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AAO Foundation Awards
Final Report

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?*

Type of Award
Biomedical Research Award

Name of Principal Investigator
Yan Jing, DDS, PhD

Title of Project
Novel roles of chondrocyte-derived bone cells in mechanical strain-induced TMJ remodeling

Period of AAOF Support
07-01-2017 to 06-[1]30-2018

Amount of Funding
$17, 000

Summary/Abstract
Introduction: The temporomandibular joint (TMJ) is the most important joint in dentistry. Many studies indicate that alteration of loading in the mandibular condyle cartilage (MCC) affects bone remodeling, but the precise mechanisms by which mechanical strain regulates TMJ remodeling remain unclear. We and other labs recently discovered that a direct transdifferentiation of chondrocytes to bone cells occurs in both development and adulthood[1-7], which challenges the current dogma that apoptosis of hypertrophic chondrocytes is a necessary step prior to the in-migration of angiogenesis and bone-forming cells. More importantly, our preliminary data revealed that altering TMJ function by the placement of an incisal cap on maxillary incisors led to a dramatic bone loss in condylar process, which was caused by the reduction of chondrocyte-derived bone cells, indicating mechanical force involves in the regulation of chondrocyte cell fate. The aim of this study is to investigate the vital roles of chondrocyte-derived bone cells in the modeling and remodeling of condylar process in response to mechanical strain.

Method: A mouse mouth-opening model was used, in which the mouth was forced to open by a spring 1 hour/day for 8 days, to likely increases loading at the TMJ[8-10]. The springs were fabricated with 0.017" X 0.025" beta-titanium wire or 0.018" stainless steel wire to generate low (50g) and high (150g) mechanical forces, respectively. To mimic the development stages for teenagers and young adults who are the main population to seek orthodontic treatment, 2-week or 3-week old compound mice (Acan-cre^{ERT2}; 2.3Col1a1-GFP; R26R^{Tomato}) were randomly divided into 50g, 150g and age-matched groups (n=4). Mechanical test gauge was used to calibrate the spring force every day. Both control and forced mouth-opening groups were anesthetized with ketamine/xylazine during the loading hour. One-time tamoxifen injections were done on the first loading day and regular pellets were provided for all mice. Multiple strategies including X-ray, Micro-CT, histological stainings, and cell lineage tracing combined with immunohistochemistry (IHC) were used to demonstrate the cellular and molecular changes.

Results: In the 2-week old mice with 50g of mouth-opening force, the X-ray image showed a smaller condylar head with lower bone density in the condylar process in the loading mice. Micro-CT quantitation further demonstrated a significant reduction of BV/TV in loading mice ($P<0.05$). These data indicate a decreased bone volume in the loading condyle. H&E staining showed no apparent change in the thickness of MCC, but less flat cells in MCC superficial layer, in which there were more differentiated chondrocytes with higher expression levels of Aggrecan and Col X (markers for chondrogenesis), indicating an increase of chondrogenic differentiation and activity in the loading mice. On the other hand, decreased expression levels of Col1 and DMP1 (markers for osteogenesis) revealed a sharp reduction of bone mass in the superior subchondral bone area in loading group compared to the control, which is in consistent with the radiology data. By using cell lineage tracing, we found many chondrocyte-derived bone cells (CBC) positive for Runx2 and Osx (early osteogenesis markers) in the superior region of MCC subchondral bone, demonstrating that MCC chondrocytes actively contributes to the osteogenesis of condylar process by transdifferentiating into bone cell in young mice. To the contrast, there were 12.6% decrease of the number of CBC in loading MCC subchondral bone ($P<0.05$).

