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Short Communication

Association of *Prx* Gene Expression with the Gingival Phenotype

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Introduction

In addition to their role in development *Prx* genes have important roles in cell proliferation and migration [1]. Smooth muscle cells transfected with rat *Prx*1 showed increased proliferation [1]. Moreover, treatment of fibroblasts *in vitro* with morpholino oligos designed to specifically inhibit *Prx*1 and *Prx*2 protein expression resulted in downregulation of Tenascin-C expression and reduction in cell migration towards fibronectin [1]. Specifically, the healing process after surgical manipulation in the oral mucosa, is determined in a high degree by the gingival phenotype. Assuming that the gingival phenotype might be regulated by gene expression, the aim of our study was to investigate if there is an association between the presence and the levels of *Prx*1 and *Prx*2 mRNA and the phenotype of human gingivae.

Materials and Methods

Subjects included in the study: Twenty two patients in need of crown lengthening were included in the study, ten with thick and twelve with thin gingival phenotype. The assessment of the gingival phenotype was done based on the clinical characteristics of the gingiva.

Surgical Procedures: All surgeries were performed by postgraduate students at the Department of Periodontology and Biology of Dental Implants at the Aristotle University of Thessaloniki. All patients were required to sign a consent form before.

Tissue samples: Connective tissue fragments 1mm x 1mm were collected from the buccal flap during the surgical procedure and immediately placed in sterile tubes containing 1mL of RNA later (Sigma). Samples were maintained in RNA later at -80°C until further processing.

RNA isolation-Reverse Transcription: Total RNA was isolated from tissue samples using the NucleoSpin RNA Kit (Macherey Nagel) following the manufacturer's protocol. The concentration and quality of RNA in each sample was determined using a SmartSpec Plus spectrophotometer (Biorad). 200ng of RNA were reversed transcribed to cDNA using the PrimeScript II 1st strand cDNA synthesis kit (Takara) using oligo-dT primer. cDNA samples were stored at -20°C until further processing.

PCR Analysis for Prx1 and Prx2 mRNA Expression: The presence and levels of *Prx*1 and *Prx*2 mRNA expression were examined by semiquantitative PCR analysis. Two microliters of the cDNA were directly used as a template for the PCR in a total reaction volume of 25μL. The reaction mixture contained 12.5μL OneTaq Hot Start 2x Master Mix with standard buffer (New England Biolabs), 1μL of each primer (10μM), 2μL cDNA template, and PCR grade water. For detecting the presence of *Prx*1 primers *Prx*1F 5'-TCCCTCCTCAAATCCTAC-3' and *Prx*1R 5'-ACTATATTCCTTGGCCTTC-3' [2] were used.

For Prx2 detection primers *Prx*2F 5'-AGCACAGTGCCACCCTACA-3' and Prx2R 5'-CTTGGCAGGCTCTTCCACC-3' [2]. Expression of

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Gadph was used as an endogenous control. The primers for Gadph detection were: Forward 5'-TGG TAT CGT GGA AGG ACT CATGAC-3' and Reverse: 5'-ATG CCA GTG AGC TTC CCGTTC AG-3' [3].

PCR conditions were as follows: initial denaturation for 5 minutes at 94°C, then 25 cycles of denaturation at 94°C for 30s, annealing at 48°C for *Prx*1, 52°C for *Prx*2 and 48°C for Gadph for 30s, extension for 45s at 68°C and final extension at 68°C for 5 min. PCR products were visualized in a 2% agarose gel using Midori green Advance (Nippon Genetics) DNA/RNA stain and a FastGene LED Transilluminator (Nippon). Semi-quantification of the PCR products was done by using the ImageJ (https://imagej.nih.gov/) analysis program. All experiments were repeated twice to ensure statistical accuracy.

Statistical analysis: The differences between thin and thick phenotypes were analyzed using t-test. Inter-group differences were analyzed by t-test and Mann-Whitney test for independent samples. A p- value <0.05 was considered statistically significant.

Results

PCR Analysis for Prx1 mRNA Expression: PCR analysis showed *Prx*1 mRNA expression in higher levels in thick gingival phenotypes compared to thin. In addition, both *Prx*1a and *Prx*1b alternatively spliced isoforms were detected and *Prx*1a was more abundant in most of the samples compared to *Prx*1b. In thin gingival phenotypes *Prx*1a was either expressed in different levels or completely absent, fact that indicates that thin gingival phenotype might be regulated by other genes' expression (Figure 1).

