FGFR3 signaling in mediating the effects of PTH on TMJ chondrocytes

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*

FGFR3 signaling in mediating the effects of PTH on TMJ chondrocytes

Award Type

Orthodontic Faculty Development Fellowship Award (OFDFA)

Period of AAOF Support

July 1, 2020 through June 30, 2023

Institution

University of Connecticut

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

Dr. Sumit Yadav

Amount of Funding

$20,000.00

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".

Final_report_AAOF_2020_ElianeDutra.pdf
Please see attached file.

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

Yes, the original specific aims and objectives were investigated. However, we modified the study design to have a better understanding of the research questions we had.

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?*

Preliminary data collected thanks to the funds provided by AAOF were invaluable for my NIH KO1 award. My goal is to continue to grow as a clinician and craniofacial scientist. The support from AAOF has helped me to obtain the necessary support to implement new research ideas, expanding my possibilities to create innovations in the field and new grants submission.

Comment: *We commend you on your project completion and the contribution of your original research to advance our specialty. We look forward to your continued successful trajectory with the publication and presentation of this work in the future.*

Accounting: Were there any leftover funds?

$0.00

Not Published

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

Yes, we have plans to publish. We are still working on the data obtained, performing quantification ad interpretation.

Not Presented

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

Yes, we have plans to present these data hopefully at AAO 2024. We are still working on the data obtained, performing quantification ad interpretation.

Internal Review

Reviewer Comments

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- Final_report_AAOF_2020_ElianeDutra.pdf

FGFR3 signaling in mediating the effects of PTH on TMJ chondrocytes

Degenerative joint diseases of the Temporomandibular Joint (TMJ) significantly impair quality of life by causing acute and chronic pain. Currently there are no effective treatments for degenerative diseases of the TMJ and total joint replacement becomes the only option. Accordingly, there is an unmet need for an effective approach to treat the degeneration of the TMJ.

We believe that the best approach to repair the joint cartilage is by exploiting the innate growth potential of the chondrocytes. Our published and preliminary data have shown that intermittent PTH (I-PTH) treatment has an anabolic effect on the cartilage of the TMJ and we posit that these anabolic effects are mediated by fibroblast growth factor receptor 3 (FGFR3). Our central hypothesis is that FGFR3 modulates the anabolic effects of I-PTH on the cartilage of TMJ. To test this hypothesis, we propose the following specific aims:

Specific Aim 1A: To determine the role of FGFR3 signaling in the anabolic effects of intermittent parathyroid hormone (I-PTH) administration in the mandibular condylar cartilage (MCC) of the temporomandibular joint (TMJ). We hypothesize that FGFR3 loss-of-function will not result in an anabolic modeling of the MCC in response to I-PTH. To test our hypothesis, we propose to delete FGFR3 in αSMA expressing cells in the MCC. Effects of FGFR3 loss-of-function with and without I-PTH will be assessed by examining chondrocyte proliferation, matrix synthesis, chondrocyte hypertrophy, mineralization, as well as biomechanical characterization of the MCC.

Specific Aim 1B: To define the molecular mechanism by which FGFR3 regulates the anabolic effects of I-PTH *in vitro*. We hypothesize that silencing/inhibiting the FGFR3 in the primary chondrocytes of murine MCC will not result in chondrocyte proliferation. Our preliminary data has shown increased chondrocyte proliferation and decreased OA markers with the administration of I-PTH *in vitro*. FGFR3 in the primary chondrocytes from the MCC of the triple transgenic reporter (Col1a1 X Col2a1 X Col10a1) mice will be inhibited using silencing RNA.

Specific Aim 1A: The mouse model for conditional deletion of FGFR3 (FGFR3 CondKO) in the αSMA expressing cells (chondrocyte progenitors) from the MCC of the TMJ of mice has been created.

- Four-week-old αSMA Cre+/FGFR3 flox/flox (FGFR3 CondKO) and αSMA Cre-/FGFR3 flox/flox littermates (control, CTRL) were injected with 5 consecutive intraperitoneal doses of tamoxifen (75 μg/kg body weight) 24 hours apart. Male and female animals are being characterized. In addition, to test our hypothesis from Specific Aim 1A, FGFR3 CondKO and CTRL mice were subcutaneously injected with I-PTH or saline solution every day for 2 weeks (**Figure 1**). After the 2 weeks period of I-PTH or saline solution injection, mice were euthanized, and mandibles were collected for histology.

