Where you originate from matters; A Comparison of Jaw and Long Bone Osteoclast Precursors

2022 Biomedical Research Awards (BRA)

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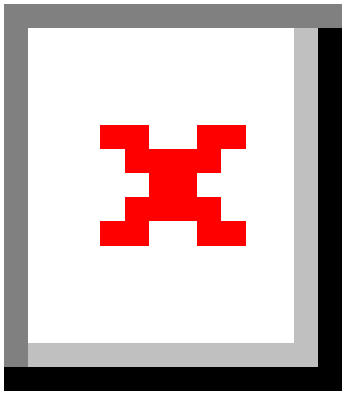
FollowUp Form

Award Information



In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*

Where you originate from matters; A Comparison of Jaw and Long Bone Osteoclast Precursors

Award Type

Biomedical Research Award (BRA)

Period of AAOF Support
July 1, 2022 through June 30, 2023

Institution
University of Minnesota

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Dr. Amy Tasca, DDS, PhD -PI, Dr. Kim Mansky, PhD- Co-I, Dr. Adam Herman, Phd consultant, Rachel Phillips- Student Investigator

Amount of Funding
$30,000.00

Abstract
(add specific directions for each type here)

Osteoclasts are multinuclear cells derived from myeloid lineage progenitors responsible for bone resorption. Osteoclast activity within craniofacial bones facilitates critical developmental processes such as tooth eruption and skull modeling; however, we lack essential knowledge about the osteoclast progenitors found in the marrow of craniofacial bones. While recent studies suggest unique molecular signatures of immune cells and myeloid derived suppressor cells within different skeletal sites, the bulk of our knowledge and understanding of osteoclast progenitors is derived from long bones. Moreover, we completely lack transcriptomic data describing craniofacial-derived osteoclast progenitors. Our data demonstrates that osteoclasts differentiated from bone marrow of the mandible are significantly larger and have increased expression of osteoclast genes compared to osteoclasts derived from the bone marrow in the femur. In this proposal we will perform single cell sequencing from mandible and femur derived bone marrow to identify gene/pathway signatures unique to the two skeletal sites. The information gained from the single cell sequencing will serve as preliminary data for a nationally competitive grant to determine how changes in the environment (medications, inflammation, injury, etc.) regulate osteoclast precursors at different skeletal sites.

Comment: *Congratulations on the completion of this project. The AAOF PARC commends you on this accomplishment and looks forward to seeing your publications in the near future.*

Respond to the following questions:

Detailed results and inferences:*
If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you

have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

AAOF Final Report Data.pdf
See uploaded file

Were the original, specific aims of the proposal realized?*

Yes. The specific aim of our proposal was realized. We proposed to determine the molecular signature of mandible and long bone derived marrow. We achieved this goal by successfully completing our proposed single cell RNA Sequencing experiment and analysis.

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

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

AAOF funding has allowed me to continue to collaborate with Kim Mansky PhD on our osteoclast research. Funds from this award supported a PhD/Orthodontic Resident candidates thesis research. The clinical and basic scientist collaboration along with mentorship is looked highly upon at our institution. I successfully went up for promotion this year. To be able to list a significant award with funding was invaluable in this process.

Accounting: Were there any leftover funds?

$0.00

Not Published

Are there plans to publish? If not, why not?*

Yes. We will publish our data after we confirm expression of differentially regulated genes between femur and mandible.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

Poster Presentation AAO Chicago 2023- Site-Specific Characterization of Mandibular and Femur Derived Osteoclasts, Rachel Clark DDS, Amy Tasca DDS PhD, Kim Mansky PhD.

Was AAOF support acknowledged?

If so, please describe:

Yes. The AAOF was listed in the acknowledgement section of the poster.

