Roles of MiR-145 in Osteocytes During Tooth Movement

2023 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:*
Roles of MiR-145 in Osteocytes During Tooth Movement

Award Type
Biomedical Research Award (BRA)

Period of AAOF Support
July 1, 2023 through June 30, 2024

Institution
The Board of Trustees of the University of Illinois

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Phimon Atsawasuwan

Amount of Funding
$40,000.00

Abstract
(add specific directions for each type here)

Please see attached document.

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".
<u>OR</u>
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".

final results AAOF.pdf

Were the original, specific aims of the proposal realized?*
Specific Aim #1. To elucidate the roles of miRNA-145 expression on Ocyte apoptosis and RANKL release and its roles in autocrine signaling in Ocyte on Ocyte-OC coupling.
Specific Aim #2. To investigate the effects of miR-145 deficiency in Ocytes on bone modeling during tooth movement in vivo.
The specific aim 1 was partially completed due to the change of approach to knock out the osteocyte using a Lentiviral system instead of knocking down Agonaute protein.
The specific aim 2 was complete and shown in the result.

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?*
We have used the funding from AAOF to successfully generate the transgenic mice, miR-145 deficiency mice under Sclerostin promoter, and the osteocyte-like cell lines underexpressing miR-145 using a Lentiviral

vector system. These tools will be used to further generate the preliminary data for the extramural funding, such as R01 or R21 NIH funds.

Accounting: Were there any leftover funds?

$0.00

Not Published

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

Yes. We are in the process of verifying preliminary results from the 2023 Biomedical Research Awards. We plan to publish the results of tooth movement in the miR-145 deficient mice in the near future. The details of bone phenotypes of the transgenic animals will be presented at the future annual meeting of AAO and submitted for publication because a resident will used the data for the MSc thesis and compete for the resident research award at AAO annual meeting.

Presented

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

A part of preliminary data was presented as a poster presentation at the 2023 Consortium for Orthodontic Advances in Science and Technology in California.
Poster presentation: 1. Atsawasuwan. P., Chen Z., Viana G.. Roles of miR-145 in osteocytes and its coupling process during bone remodeling. 2023 Consortium for Orthodontic Advances in Science and Technology. Lake Arrowhead, CA, October 2023.
In addition, the support from the AAOF was used to generate the data for the UIC orthodontic residents and one of the residents received Thomas M Graber award of special merit in 2024 annual meeting of AAO and 2023 Dr. WIlliam R Proffitt Resident award.
2. Karkazis EM, Lown, S, Viana G, Nicholas C, Oubaidin M, Reed D, Atsawasuwan P. Bone Characterization in a Novel Dicer-Deficiency in Osteocyte Transgenic Mouse Model. Dr. William R Proffitt Resident Scholar Awards competition. 124th Annual Session-American Association of Orthodontists, New Orleans, LA, May 2024.
3. Karkazis EM, Lown, S, Viana G, Nicholas C, Oubaidin M, Reed D, Atsawasuwan P. Bone Characterization in a Novel Dicer-Deficiency in Osteocyte Transgenic Mouse Model. 125th Annual Session-American Association of Orthodontists, Philadephia, PA, April 2025.

Was AAOF support acknowledged?

If so, please describe:

Yes. The 2023 Biomedical Research Award from the AAOF was acknowledged in the poster and will be acknowledged in future presentations.

