

Mechanisms of PTH action in Cartilage of Temporomandibular Joint

2022 Orthodontic Faculty Development Fellowships (OFDFA)

Dr. Po-Jung Chen

pchen@unmc.edu
O: 860-328-4843

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*

Mechanisms of PTH action in Cartilage of Temporomandibular Joint

Award Type

Orthodontic Faculty Development Fellowship Award (OFDFA)

Period of AAOF Support

July 1, 2022 through June 30, 2023

Institution

University of Nebraska Medical Center

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

Sumit Yadav

Amount of Funding

\$20,000.00

Abstract

(add specific directions for each type here)

Career Development: This fellowship application describes 1 years training program to further my career aspirations to become an independent clinician-scientist. After gaining extensive research and didactic experience for the past decade, and training under the leading scientist in the field of craniofacial biology, I have set clear goals and objectives for the rest of my scientific career. My long-term goal is to pursue an

independent investigator in an academic environment. A component of the role will include training the next generation of orthodontist and craniofacial researches. My short-term objectives during the fellowship award are to gain additional areas of expertise and training that will provide a strong foundation for conducting this interdisciplinary research. Expanding my expertise will allow me to accomplish my proposed research during the fellowship period, and will increase the likelihood of securing my first extramural grant as a principal investigator. Educational Goal: To be a knowledgeable orthodontic educator and independent scientist in the field of craniofacial biology, I will continue to attend didactic course and scientific meeting as well as laboratory-based research and technical skill training. Teaching Goal: To improve my teaching skills, I plan to enroll for the Institute for Teaching and Learning program (ITL) held by the Academy for Academic Leadership program (AAL). Clinical Skill Goal: My goal is to continue achieving excellence in orthodontic care of the patients under the most recent evidence based and to enhance my clinical skills by utilizing the most recent technological advances.

Science: There is currently an unmet need for a clinically effective approach to treat and regenerate osteochondral tissues of TMJ. Our recent preliminary data suggests that the administration of intermittent (I-) Parathyroid Hormone (PTH) enhances cartilage repair and regeneration, and may cause chondrocyte de-differentiation, generating a novel pool of chondroprogenitors. Additionally, we have associated Fibroblast Growth Factor 2 (FGF2) as a major modulator of this observed de-differentiation. Our central hypothesis is that FGF2 is master regulator of chondrocyte de-differentiation with I-PTH administration. The hypothesis will be tested by following specific aims: Aim 1: Evaluate whether I-PTH causes chondrocyte de-differentiation. Our hypothesis for this aim is that I-PTH administration will lead to de-differentiation of chondrocytes, thus increasing the chondroprogenitor population. We will use a Col10a1-CreERT2 X R26-LSL-tdTomato X Col1a-eGFP mouse model to perform lineage tracing in the MCC following I-PTH administration. We will use both fluorescence microscopy and fluorescence-activated cell sorting (FACS) and histomorphometry to determine the percentage of eGFP cells with and without I-PTH. Aim 2: To define the molecular mechanism by which FGF2 regulates de-differentiation. We hypothesize that conditional deletion/silencing of FGF2 in mature chondrocytes will not result in chondrocyte de-differentiation. In-vitro: Primary chondrocytes from triple transgenic reporter mouse model (Col1a1-eGFP X Col2a1-cyan X Col10a1-mCherry) will be transfected with siRNA FGF2 then treated with I-PTH or PBS for 2-weeks. Cell imaging and FACS sorting will be done to determine the outcome. In-vivo: FGF2 will be conditionally deleted from aggrecan expressing cells (mature chondrocytes) and PTH or PBS will be injected. The histomorphometry will be done to assess the outcome.

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".

To investigate the effects of PTH on chondrocyte de-differentiation and assess the effect of fibroblast growth factor 2 (FGF2) expression on temporomandibular joint (TMJ) mandibular condylar cartilage (MCC).
Materials and methods: For the in vitro experiments, primary chondrocyte cultures from the MCC of eight 10-week-old triple collagen transgenic mice (Col1a1 X Col2a1 x Col10a1) were treated with I-PTH for 14 days. We examined the effects of PTH on the expression of Col1a1-green cells, Col2a1-blue cells and Col10a1-red cells in comparison to control. For the in vivo experiments, sixteen 10-week-old mice were divided into 2 groups: (1) I-PTH (n = 8)-subcutaneous daily injection of PTH; (2) control group (n = 8)-subcutaneous daily

injection of saline solution. Experiments were carried out for 2 weeks. Mice were injected with calcein, alizarin complexone, and cell proliferation marker before euthanasia. Result: in vitro: The presence of PTH leads to significantly increased expression of Col1a1, Col2a1 and Col10a1 positive cells. Quantitative polymerase chain reaction analysis of the I-PTH treated chondrocytes revealed significantly decreased relative expression of collagen type X (Col10a1), alkaline phosphatase (Alp), and Indian Hedgehog (Ihh) and remarkable increased expression of Sox9, and fibroblast growth factor 2 (Fgf2). In vitro: There was a significant increase in bone volume, tissue density, mineral deposition, osteoclastic activity, cell proliferation in the cartilage, and cartilage thickness in the I-PTH-treated mice when compared with the control group. In addition, immunohistochemistry in cartilage revealed that I-PTH administration led to an increase in expression of vascular endothelial growth factor and to a decreased expression of sclerostin, matrix metalloproteinase 13, and aggrecanase-1 (ADAM-TS4). Conclusion: The present study showed that I-PTH treatment increased proliferation and differentiation in vitro. Our in vivo experiments also showed increased cellular proliferation and differentiation.

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

Yes

Were the results published?*

No

Have the results of this proposal been presented?*

No

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

The funding has helped me on setting up my research lab and supported me traveling to present my research result. Additionally, the funded research allowed me to generate data for future grant application.

Accounting: Were there any leftover funds?

\$0.00

Not Published

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

Yes, we are finalizing the data and manuscript for publication

Comment: *The AAOF PARC commends the completion of your OFDFA project and looks forward to seeing the results of this study in the public domain through publications and presentations. We encourage you to continue your research and education development in the years to come.*

Not Presented

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

Yes, we are planning to present in next TMJ research meeting.

Internal Review

Reviewer Comments

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

No files were uploaded