Internal Review

Reviewer comments

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- AAOF Final Report Data.pdf

CD11b^{+} cells were isolated from femur or mandible derived bone marrow of 2- month-old male C57Bl/6J mice. Cd11b microbeads from Miltenyi Biotec were used to separate the Cd11b+ cells from the bone marrow. Cd11b isolation was verified by flow cytometry. Samples from Cd11b^{+} femur derived cells or mandible derived cells (N=3 each) to the UMN Genomics Center for single cell RNA sequencing. CD11b^{+} cells include monocytes/macrophages, dendritic cells, neutrophils, natural killer cells and granulocytes. Six single cell captures targeting 10,000 cells each were performed (one for each sample) by ST G chip for 3' gene expression. Of the cells captured, cell viability ranged from 91-97.8 percent. From the gel bead emulsions, libraries were prepped. Sequencing was performed on the Illumina Nova Seq system. Femur gene expression data as was used as the baseline for analysis. Preliminary single cell data was processed in Seurat v4.3.0 in R v4.1.0. Number of genes detected (nFeatures), numbers of UMIs/unique molecular identifiers (nCount_RNA), which is representative of the number of unique transcripts, and percent mitochondrial UMIs were plotted. Mean genes per cell detected across all datasets was 2,310 genes (median=2,013), mean UMI counts per cell was 10,379 (median=7,901). Filters were applied to remove low quality cells. Cells with < 600 (low quality) or > 6000 genes (likely multiplets) detected, or more than 20% mitochondrial reads (apoptosis cells) were removed. An average of 5% of cells were removed from each data set.

From this processed data, subpopulations were identified using SingleR and ImmGenData references. Using this data two-dimensional t-SNE representations of the expression levels of each of the samples was plotted and unique cell populations identified as well as differentially expressed genes. At a resolution of 0.1, nine unique subpopulations (0-8) of cells were identified (Fig 1). Using the mostly highly expressed genes in each cluster, we identified the cell type found in that cluster.

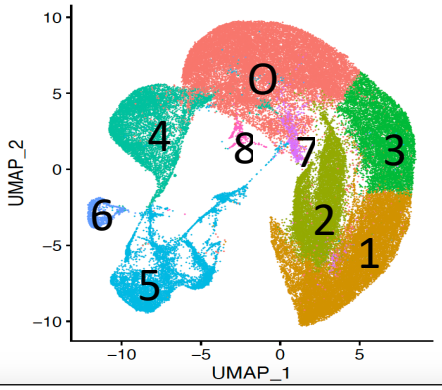
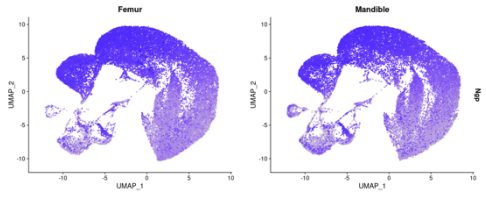
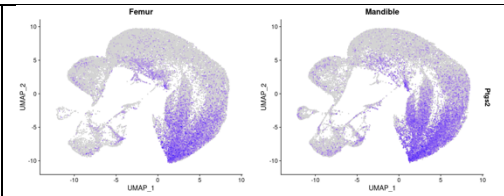
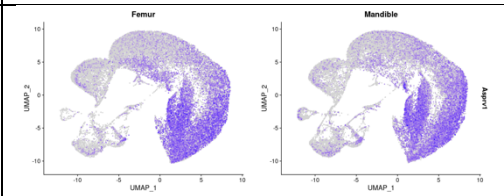
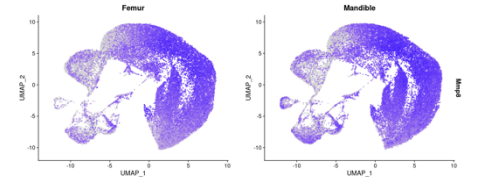
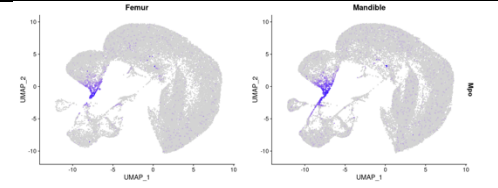
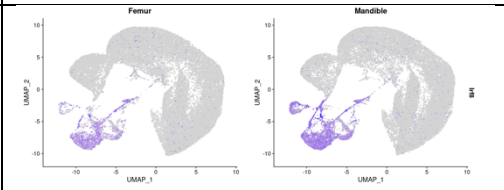
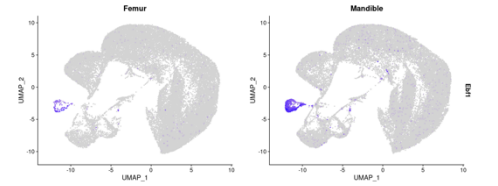
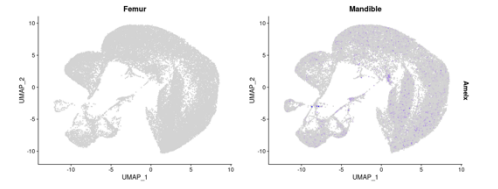