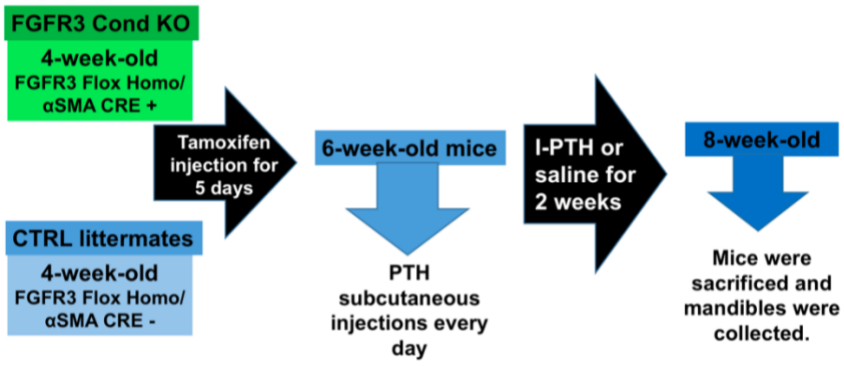

Figure 1: ***Experimental Design for Aim 1A.*** FGFR3 CondKO and CTRL mice received 5 daily injections of tamoxifen. Two weeks after the last injection of tamoxifen, mice received daily injection of I-PTH or saline for 2 weeks.

- Frozen sagittal sectioning of the mandibular condyle of control and experimental mice was performed. Sections were stained for Toluidine Blue, Safranin O, TUNEL and TRAP.
- Histological characterization of the FGFR3 condKO mice revealed a striking phenotype characterized by substantial cellular changes in comparison to CTRL. We observed a change on the shape of the chondrocytes; cells seem to be larger in size, presenting with a cellular phenotype we are yet to understand **(Figure 2A, 2B, 2E and 2F)**.

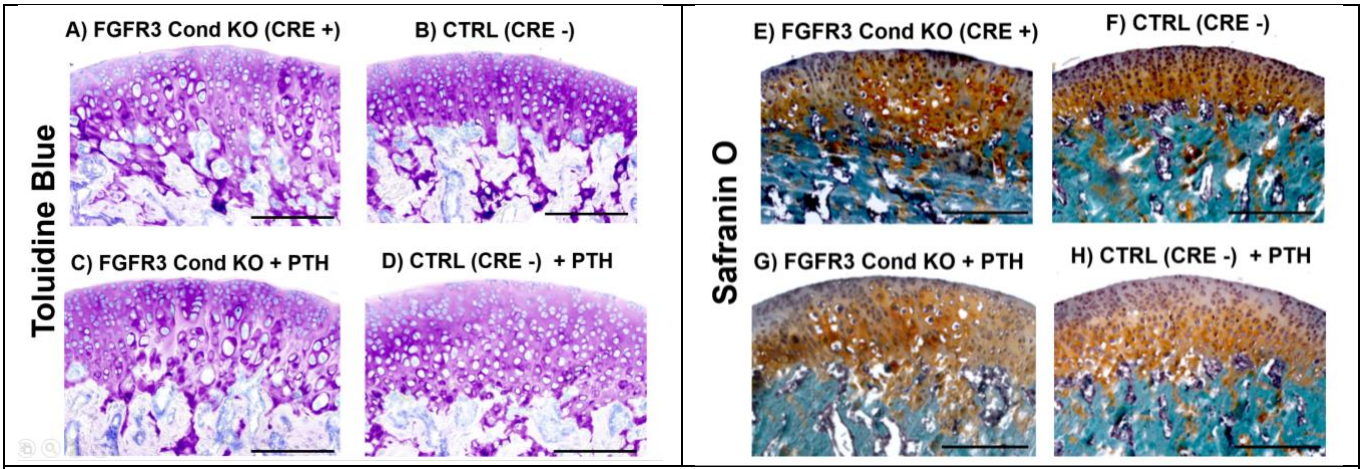

Figure 2: ***FGFR3 CondKO mouse model and the effects of I-PTH.*** Sagittal sections of mandibular condyles of **A,E)** FGFR3 ConkKO (CRE+) and **B,F)** CTRL (CRE-) injected with saline, and **C,G)** FGFR3 ConkKO (CRE+) and **D,H)** CTRL (CRE-) injected with I-PTH. Toluidine Blue (A-D) and Safranin O (E-H). Scale bar = 200µm.

- In addition, although the cells seem to be larger, a lower number of cells seem to be present in comparison to controls **(Figure 2A, 2B, 2E and 2F)**.
- Our previous data shows that I-PTH treatment induces an anabolic effect on the MCC of the TMJ. Interestingly, I-PTH caused increase in cartilage thickness and cellularity on the CTRL (CRE -) mice **(Figure 2D and 2H)**, but it did not seem to

change the phenotype caused by the conditional deletion of FGFR3 (FGFR3 condKO) **(Figure 2A, 2C, 2E and 2G)**.

