |                |
|--------|---------------------------|----------------|
|        | Microarray analysis       | Real-time PCR  |
| PG1005 | 2.47↑                     | 12.24 ± 2.12↑  |
| PG1006 | 2.11↑                     | 11.57 ± 1.06↑  |
| PG1007 | 3.68↑                     | 24.67 ± 3.67↑  |
| PG1008 | 3.33↑                     | 28.99 ± 4.56↑  |
| PG1009 | 3.03↑                     | 27.56 ± 2.66↑  |
| PG1010 | 2.49↑                     | 19.88 ± 3.41↑  |
| PG0874 | 2.43↑                     | 18.58 ± 2.08↑  |
| PG1513 | 2.00↑                     | 15.86 ± 2.12↑  |
| PG0684 | 0.36↓                     | 0.036 ± 0.004↓ |
| PG0682 | 0.39↓                     | 0.047 ± 0.005↓ |
| PG1975 | 0.49↓                     | 0.067 ± 0.011↓ |
| PG1982 | 0.49↓                     | 0.058 ± 0.007↓ |
| PG2008 | 0.22↓                     | 0.011 ± 0.002↓ |
| PG0001 | 0.50↓                     | 0.068 ± 0.008↓ |
| 16sRNA | -                         | 1              |

cell division, and *PG1975* encodes hemagglutinin HagC related to pathogenicity of *P.gingivalis* W83. In addition, *PG1982* encodes a CRISPR protein related to CAS1 family. CRISPR/CAS system can protect bacteria against the encroachment by phage, and resist other chromosome genetic material and prevent from the expression of their genes [19–21]. Downregulation of *PG1982* suggests a decrease in the defense capability of *P.gingivalis* W83.

It should be noted that gene expression observed in this study was in mRNA level. As we have known, alterations in mRNA expression are not always consistent with those in protein expression. Therefore, observations in protein level of gene expression will be more convincing. However, it is impracticable to analyze the protein expression of all 64 genes with RNA expression alteration. Moreover, some products of these genes are still hypothetical proteins. Because the inoculated *P.gingivalis* was cultured outside the rat oral cavity for some days before RNA extraction, the RNA samples cannot exactly reflect the changes in gene expression after inoculation,

although the results can still indicate which genes are upregulated or downregulated.

## Conclusions

Our study shows that *P.gingivalis* W83 has pathogenic effects on host, and local inflammatory oral environment alters the gene expression profile of *P.gingivalis* W83. Products of these upregulated and downregulated genes are mainly related to transposon functions, cell transmembrane transportation, protein and nucleic acid metabolism, energy metabolism, cell division and bacterial pathogenicity. These changes may lead to decreased proliferation and pathogenicity of *P.gingivalis* W83, and favor themselves to adapt local environment for survival.

## Abbreviations

*P.gingivalis*: Porphyromonas gingivalis; PCR: Polymerase chain reaction; SPF: Specific pathogen free; ATCC: American Type Culture Collection; CFU: Colony forming unit; CEJ: Cementoenamel junction; ABC: Alveolar bone crest; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; CAS: CRISPR associated genes.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

JZ carried out microarray analysis, analyzed and interpreted the data, and drafted the manuscript. QL performed *P.gingivalis* W83 culture and inoculation. CLP performed real-time PCR analysis. JCL and HYW were responsible for the isolation and identification of the strain. LST performed alveolar bone loss analysis. YPP designed the study and drafted the manuscript. All authors read and approved the final manuscript.

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