Internal Review

Reviewer comments

Reviewer Status*

File Attachment Summary

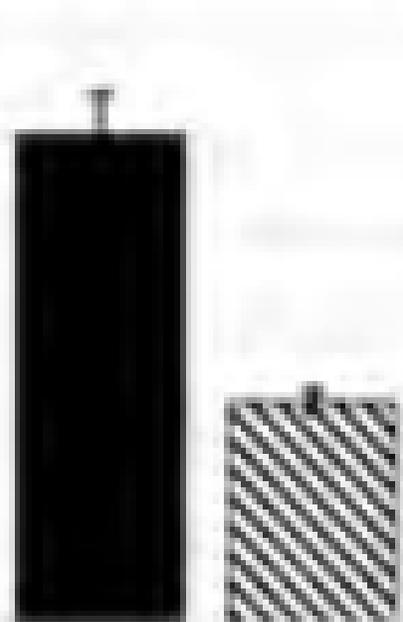*Applicant File Uploads*

- final results AAOF.pdf

Results

The novel miRNA-145 deficiency under the Sclerostin promoter transgenic mice have been successfully established and their bone phenotypes and miRNA-145 expression levels in bone matrices are continuously characterized and compared with their control animals. The miR-145 expression levels in bone matrices of harvested femurs from the Dicer homozygous and Sost+ were suppressed 0.39 folds compared to the Dicer homozygous and Sostcre transgenic mice. In males, the suppression was 0.33 folds, while in females was 0.45 folds compared to their controls (Figure 1). We have performed tooth movement in male miR-145 deficient mice and found that tooth movement was significantly accelerated in the homozygous miR-145 fl/fl/Sostcre+ group (220+45µm) (Figure 2 D, E) compared to homozygous miR-145 fl/fl/Sostcre- control group (80+70µm) (Figure 2A, B) and heterozygous miR-145 fl/-/Sostcre+ (110+90µm) (Figure 2C).   For *in vitro* experiments, we have tested the cellular behaviors of OCY454 osteocyte cell line and OCY454 scramble and Argonaute (AGO) deficient OCY454 cells under the fluid shear stress to establish the baseline behavior of the control cells and AGO deficient cells. We discovered that OCY454 under fluid shear stress expressed higher levels of Opg, Rankl (osteoclastic differentiation factors), and Rock1 (apoptosis-related gene) and podoplanin (E11), Dmp1, and Phex (early osteocytic markers) and lower levels of Sclerostin, Mepe (late osteocytic markers) compared to non-loaded controls (Figure 3). In addition, the protein levels of OPG and sRANKL in the spent cell media of OCY454 under fluid shear stress were higher compared to non-loading control groups. The Rankl/Opg ratio of loaded cells was higher in wild-type OCY cells but not markedly increased in AGO-deficient OCY cells (Figure 4). We attempted