Since the teenage patients have great probability to be exposed under orthopedic treatment or strong intermaxillary traction that may result in a high mechanical strain in TMJ, we subsequently increased the mouth-opening force to 150g by using the same mice line and time frame. Both X-

ray and Micro-CT showed a significant bone loss in the 150g loading condyle. By using cell lineage tracing, we found a thinner MCC with much less Tomato^{+} chondrocytes in the 150g-loading mice, in which Aggrecan expression was significantly reduced. The high level of mechanical force also caused a great reduction of Col X expression, indicating a defect of chondrocyte maturation. On the other hand, the decreased DMP1 expression reflected a dramatic bone loss in the condylar process, especially the superior region beneath the MCC, in which there were abundant CBC in control but few in 150g loading condyle (18.3% decrease in CBC number, $P<0.01$). Taken together, our findings reveal that 1) moderate alternation of loading environment in 2-week old mice slightly accelerates the chondrogenesis but obviously reduces the subchondral bone formation via the inhibition of transdifferentiation from chondrocytes into bone cells; 2) dramatic change of TMJ loading during development stage leads to severe defect of chondrogenesis and a great bone loss caused by the reduction of chondrocyte-derived osteogenesis.

Next, we investigated how the mechanical loading affects the condyle remodeling in young adult mice. Same compound mice and loading strategies were used on 3-week old mice. Compared to the 2-week old group, 50g loading force led to more decrease in chondrogenesis and bone density ($P<0.05$) with fewer CBC in condylar process (16.9% reduction in the number of CBC, $P<0.05$). In further, 150g of mechanical force caused more severe loss of chondrogenesis with much reduction in bone density and chondrocyte transdifferentiation (21.2% reduction in the number of CBC, $P<0.05$).

Taken together, our studies reveal that: 1) MCC chondrocytes actively contribute to the osteogenesis of condylar process by transdifferentiating into bone cells during TMJ modeling and remodeling; 2) the chondrocyte transdifferentiation is subject to the alteration of MCC mechanical loading; 3) during development stage, an alteration of loading via 50g mouth-opening force on MCC slightly increases chondrogenesis but obviously inhibits the chondrocyte-derived osteogenesis, leading to a bone loss in condylar process; 4) a dramatic change of mechanical loading via 150g mouth-opening force during TMJ growth severely defects the MCC chondrogenesis and chondrocyte transdifferentionation, resulting in significant reduction of subchondral bone volume; 5) compared to 2-week old group, 3-week old mice with 50g loading had fewer CBC in condylar subchondral bone, and the chondrocyte transdifferentiation was dramatically inhibited in 150g loading group. Our findings indicate that chondrocyte-derived osteogenesis plays a key role in the modeling and remodeling of condylar process in response to TMJ loading changes. These may provide greater understanding of how TMJ development and remodeling are regulated by mechanical loading, and explore a novel role that mandibular condylar chondrocytes may play during orthodontic and orthopedic treatment.

Reference

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Response to the following questions:

1. Were the original, specific aims of the proposal realized?

Yes.

2. Were the results published?

The results are not published yet, but we have started the manuscript organization. Additionally, some of the data have been used in my recent NIH R01 application in June 2018.

3. Have the results of this proposal been presented?

a. If so, list titles, author or co-authors of these presentation/s, year and locations

Yes. Part of the results have been presented by Dr. Yan Jing in April 2018 ASBMR/AIMM meeting at Snowmass, CO.

Title: Novel Roles of Endochondrogenesis in Mandibular Condyle Formation and Remodeling

b. Was AAOF support acknowledged?

Yes.

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

The main goal of this proposal is to focus on my research plan and collect preliminary data for a future external grant. As mentioned before, some of the data obtained in this project have been used in my recent NIH R01 application in June 2018. In addition, my BRA award supported one of my publications about the contribution of chondrocyte-derived

osteogenesis in limb bone development and remodeling under the regulation of BMPr1a signal (Jing et al., Scientific reports, 2018). I am also preparing the manuscript with the data generated in this proposal. So far, as a junior faculty, I sincerely acknowledge the great supports from AAOF on my research career development. In the future, I will continue to apply for new AAOF funding, which will definitely help me to harvest more achievement.