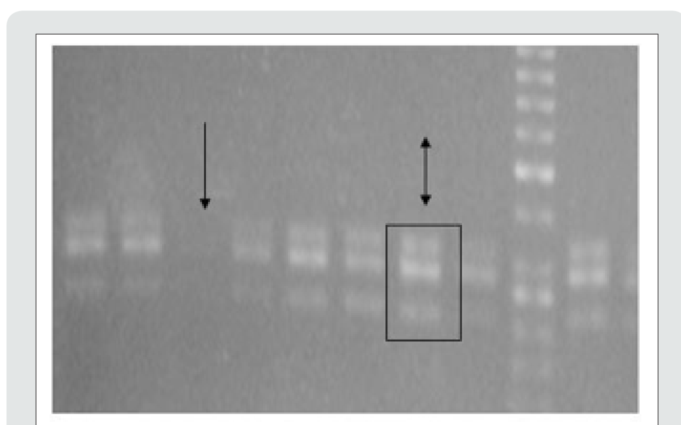

Figure 1: *Paired related homeobox* 1a and b gene isoform expression. The single arrow indicates the absence of *Paired related homeobox* 1 *Paired related homeobox* expression in a sample with thick gingival phenotype and the double arrow indicates the presence and abundance of both isoforms of *Paired related homeobox* 1 gene.

PCR Analysis for *Prx*2 mRNA Expression: PCR analysis showed that *Prx*2 mRNA expression was absent in thick gingival phenotypes except for one sample. On the contrary, *Prx*2 was highly expressed in all samples with thin gingival phenotype (Figure 2).

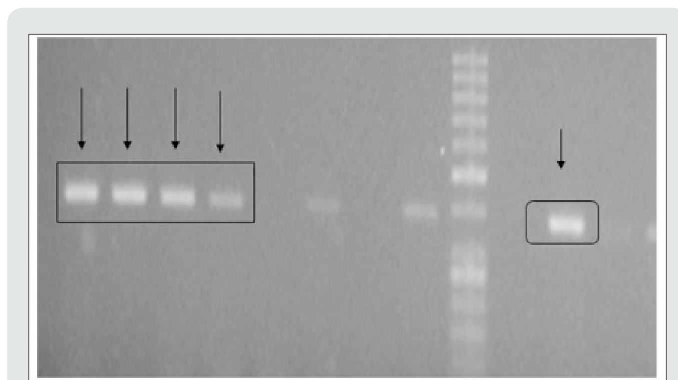

Figure 2: *Paired related homeobox* 2 gene expression. The single arrows indicate the high levels of *Paired related homeobox* 2 gene expression in samples with thin gingival phenotypes.

Discussion

Recent studies determine the gingival phenotype based only on clinical characteristics of the gingiva. Placing the periodontal probe in the gingival sulcus can give the appearance of transparency of the gingiva and this biotype is characterized as 'thin'. If the gingiva is not transparent when placing the probe, the gingiva is characterized as 'thick' [4,5]. In this study we aimed to associate the expression of *Prx* genes with the gingival phenotype. Our results indicate that *Prx*2 is expressed in thin phenotypes, whereas *Prx*1 is expressed in thick phenotypes. It is well established that *Prx* genes play an important role in craniofacial development. Our study indicates that *Prx* genes are not only expressed in tissues of ectomesenchymal origin, but they might also play an important role in determining the gingival phenotype at a molecular level. It is interesting t that *Prx*2 was almost exclusively expressed in thin phenotypes and in the same samples *Prx*1 was absent, implying opposing roles of the two genes in adult gingival tissues. Overall, our study indicates a strong association of *Prx*1 and *Prx*2 gene expression with the gingival phenotype. It is possible that *Prx* gene expression can be used as a molecular screening test to define more accurately the gingival phenotype.

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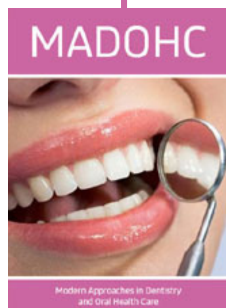

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