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TITLE: Targeting Piezo Ion Channels for Mitigation of Osteoarthritis Pain and Disease Progression

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14. ABSTRACT

The purpose of this project is to determine the roles of Piezo mechano-sensing channels in osteoarthritis (OA) and pain associated with OA using mouse genetic models. Two major aims are proposed.1) assessment of the effect of cartilage-specific loss of Piezo1 and Piezo2 in OA progression in surgically created OA in mice, and 2) assessment of OA-associated pain in Piezo2 haplo-insufficient mice.
The scope of this project for the second year is generation of genetic mouse modles and validation, and creation and analysis of OA in these models for Aim 1 (neuron specific Piezo KO) and Aim 2 (cartilage-specific Piezo KO).
Major findings: The Aim2 to assess OA progression in mice with joint-specific Piezo conditional KO [cKO, *Gdf5-Cre:Piezo1(fl/fl):Piezo2(fl/fl*)] was completed. Piezo 1/2 deletion in developing joints does not disturb joint development when assessed the joint morphology at 3 and 6 months of age. Therefore, Piezo channels are not essential for joint cartilage development. Induction of OA via destabilization of medial meniscus (DMM) surgery caused OA in both Piezo cKO and control mice. The cohort of Piezo cKO included a greater number of mice that showed lower grade OA compared with the control cohort, although it does not reach the statistical significance. This result suggesting that Piezo genetic deletion might have modest beneficial effects on OA progression. Using fluid flow shear stress (FFSS), we assessed expression of mechanical stress-induced OA-associated genes in control and Piezo cKO articular chondrocytes. Both control and Piezo cKO chondrocytes showed similar upregulation of OA-associated genes, suggesting that different mechanotransducer other than Piezo 1/2 channels dominantly mediate FFSS. The Aim 1. we have generated neuron-specific Piezo KO mice. They showed overt neurological deficits. Unfortunately, when we performed DMM surgery on mice. After one measurement, the incapacitance meter broke down, and the company (Bioseb) did not respond to our repeated requests for device repair, and the device was not repaired during the study period.

The significance of these findings indicate that 1) Piezo1 and 2 are not required for normal articular and growth plate chondrocytes *in vivo* (at least up to 6 months) and suggest that 2) Piezo 1 and 2 might modestly contribute to OA progression and that different mechanotransducers other than Piezo1 and 2 dominantly mediate mechanical stress *in vitro*. 3) These in vivo results are not fully compatible to the suggested role of Piezo channels in articular chondrocytes based on in vitro study (Lee W et al 2014 PNAS, ;111(47):E5114-22).

15. SUBJECT TERMS
Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research

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1. Introduction

Osteoarthritis (OA), associated with joint injuries and aging, is a prevalent condition. One of the theories for OA development is culumative mechanical stress. It is known that in response to mechanical stress chondrocytes, a major cell population of joint cartilage, produces diverse molecules to affect the microenvironment including cytocyines and matix proteases. We hypothesize that suppressing response chondrocytes to mechanical stress inhibits progression of mechanical-stress-induced OA. Piezo mechano-sensing channels mediates mechanical stress in chondrocytes and mediates sensation in sensory nerves including nociception. This project aims to determine the role of Piezo mechano-sensing channels in OA progression and chronic pain using genetic mouse models. OA is surgically induced in mice with cartilage-specific and neuron-specific Piezo channel deletion to assess OA and pain. The purpose is to provide scientific basis whether Piezo channels can be therapeutic targets for OA.

2. Keywords

Osteoarthritis, mouse, genetic models, mechano-sensing ion channel, Piezo1, Piezo2, pain, joint, synovial.

3. Accomplishments

Major goals

The following tasks are proposed in SOW during the entire research period:

Major Task 1 (Aim1) : Establish animal model and system validation (neuron-specific Piezo2 and Piezo1 ablation) (original plan 6-12 months)

Major Task 2 (Aim1) : Evaluation of Piezo deletion effects on OA (neuron-specific Piezo2 and Piezo1 ablation). (original plan 6 – 18 months)

Major Task 3 (Aim2) : Establish animal model and system validation (cartilage-specific Piezo deletion) (original plan 6 – 12 months)

Major Task 4 (Aim2) : Evaluation of Piezo deletion effects on OA (cartilage-specific Piezo deletion). (original plan 6 – 18 months)

Accomplishments under major goals

Major Task 1 and 2 (Aim1) : Establish animal model and system validation (neuron-specific Piezo2 and Piezo1 ablation)

1) Major activities: The reporting period was spent on the subtask1 establish mouse colonies.

