AAO Foundation Award Final Report

Type of Award: Biomedical Research Award

Principal Investigator: Wellington J. Rody Jr.

Title of the project: Gene expression profile analysis of osteoclast and odontoclasts

Period of AAOF support: 07-01-15 to 06-30-17

Amount of Funding: $ 30,000

Summary/Abstract

Introduction and objectives: Although it is believed that 'odontoclasts' possess properties common to 'osteoclasts', the regulatory mechanisms that mediate odontoclastic dentin resorption may differ from osteoclastic bone resorption. Thus, the goal of this pilot study was to identify gene clusters that were specifically induced by the interaction of odontoclasts with dentin. Our hypothesis is that differential gene expression exists between those two cell types that would allow us to identify specific markers of dentin resorption in future studies. **Material and Methods**: Interrogation of odontoclasts and osteoclasts was performed using primary bone marrow cells from C57BL6 mice cultured on either dentin or bone slices for one week. Cell culture experiments were conducted in triplicate to reduce random error and the supernatants from each experiment were pooled together to gather enough RNA for a robust downstream analysis. Total RNA was purified from the cultures at days 3 and 7 and subsequently used for RNA-seq. analysis. Reads acquired from the illumina HiSeq system were cleaned up with the Cutadapt program to trim off sequencing adaptors and low quality bases with a quality phred-like score<20. Reads <40 bases were excluded from RNA-seq analysis. The transcripts of the garden tomato mus musculus (C57BL6) from the NCBI database were used as reference sequences for RNA-seq analysis. The cleaned reads of each sample were mapped independently to the reference sequences using the mapper of bowtie2 with a maximum of 3 mismatches for each read. The mapping results were processed with the samtools and scripts developed in house to remove potential PCR duplicates and select uniquely mapping reads for gene expression estimation. The number of mapped reads for each individual gene were then counted and analyzed between samples. In each comparison, only genes with log2 transformed folder change >=2 and the mapped reads >100 in either sample were selected for further analysis. **Results:** Approximately 452 genes were differential expressed in odontoclasts at day 3 in comparison to osteoclasts on bone at the same time point, including 23 up-regulated genes and 429 down-regulated genes. Similarly, 450 genes were differentially expressed in odontoclasts at day 7 in comparison to osteoclasts at day 7, including 39 up-regulated genes and 411 down-regulated genes. The comparison between odontoclasts vs. osteoclasts at both days 3 and 7 revealed 243 genes that were uniquely expressed in dentin. The selection of meaningful gene candidates for clinical application is still an ongoing endeavor for our group; however, some interesting genes linked to dentin resorption were identified. Included amongst the genes that were more expressed by odontoclasts was LGALS3. This gene encodes the protein galectin-3, a member of the galectin family that seems to play regulatory roles in mineralized tissue remodeling. While the expression of this gene went up 3-4 fold in dentin over time (from day 3 to day 7), our results did not show relevant expression of this gene at any of the time points in bone. Moreover, our previous human proteomic study indicate that galectin-3 can be found in gingival crevicular fluid collected from teeth undergoing root resorption, making it even more attractive as a diagnostic

tool. **Conclusion**: Our results depict a difference in gene expression profile between osteoclasts and odontoclasts that may have potential clinical application for assessment of root resorption.

1. Were the original, specific aims of the proposal realized?

Yes. We accomplished the proposed specific aim.

2. Were the results published? If so, was AAOF support acknowledged? If not, are there plans to publish? If not, why not?

Results have not been published yet. I am planning to publish this study in an orthodontic or bone biology journal.

3. Have the results of this proposal been presented? If so, when and where? If not, are there plans to do so? If not, why not?

Yes. Preliminary findings were discussed at the 2017 AADR/IADR meeting in San Francisco CA and at the 2017 AAO annual session in San Diego CA. We intend to present the final results at future meetings.

4. **To what extent have you used, or how do you intend to use, AAOF funding to further your career?**

I have used AAOF funding to foster my career development, consolidate research collaborations as well as to generate preliminary data for publications and NIH grant applications.