to establish a stable OCY454 clone, but we could not obtain any stable clones due to the low transfection efficiency of the plasmid and the transfection technique. We decided to establish a Lentiviral vector from the University of Iowa to suppress miR-145 and use the Lentiviral vector to infect the OCY 454 cells and establish stable clones. We obtained 2 stable clones of OCY underexpressing miR-145 with miR-145 expression levels at 0.4 and 0.7 folds compared to the control cells (Figure 5). We subjected the clones to the fluid flow loading platform (Streamer, Flexcell Int) and found increased expression levels of all osteocytic markers, Dmp1, E11, Sost, Phex and Mepe in the loaded groups compared to the unloaded group. The patterns of upregulation of the osteocytic gene markers were less striking compared to those of controls. However, only E11 demonstrated a significant difference (P<0.05) (Figure 6A). As the report showed that osteocytes are the major source of osteoclast-differentiating factors, all the test cells under loading expressed higher levels of Rankl and Opg, compared to the unloaded groups (P<0.05) (Figure 6B). As the apoptosis of the osteocytes is needed in the process of Rankl and Opg secretion, we investigated the apoptosis-related gene markers in the tested cells and found that the apoptosis-related genes, Rock1, Rock2, and Pcdc4, in the loaded cells were up-regulated compared to the unloaded groups (P<0.05) (Figure 6C). When compared to the controls, only OPG, Rankl and Rock2 expression of miR-145 underexpressing clones demonstrated the significant differences (P<0.05). The preliminary results demonstrated that fluid sheer stress affected the osteocyte-like cells and the underexpression of miR-145 did not affect the cell behaviors under loading; however, miR-145 underexpression may affect the degree of cell apoptosis which will need further investigation.
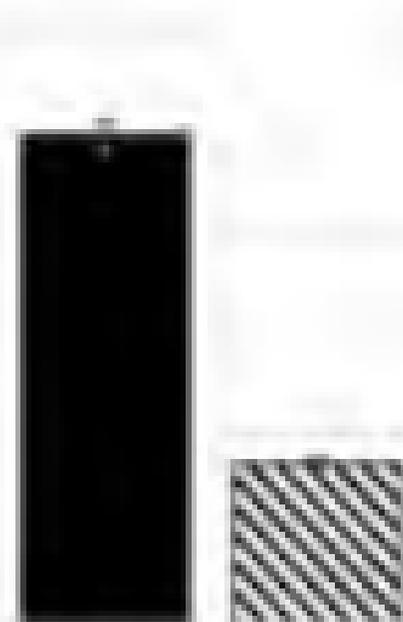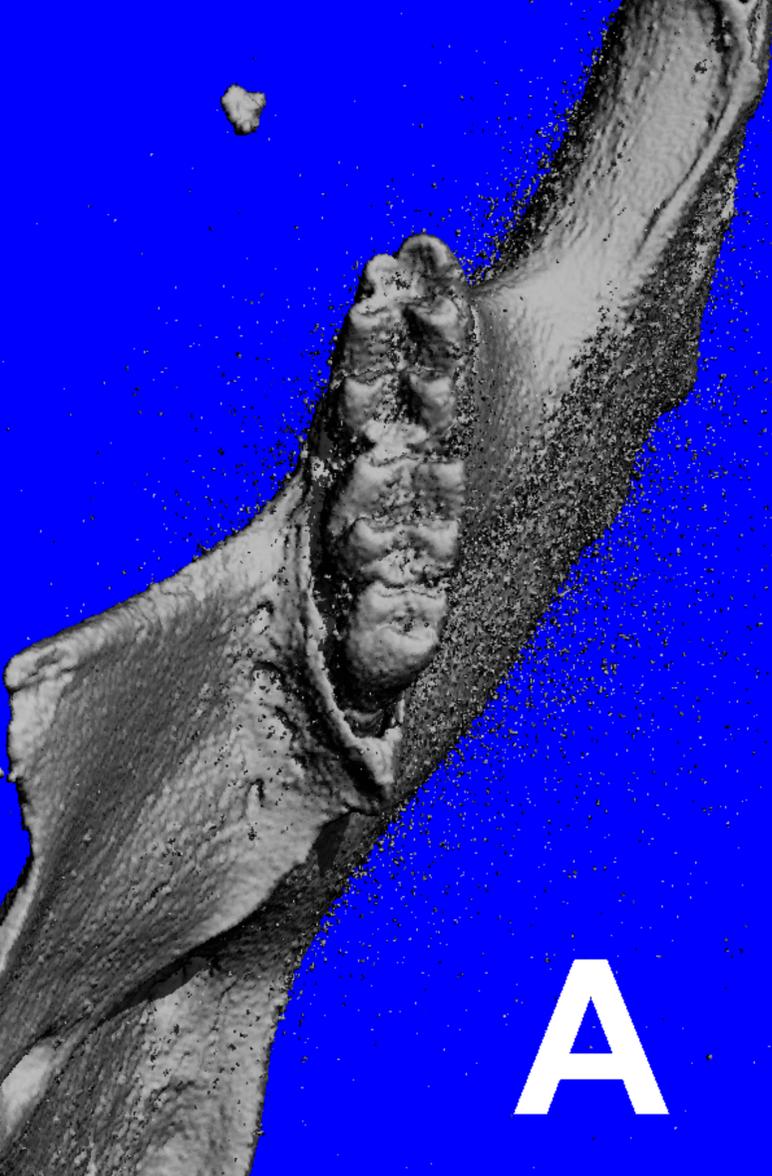
miR-145