2) Specific objectives: Subtask 1) To generate sensory nerve specific Piezo2 (heterozygous) with Piezo1 (homozygous) deletion in mice. Subtask 2) confirmation of gene deletion and the absence of an overt basal phenotype. Subtask 3) confirmation of the absence of basal cartilage phenotype.

3) Significant results: We have generated tamoxifen-inducible neuron-specific Piezo2 heterozous, Piezo1 homozygous deletion (cKO) and confirmed that these mice do not have an abnormal basal phenoytpe. We performed DMM surgery at 3 months on a rolling basis. At one month after the DMM surgery, we performed static wight-bearing analysis on two cKO mice. This preliminary measument showed a prommising tendency (**Figure 1**). Unfortunately, before the second measurement, the incapacitance meter started malfunctioning. The manufacturere was not able to repair the device or providing a loaner device in a timely manner. and we were not able to collect no further data in this Aim.

4) Other achievements. Nothing to report.

Major Task 3 (Aim2) : Establish animal model and validation (cartilage-specific Piezo2 and Piezo1 ablation).

1) Major activities: Generation of Piezo1 and 2 doubly homozygous conditional knockout mice, evaluation of Piezo knockout efficiency, and characterization of the basal cartilage phenotype.

2) Specific objectives: Subtask 1) To generate cartilage-specific Piezo KO mice. Subtask 2) To validate the anmal model. Subtask 3) Assessment of basal cartilage phenoytype.

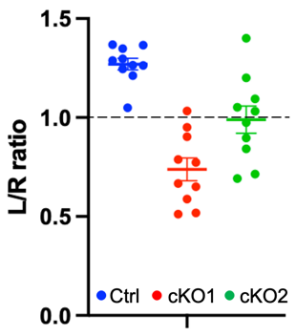

Figure 1. Preliminary pain assessment by the Incapacitance meter test on one control and 2 cKO mice at 1 month after DMM surgery. These 2 cKO mice did not show increased left hindlimb weight bearing like control (also see control data in Fig. 3)

3) Significant results: We assessed the cartilage phenotype of Piezo1/2 double cKO mice. Knee joints were fixed, decalicified, processed, sectioned, and stained with H/E or Safranin O. We found that deletion of Piezo1 and 2 in joint cartilage using Gdf5-Cre transgenic mice [Gdf5-Cre:Piezo1(fl/fl):Piezo2(fl/fl)] causes no overt abnormalities at 3 and 6 months of age (**Figure 2**). This result suggests that Piezo suppression itself has no negative effects in cartilage development or homeostasis. We also confirmed that the expression of Piezo1 and Piezo2 was efficiently reduced.

4) Other achievements. Nothing to report.

Major Task 4 (Aim2) : Evaluation of Piezo deletion effects on OA (cartilage-specific Piezo2 and Piezo1 ablation).

1) Major activities: We generated Piezo1/2 KO mice and OA was induced by DMM surgery at 3 months of age. Pain is analyzed by the incapacitance test, and histology is analyzed at 6 months of age (3 months after OA induction). We have started obtaining data. We harvested and processed two KO and one control samples. As shown in Fig. 1. DMM surgery induced OA in two Piezo1/2 KO with the OARSI score of 3 at the posterior side of the tibial plateau, and in the control with the OARSI score of 5.

2) Specific objectives: Subtask 1) To evaluate pain in cartilage-specific Piezo KO mice with OA. Subtask 2) To evaluate the cartilage phenotype of cartilage specific Piezo KO mice with OA.

3) Significant results:

For pain assessment performed by the incapacitance meter test that measures left limb/right limb weight bearing balance, we did not observed beneficial effects of Piezo1/2 deletion in cartilage (**Figure 3**).

With regard to DMM-induced OA, both control and Piezo cKO mice showed moderate to severe OA upon DMM surgery. Piezo1/2 KO might show less severe OA damage (**Figure 4**).