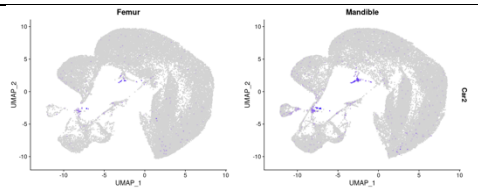
Figure 1. UMAP of Individual clusters.

Table 1 lists the cluster and the cell that we have determined to be contained within that cluster. The table also includes an example of one of the highly expressed genes that was used to determine cell identity. In the last column is a UMAP of that gene indicating cluster location of expression.

Table 1. Cell Types found within Single Cell Sequencing of CD11b^{+} Cells

Cluster	Cells contained within Cluster	Example of Gene used to identify cell type	UMAP of Gene
0/4	Neutrophils	*Ltf*	

1	Macrophages	*Ptgs2*	
2	Myeloid Suppressor Cells	*Asprv1*	
3	Macrophage	*Mmp8*	
4	Proliferating Neutrophils	*Mpo*	
5	Inflammatory Macrophage	*Irf8*	
6	B cell	*Ebf1*	
7	M2 macrophage	*Amelx*	

8	Natural Killer Cells	*Car2*	

Differentially regulated genes: The goal of this project was to begin to identify mechanisms to explain why osteoclasts in the mandible are enhanced in size compared to femur derived osteoclasts. We have identified several differentially regulated gene pathways in the mandible compared to the femur. Using pathway analysis of these genes, we focused on clusters 1-3, 5, 7 and 8, as they should contain osteoclast precursors or cells that will affect osteoclast differentiation. Upregulated pathways in these clusters contained genes know to regulate inflammation, metabolism and osteoclast differentiation (Fig 2). Examples of genes involved in inflammation include the cytokines C-C motif chemokine ligand 2 and 3 (*Ccl2* and *Ccl3*). C-X3-C motif chemokine receptor 1 (*Cx₃cr1*) has been demonstrated to be a receptor expressed on osteoclasts involved in aging. The gene *Cd86* is a marker for inflammatory macrophages. In cluster 7 we saw increased expression of the gene *amelogenin X-linked Amelx* which is known to enhance tooth mineralization. In those same clusters we identified gene pathways involved in adhesion and hemopoietic cell lineage as down regulated.

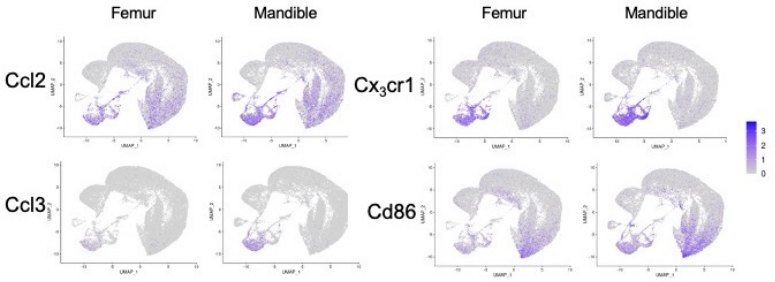


Figure 2. UMAP of Differentially Regulated Genes in Mandible Compared to Femur

Future Directions: The next steps going forward will be to confirm expression of differentially regulated genes between femur and mandible. We will also begin designing biological experiments to determine how differences in gene expression affect function.