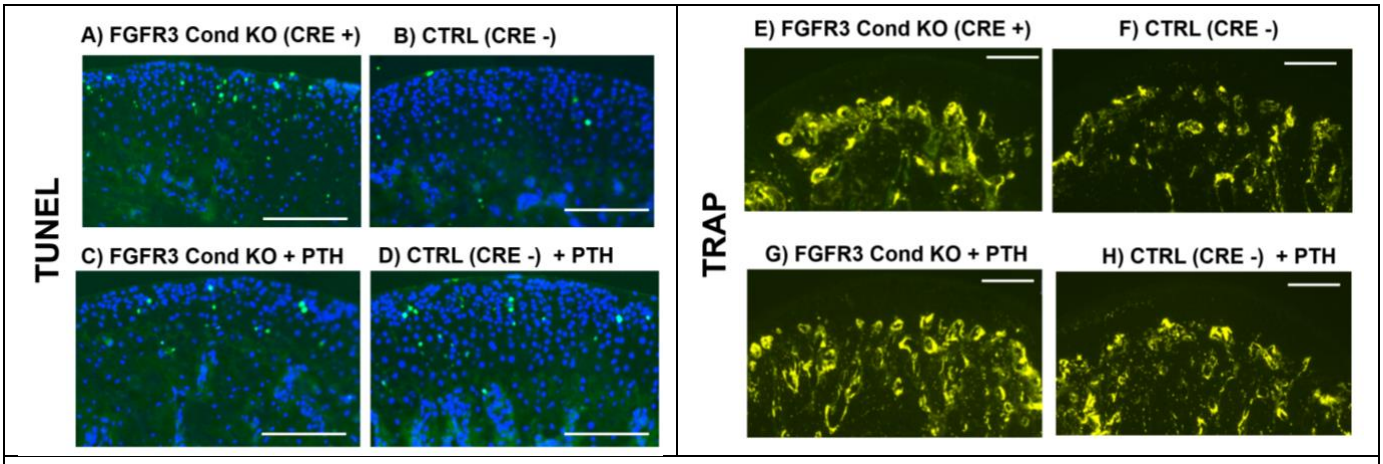

Figure 3: ***FGFR3 CondKO mouse model and the effects of I-PTH.*** Sagittal sections of mandibular condyles of **A,E)** FGFR3 ConkKO (CRE+) and **B,F)** CTRL (CRE-) injected with saline, and **C,G)** FGFR3 ConkKO (CRE+) and **D,H)** CTRL (CRE-) injected with I-PTH. TUNEL staining (A-D), Scale bar=180µm. TRAP staining (E-H), Scale bar=100µm.

- Next, we performed fluorescent TUNEL staining to analyze if the increased size of chondrocytes and reduced number of cells would be correlated with cellular apoptosis. TUNEL staining revealed no substantial difference between FGFR3 CondKO and CTRL mice **(Figure 3A-D)**, suggesting cellular apoptosis may not associated with the observed cellular phenotype.
- Furthermore, in TRAP staining was performed to evaluate subchondral bone remodeling. We found an increased TRAP activity in the FGFR3 CondKO in comparison to CTRL **(Figure 3E and 3F)**. I-PTH treatment has increased TRAP expression in the CTRL mice **(Figure 3F and 3H)**, but we did not identify an expressive difference between FGFR3 CondKO saline injected **(Figure 3E)** and FGFR3 CondKO I-PTH treated **(Figure 3G)**. Interestingly, the larger cells that we identified seem to be expressing TRAP, suggesting those cells could possibly either be fused hypertrophic chondrocytes or chondroclasts, osteoclasts within the cartilage.

- Preliminary conclusion: Conditional deletion of FGFR3 in αSMA cells (chondrocyte precursors from the MCC) causes a cellular phenotype characterized by decreased number of cells and increased size of cells. We are still trying to understand this phenotype. I-PTH does not seem to exert the anabolic effects observed in the control mice.

<u>Specific Aim 1B:</u> Conditions for shRNA of FGFR3 in primary chondrocytes isolated from mandibular condyle of mice are still being tested.

We have successfully obtained deletion of FGFR3 in primary chondrocytes (**Figure** 4**)**, but we are having issues to achieve consistent FGFR3 deletion for the period that we intend to carry on our primary chondrocytes cell culture and I-PTH treatment. We are

considering to isolate chondrocytes from the FGFR3 CondKO mouse model tagged to Ai9 to perform the cell culture experiments planned in Aim 1B.

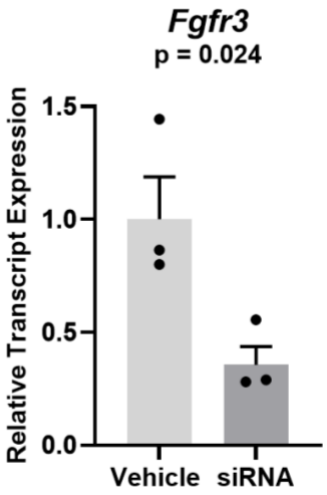

Figure 4: qPCR illustrating relative expression of *Fgfr3* after siRNA in primary chondrocytes isolated from the mandibular condyle of mice.