ROLES OF MICRORNAS IN OSTEOCYTES DURING TOOTH MOVEMENT

2020 Grants

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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 MICRORNAS IN OSTEOCYTES DURING TOOTH MOVEMENT

Award Type

Biomedical Research Award (BRA)

Period of AAOF Support

July 1, 2020 through June 30, 2023

Institution

University of Illinois, Chicago

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

Anne George

Amount of Funding

$30,000.00

Abstract

(add specific directions for each type here)

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".

detailed result.pdf
attached

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

Yes. The specific aims of the proposal were realized and the characterization of the Dicer/dmp-1 animals was completed. There was not difference of tooth movement in these animals, however the bone phenotypes showed sexual dimorphism. The spent media from loaded osteocytes with miR-29 inhibitor did not exhibit any significant effects on the induction of osteoclast differentiation.

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?*

With the generous funding from AAOF, we have investigated the effect of dicer deficiency in the cells which expressed DMP-1 promotor and did not find any difference of tooth movement however, the bone phenotype showed sexual dimorphism which may lead to the future investigation on the roles of miRNAs in bones and sexual dimorphism. The absence of miR-29 in osteocyte cell line induce the secretion of RANKL differently but the loading on these cells did not change the level of RANKL leading to no difference in osteoclast induction. The manuscript of these findings are the preparation process and will be submitted for publication. L hope the publications from this study will be used to demonstrate as scholarly products for my promotion and tenure process.

Accounting: Were there any leftover funds?

$0.00

Not Published

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

Yes. The manuscripts are in the preparation process for submission. We will notify the AAOF once the manuscript is accepted for publication.

Presented

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

1. P. Atsawasuwan, Z. Chen, S. Shirazi, X. Zhou MicroRNA expression profile in osteocytes under fluid-flow shear stress, Annual meeting American Association for Dental Research, e-poster #2383, Virtual meeting, July 2021. The fund from AAOF was acknowledged during the presentation
2. Chen Z, Zhou X, Atsawasuwan P. The effect of microRNA-29 over and underexpression under fluid-flow shear stress. Poster #121, 2022 UIC Clinic & Research day, March 2022. The fund from AAOF was acknowledged in the poster.

Was AAOF support acknowledged?

If so, please describe:

Yes. the AAOF support was acknowledged in the posters.

Internal Review

Reviewer Comments

Reviewer Status*

File Attachment Summary

Applicant File Uploads

- detailed result.pdf

Specific aim#1: **To investigate the absence of functional miRNAs in osteocytes during tooth movement *in vivo*.**

This specific aim is to compare the distance of tooth movement and the bone phenotypes of conditional deficient Dicer under Dmp1 promotor mice and their control wildtypes. The animals in both groups which consisted of males and females have been subjected to orthodontic tooth movement model using 2 cN springs. The orthodontic force was applied for 14 days. Then the animals were sacrificed and the samples from long bone and hemimandible have been subjected to microCT. The results showed comparable tooth movement in the conditional *Dicer* deficient under *Dmp1* promoter transgenic mouse group compared to wildtype controls and *Dmp1 cre* controls in both sexes (Figure 1). The distance of tooth movement did not show the statistically significance difference ($P<0.05$); however, the trend of the tooth movement demonstrated accelerated tooth movement in males of the *Dicer/Dmp1 mice (Df/df/Dmp1)* (0.218±0.084 mm, Figure 1 MALE: right) group compared to control littermate (Df/df) and *Dmp1*cre mice respectively (0.190±0.104 mm, Figure 1 MALE: middle and 0.185±0.110 mm, Figure 1 MALE: left). There was no trend of tooth movement in females group (0.188±0.089 mm: Df/df/Dmp1, 0.190+0.110 mm: Df/df, 0.195±0.119 mm: Dmp1 cre). The analysis of microCT of hemimandible did not show any difference in both males and females. Bone parameters in the mandible did not show any differences. The analysis of microCT from femurs exhibited a difference in average cortical bone thickness phenotype and the trabecular bone phenotype in the long bone exhibited some differences for bone volume fraction and trabecular bone mineral density ($p<0.05$) (Figure 2). The significant differences were detected in bone volume fraction and trabecular bone mineral density (BMD) (Figure 2).