Relative fold changes

Females   Males

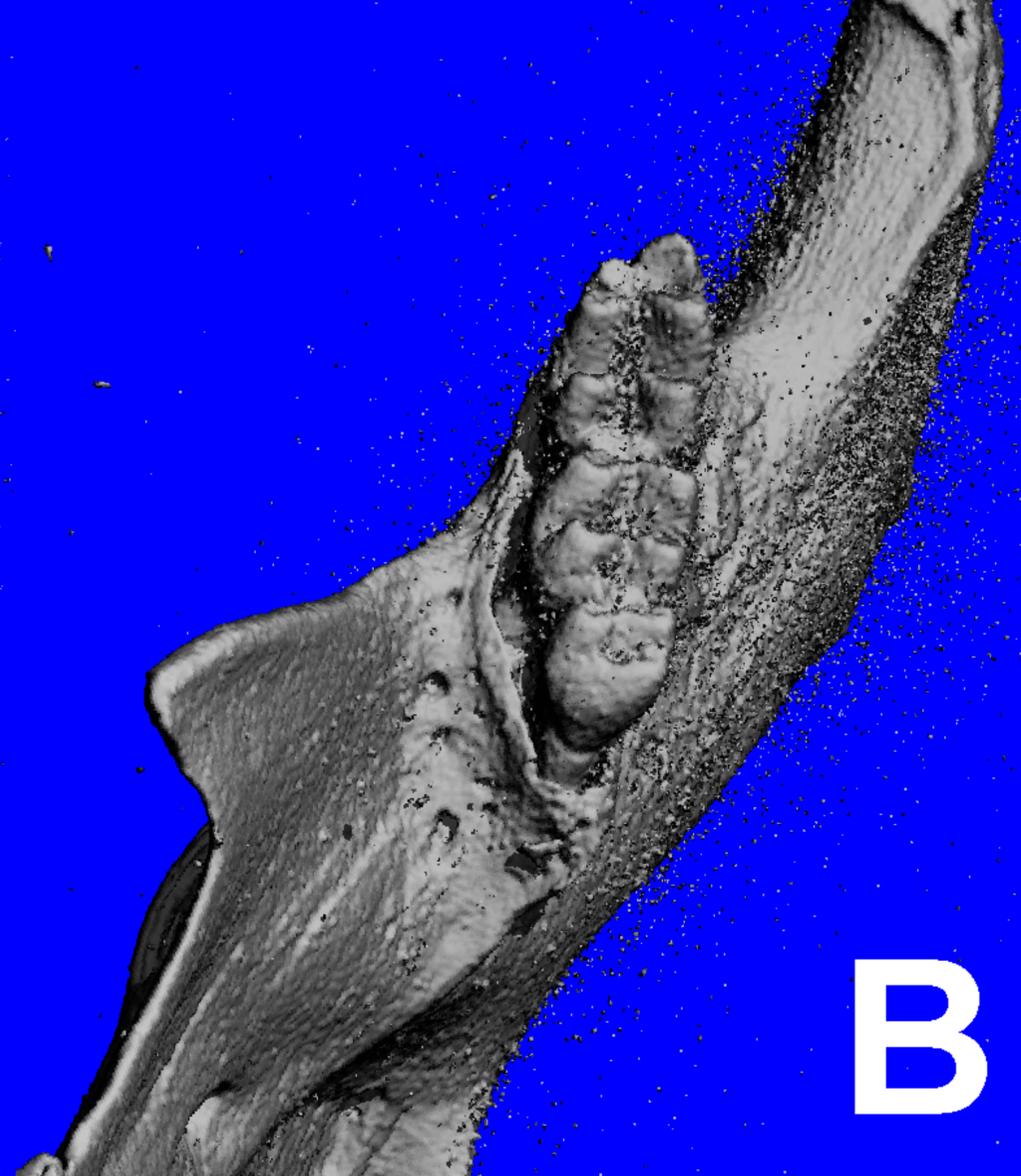■ miR-145 df/df/sost-   ☒ miR-145 df/df/sost+

Relative fold changes for Phex, Podoplanin (E11), Dmp1, Sost, Mepe, Opg, Rankl, Igf1, and Rock1.

