Analysis of Oral Neutrophil Transcriptome Changes before and after Treatment in Periodontal Patients with and without Type 2 Diabetes

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Abstract

Periodontitis is a significant oral disease affecting about half of American adults. It is associated with inflammations caused by immune cells in response to changes in the subgingival microbiome. Type 2 Diabetes (T2D) upregulates inflammatory response and promotes tissue destruction and thus increases the risk of developing periodontitis. It has been suggested that oral neutrophils are the main immune cell responsible for periodontitis progression. However, how the neutrophils function differently in T2D patients compared to non-diabetic individuals (ND) remains to be understood. In this study, oral rinse samples were collected from periodontitis patients with or without T2D before and after periodontitis treatment. Oral neutrophils were isolated and RNA sequencing (RNA-Seq) was performed. PCA and hierarchical clustering analyses showed that the neutrophil transcriptome profiles were different between T2D and ND patients. Using DESeq2, we identified a set of genes that were differentially expressed between T2D and ND patients, including TCF7L2, CAPN10, and HHEX, which have been associated with T2D. By using Gene Set Enrichment Analysis (GSEA), we found two pathways, epithelial mesenchymal transition and interferon gamma response, significantly enriched in the differentially expressed genes. This study provides important molecular insights on the immune response differences by neutrophils between T2D and ND patients in the context of periodontitis. It will help us better understand the interplay between periodontitis and T2D.

Introduction

- Periodontitis is a common oral disease in adults. It is associated with inflammations caused by immune cells in response to changes in the subgingival microbiome.
- T2D patients have upregulated inflammatory response and increased risk of developing periodontitis.
- Oral neutrophils have been suggested as the main immune cells responsible for periodontitis development.
- It is not well understood whether the neutrophils act differently between T2D and ND individuals with periodontitis.

Methods

- 7 T2D and 5 ND patients with periodontitis were recruited in the study. Oral rinse samples were collected at two visits: before periodontitis treatment (D1), and after treatment (D2).
- Neutrophils were isolated from oral rinse samples, and RNA-seq was performed.
- Genes with less than 300 total read counts were filtered. For unsupervised hierarchical clustering and GSEA, genes with < 25th percentile variance were excluded.
- Data normalization and transformation were performed using DESeq2.
- PCA and hierarchical clustering analyses were performed.
- Differentially expressed genes (DEGs) were identified with adjusted p-value < 0.05.
- Gene enrichment analysis were performed on the differentially expressed genes.

Results

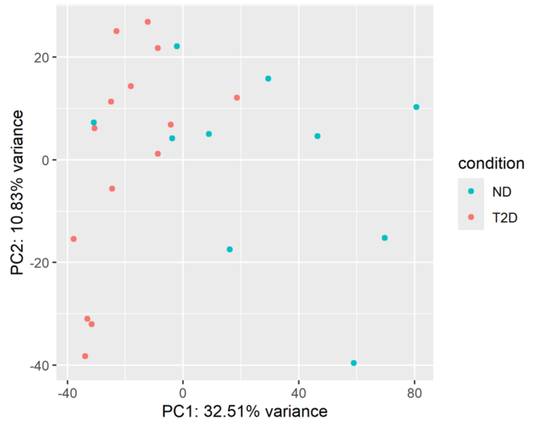

Figure 1. PCA shows that the gene expression profiles of T2D and ND were different.

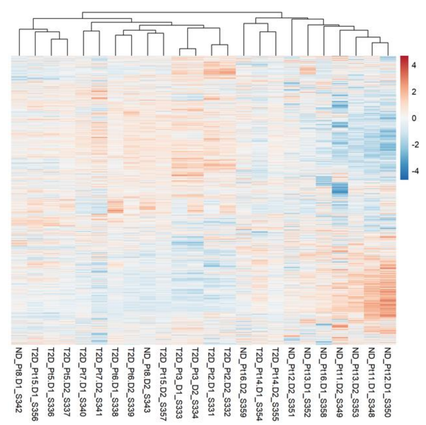

Figure 2. Unsupervised hierarchical clustering groups T2D and ND patients separately.

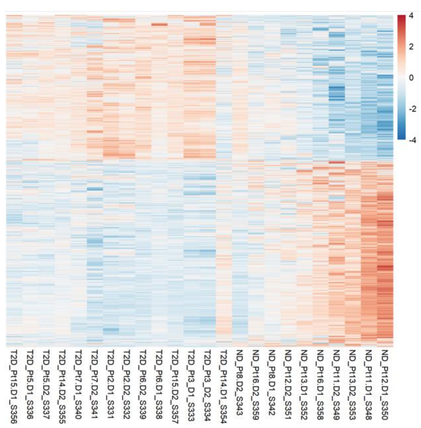

Figure 3. Differentially expressed genes between T2D and ND patients.

Table 1. Two pathways significantly enriched in the differential expressed genes identified by GSEA.

ID	setSize	enrichmentScore	NES	pvalue	p.adjust	qvalue	rank
Epithelia Mesenchymal Transition	17	0.562305	2.148876	0.001515	0.029791	0.025582	284
Interferon Gamma Response	31	0.444484	2.083532	0.001568	0.029791	0.025582	698

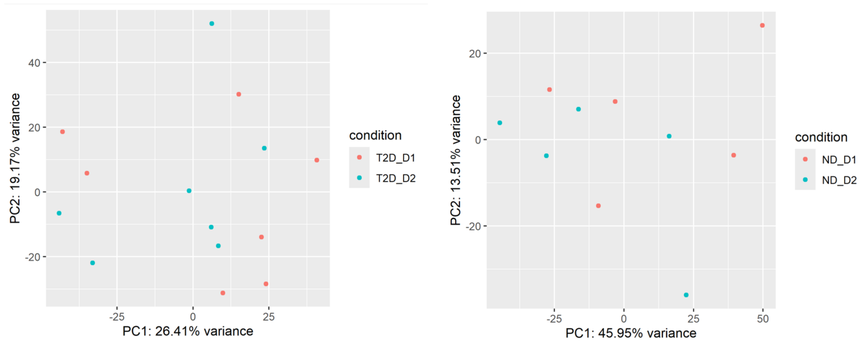

Figure 4. The neutrophil expression profiles are not significantly different between the two visits in both T2D and ND patients.

Discussion

- PCA and hierarchical clustering analyses suggested that the neutrophil transcriptome profile of T2D patients was different from those of ND patients (Figures 1 and 2).
- We identified a set of genes that were differentially expressed in neutrophils between T2D and ND patients such as TCF7L2, CAPN10, and HHEX. These genes were indicated in T2D from previous studies (Figure 3).
- By using GSEA, we found two pathways, epithelial mesenchymal transition and interferon gamma response, significantly enriched in the differentially expressed genes (Table 1).
- Based on PCA, we did not find significant differences in neutrophil gene expression between the two visits before and after periodontitis treatment. This is different from the findings by Lakschevitz *et al.* (Figure 4).

References

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Lakschevitz FS, Aboodi GM, Glogauer M (2013) Oral Neutrophil Transcriptome Changes Result in a Pro- Survival Phenotype in Periodontal Diseases. PLoS ONE 8(7): e68983. https://doi.org/10.1371/journal.pone.0068983.g006

Acknowledgement

We thank Dr. Renate Lux, Dr. Flavia Pirih, Dr. Matthew Freeby, Dr. Brittany Wall, and Dr. Syeda Masooma for their collaboration and for providing patient samples and data for this analysis.