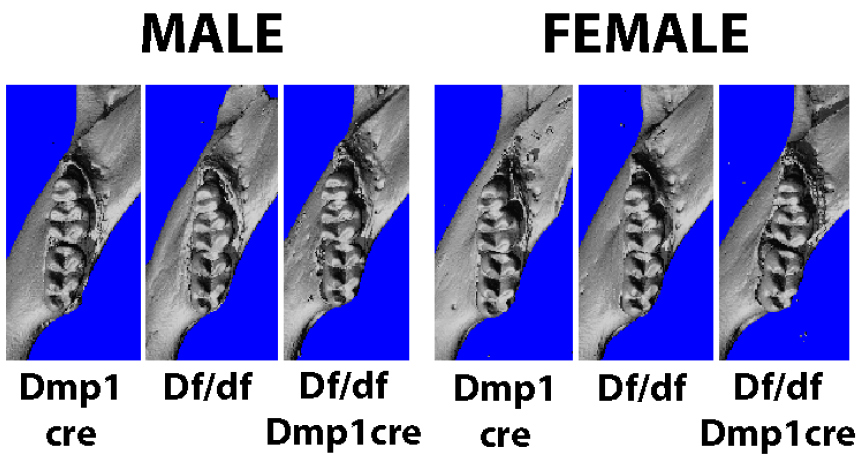
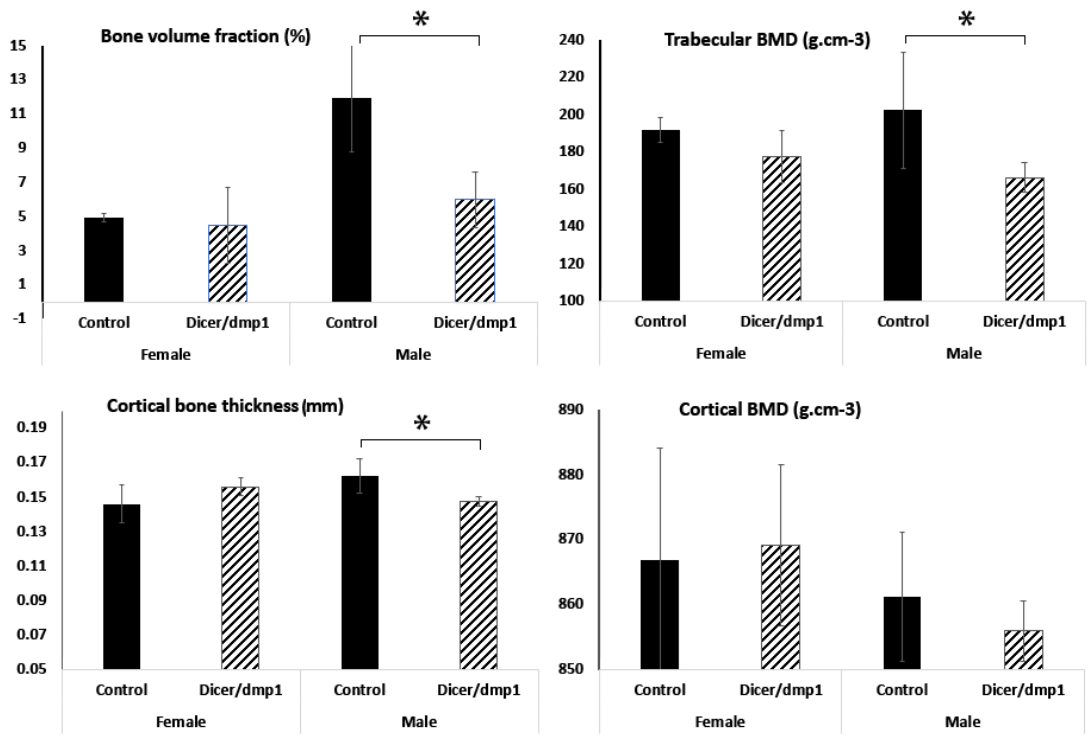


Figure 1. 3D Representatives of the comparison of first mandibular molar tooth movement among Dicer/Dmp1 mice (Df/df/Dmp1 cre), control littermates (Df/df) and Dmp1 cre mice for both males and females. No differences of tooth movement were found among these mice; however, there was a trend of tooth movement in male groups.

Figure 2. Comparison of cortical and trabecular bone phenotypes between wildtype controls and conditional Dicer deficient under Dmp1 promotor mice (Dicer/dmp1) groups by sex (male and female; n=3). *: $P<0.05$.



We performed TRAP staining on all sexes and genotypes of the animals and found no difference among the animals. The number of osteoclasts were comparable among the genotypes of animals.

Specific aim#2: **To investigate the effect of loading on osteocytes with altered miRNA-29 expression *in vitro*.**

The murine osteocyte-like cells both Ocy454 and IDG-SW3 were subjected to a fluid shear stress loading module and the spent media has been collected and stored at -80C for the osteoclast differentiation experiment. We collected the cells and extracted total RNA for next-generation sequencing for further information to confirm the directional changes of miRNA-29 family in both osteocyte-like cell lines after exposure to the fluid shear stress loading module. The up-regulated expression changes of all miRNA-29 family member expression were observed in both cell lines (Table 1). Some of the miRNA-29 family members demonstrated significantly up-regulated changes after fluid shear stress exposure (P<0.05).

