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Validation of cell-specific decomposition of gingival tissue transcriptomic signatures in periodontal health and disease

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BACKGROUND

Periodontitis is a chronic inflammatory disease that is associated with microbial dysbiosis and characterized by loss of connective tissue attachment and alveolar bone. Susceptibility and disease progression depend on the complex interactions among microbial challenge, the host's genetic factors, and acquired environmental stressors. Identification of transcriptomic signatures in the different cellular constituents of the gingival tissue has the potential to elucidate key molecular mechanisms underlying the initiation and progression of periodontitis. In the first phase of this project, we applied Population-Specific Expression Analysis (PSEA), a computational method that deconvolutes whole tissue transcriptomes into cell-specific signatures, to predict cell-specific differential gene expression in gingival tissues in states of periodontal health and disease.

OBJECTIVE

The current work focused on the validation of the PSEA-predicted transcriptional signatures in gingival epithelial and B cells in healthy and periodontitis-affected gingival tissues using independent, non-computational methodologies.

METHODS

- PSEA of gingival tissue samples identified several genes as being differentially expressed in periodontal health and disease in epithelial cells (COL4A2, C16orf58, CYGB, TGFB1, RORA, WDR45, YOD1, SH3GL3, EPN3, DUSP14, OAF), and in B cells (CERS3, CAMSAP1,HMGCR, INPP4A, THEMIS2, FAM46C, GNG7, IL10RA).
- Pairs of gingival tissue samples (each from a periodontally healthy and a periodontitis-affected site of the same patient) were harvested from 15 patients undergoing periodontal surgery (IRB protocol: AAAR0526).
- Periodontitis-affected gingival tissue samples showed bleeding on probing (BOP), probing depth (PD) ≥5mm, and clinical attachment loss (CAL) ≥ 3mm; periodontally-healthy control sites showed no BOP, PD ≤3mm, and no CAL. All patients were non-smokers.
- Tissue samples were processed to form a single-cell suspension, using a gentle MACS dissociator. **(Figure 1)**
- The Immuno-magnetic separation of epithelial cells and B cells were performed using CD326 (EpCAM) and CD19 magnetic MicroBeads respectively.
- RNA was extracted and quantified using NanoDrop 1000 device and cDNA synthesis was performed using a High Capacity cDNA Reverse Transcription Kit.
- Finally, a quantitative real-time PCR assay was performed to validate differential expression of selected predicted candidate genes in epithelial cells (TGFB1, RORA) and B cells (CERS3, CAMSAP1).
- One-tailed paired and unpaired t-tests were used for statistical analysis. Statistical significance was defined at $p<0.05$.

RESULTS

- The mean age of the patients was 53 years (range, 35 to 72 years). On average, study participants had 25 teeth present.
- Among the harvested gingival tissue samples, mean PD was 2.7 ± 0.6 mm at healthy sites and 5.4 ± 2.1 mm at periodontitis-affected sites; the latter showed a mean CAL of 6.9 ± 2.2 mm.
- Analyses of paired samples from periodontitis-affected and healthy sites obtained from the same donor showed significantly lower levels of RORA (p=0.03) and TGFb (p=0.01) in epithelial cells isolated from periodontitis-affected sites, consistent with PSEA predictions (n=8 pairs for RORA, and n=7 pairs for TGFb; Figures 2 & 3).
- In unmatched analyses that included the total number of samples that were successfully processed irrespective of donor, RORA (p=0.03) and TGFb (p=0.02) were significantly lower in epithelial cells isolated from periodontitis-affected sites compared to healthy sites (n=22 for RORA, and n=18 for TGFb; Figures 2 & 3).

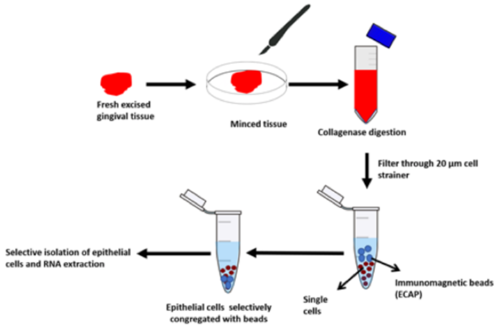

Figure 1. Schematic representation of gingival epithelial cell isolation from surgically excised gingival tissue.

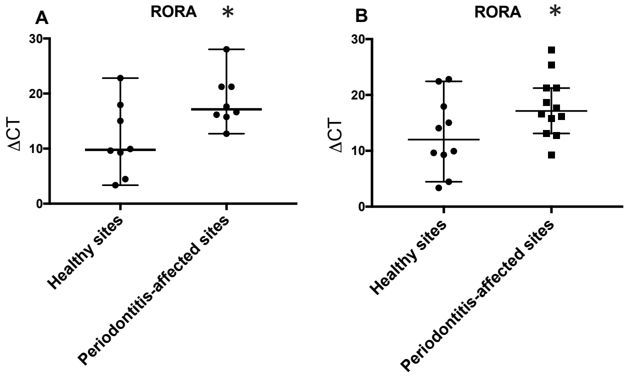

Figure 2. Relative expression levels of RORA assessed through quantitative real-time PCR in matched (n=8 pairs; panel A) and non-matched analyses (n=22; panel B). Data is presented as median and 95% confidence interval.18s was used as normalizer.
* indicates statistically significantly different from healthy sites.

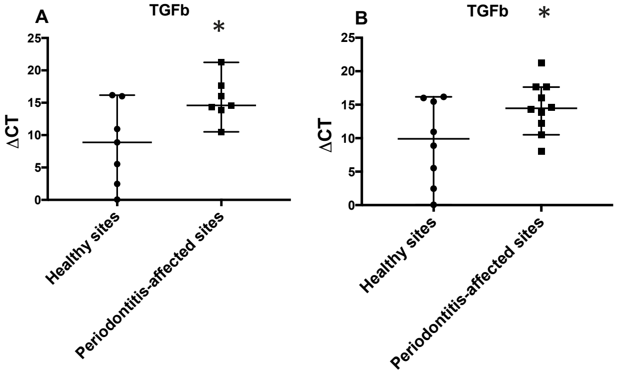

Figure 3. Relative expression levels of TGFb assessed through quantitative real-time PCR in matched (n=7 pairs; panel A) and non-matched analyses (n=18; panel B). Data is presented as median and 95% confidence interval.18s was used as normalizer.
* indicates statistically significantly different from healthy sites.

CONCLUSION

The successful validation of PSEA-predicted differentially-expressed genes in two cell types (epithelial cells and B cells) by quantitative RT-PCR suggests that PSEA is suitable for the deconvolution of the human gingival transcriptome and offers a viable computational alternative to experimental separation methods.

ACKNOWLEDGEMENT

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