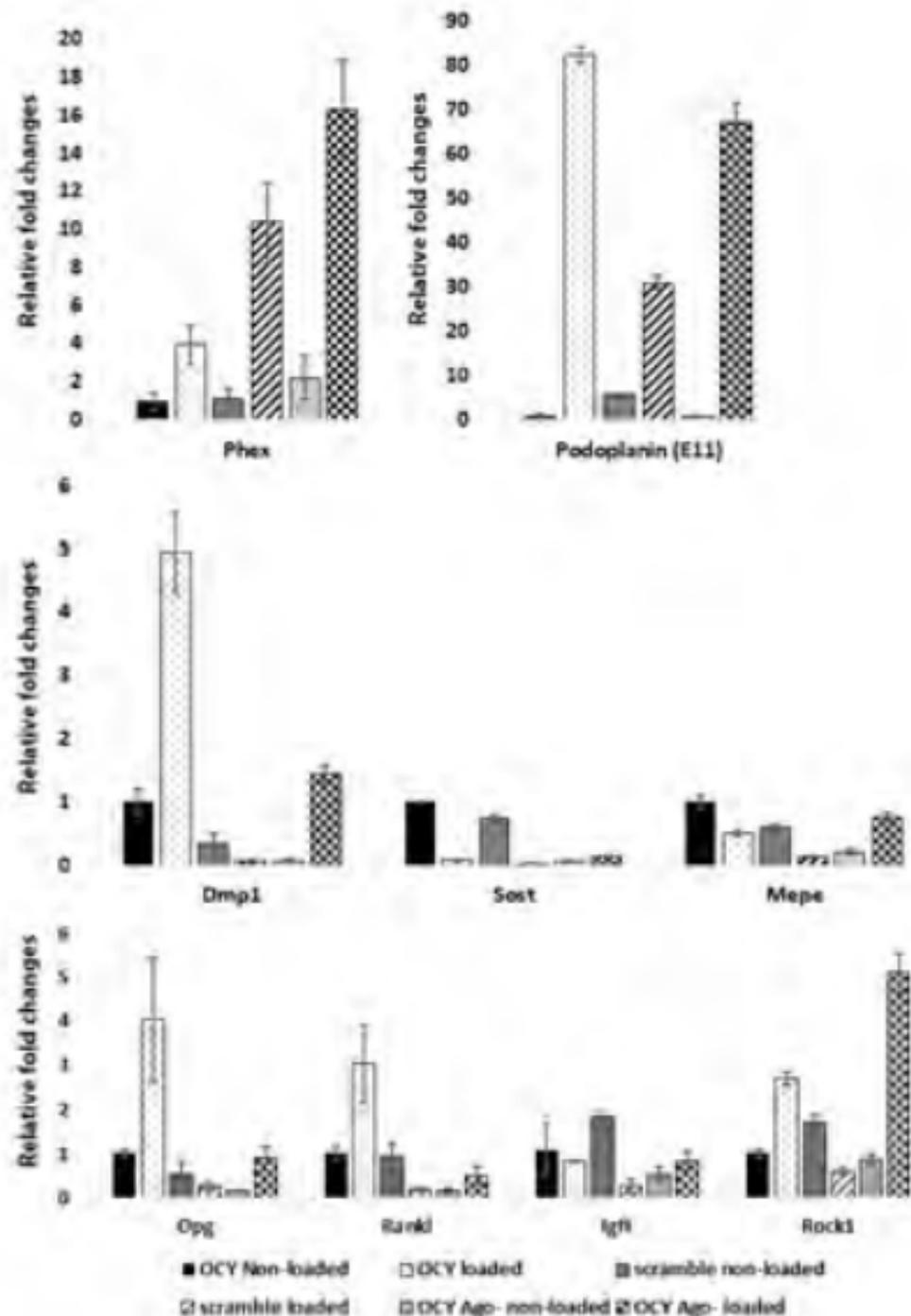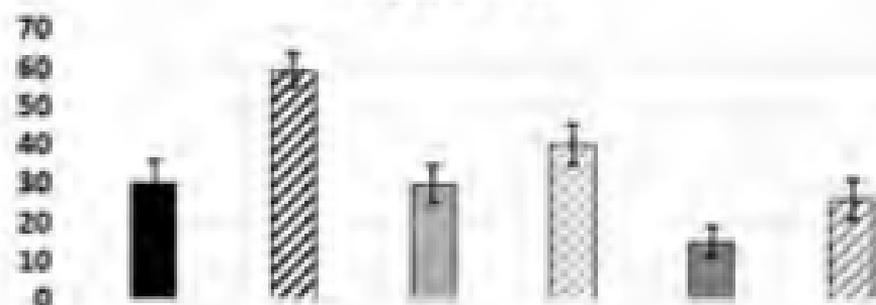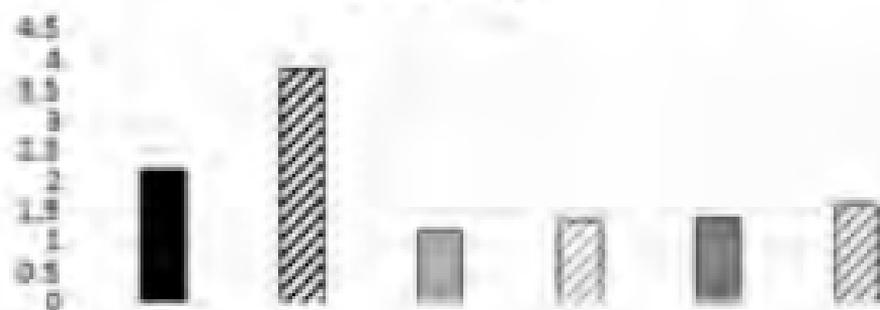Legend: ■ OCY Non-loaded □ OCY loaded ▨ scramble non-loaded ▧ scramble loaded ▨ OCY Ago- non-loaded ▨ OCY Ago- loaded

Rankl (pg/ml)

Opg (pg/ml)

Rankl/Opg

Scramble non-loaded
Scramble loaded
OCY Agp-1 non-loaded
OCY Agp-1 loaded
OCY Agp-2 non-loaded
OCY Agp-2 loaded

Expression level of miR-145

Relative fold changes

1.2	
1	
0.8	
0.6	
0.4	
0.2	
0	

Ctrl   miR-145 underexp clone1   miR-145 underexp clone2

A Osteocyte markers

B Osteoclast-related differentiation genes

C Apoptosis-related genes

■ Ctrl-unloaded ▧ Ctrl loaded
▤ miR-145 underexp clone1 unloaded ▧ miR-145 underexp clone1 loaded
▨ miR-145 underexp clone2 unloaded ▥ miR-145 underexp clone2 loaded