Table 1. The copy numbers and changes of miRNA-29 family in Ocy454 and IDG-SW3 cell lines. *P<0.05

	miR_name	miR_seq	up/down	fold_cha	log2(fo	pvalue(t	OCY_ctrl (me	OCY_exp (mean)
Ocy454	mmu-miR-29a-3p	TAGCACCAT	up	1.89	0.92	**0.009***	53,735	101,398
	mmu-miR-29b-3p	TAGCACCAT	up	1.85	0.89	0.081	1,275	2,365
	mmu-miR-29c-3p	TAGCACCAT	up	1.14	0.19	0.202	439	502
IDG-SW3	mmu-miR-29c-3p	TAGCACCAT	up	1.76	0.81	**0.003***	207	364
	mmu-miR-29b-3p	TAGCACCAT	up	1.33	0.41	0.117	1,801	2,389
	mmu-miR-29a-3p	TAGCACCAT	up	1.22	0.28	0.236	126,485	153,718

We have performed the fluid flow shear stress on the Ocy454 and collected the cultured media after fluid flow and determined the levels of sRANKL and OPG using mouse sRANKL and OPG ELISA kits. We have shown that Ocy454 exhibited apoptotic cell appearance after exposure to fluid flow shear stress; however we also performed the cell vitality assay using trypan blue staining to elucidate the numbers of cell death in the cultures. The spent culture media from the no-load and loaded Ocy454 were collected and cultured with RAW264.7 cells to evaluate the osteoclast differentiation induction of the sRANKL and OPG level in the spent media. The results showed that fluid flow shear stress induced cell death and increased level of sRANKL and OPG in Ocy454 (**Figure 3A, B**). Higher numbers of dead cells in the loaded group was found compared to no loaded and wildtype group (**Figure 3C**) and the spent media from the loaded cells induced higher numbers of multinucleated giant cells from the RAW264.7 cells (**Figure 3D**). We verified the response of miR-29 family after subjecting to fluid flow loading using miR realtime PCR and check the transfection efficiency of miR-29 mimics and power inhibitors in Ocy454 as shown in **Figure 4 A and B.** Fluid flow loading influenced the expression pattern of miR-29 family by significant increasing the expression of this miRNA family (**Figure 4A**)and the miR-29b mimic increased the expression of miR-29b while the power inhibitor of miR-29 significantly decreased the expression of miR-29a, b and c. (**Figure 4B**)

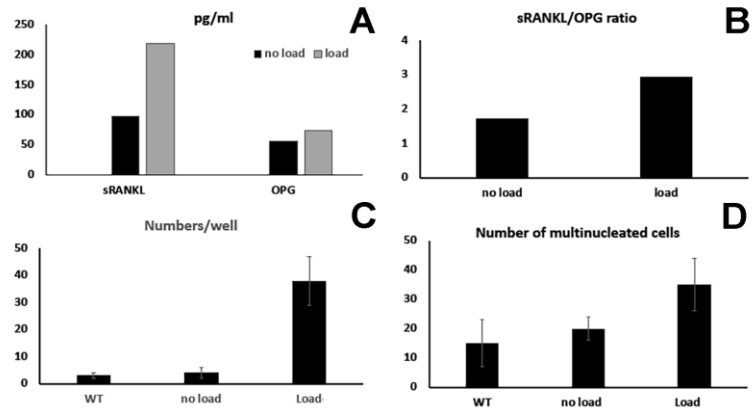


Figure 3. The levels of sRANKL and OPG in the cultured media from Ocy454 of loaded and no loaded groups (A). The ratio of sRANKL/OPG in the OCy454 spent cultured media (B). The numbers of dead cells in each group of Ocy454 (WT, no load and load)(C). The numbers of multinucleated giant cells after RAW264.7 cells were cultured in the Ocy454 spent media

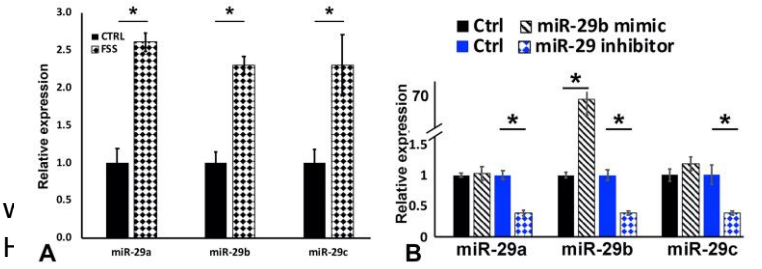


Figure 4. The level of miR-29 a, b and c in Ocy454 after subjected to fluid flow shear stress demonstrated increase in all isoforms (A) and miR-29b mimic increase expression of miR29b and power inhibitor of miR-29 decrease expression level of all member of miR-29 family in Ocy454 (B).

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the numbers of osteoclasts in the control group.