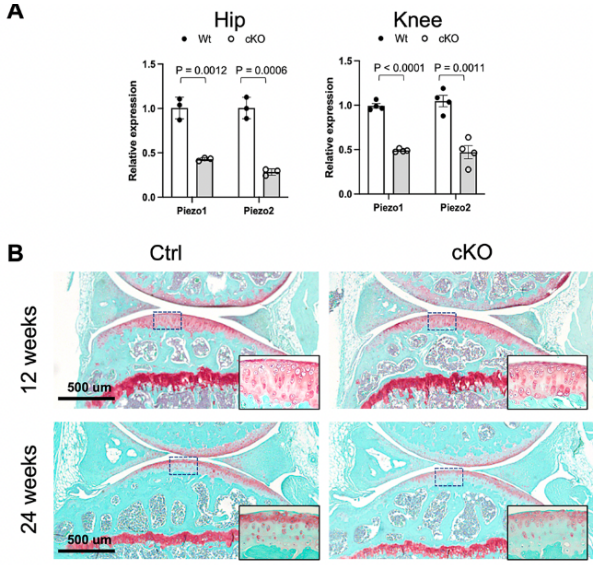

Figure 2. (A) Reduced expression of Piezo1 and 2 in manually dissected articular cartilage of the hip and knee of 10 day-old cKO (*Gdf5-Cre:Piezo1* $^{fl/fl}$ *:Piezo2* $^{fl/fl}$)mice. The inperfect reduction is likely due to the contaminination of non-articular chondrocytes like growth plate chondrocytes and sub chondral bone. (B) Safranin-O staining of the knee joint sections at indicated ages. No overt abnormalities in cKO mice.

4) Other achievements: Related to Aim2, in order to test whether Piezo channel inhibition reduces mechanical stress-induced OA-associated gene expression in chondrocytes, in vitro experiments were performed using primary articular chondrocytes from cKO mice. Fluid flow shear stress (FFSS) was applied to control and cKO cells using a shaking platform. We found that cKO chondrocytes showed similar reponses to FFSS as control chondrocytes (**Figure 5**)

Opportunities for training and professional development

This project provided learning opportunities for two US college graduates (BS) and a Chinese MD, PhD student and a Russian MD, PhD student. This project and its progress are also presented at departmental meetings.

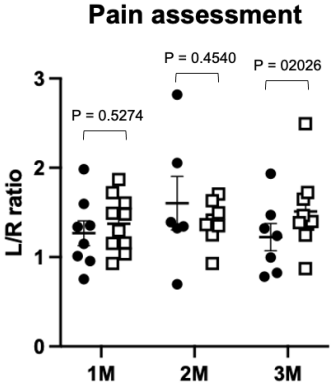

Figure 3. Pain assessment measuring weight-bearing ratio of the left and right hindlimbs. cKO mice showed similar L/R ratios with control mice, suggesting Piezo ablation in the joint has litte effects on pain.

Results dissemination

These findings are published as preprint (BioRxiv **doi:** https://doi.org/10.1101/2022.10.07.511314). The manuscript is also currently under review by Ostoarthritis and Cartilage.

4. Impact

Impact on the development of the principal discipline of the project: It has been known that mechanical stress is the central mechanism for OA and it has been hypothesized that cells in the joint express OA-promoting factors, including inflammatory cytokines and metalloproteinases in response to mechanical stress. However, critical mechanotransducers that mediate this process are not known. Among several different mechano-sensing systems, Piezo channels have been ones of the top candidates of such mechanotransducers because they are activated by high-level, injurious mechanical load, and that in vitro and ex vivo data havesuggested that Piezo inhibition has chondroprotective effects. Until this present study, direct in vivo data regarding the effect of Piezo inhibition on OA have not been available.

Using mouse genetic model, this study rigorously tested this hypothesis. The results are not very supportive the hypothesis that Piezo inhibition has significant beneficial effects on OA. However, the preliminary data that two sensory-nerve specific Piezo cKO mice showed no pain-associated weight bearing assymmetry suggest that Piezo inhibition in sensory nerves may have pain-relieving effects in OA. In addition, some joint-specific Piezo cKO mice showed milder OA. Although cKO and control mice both show moderate to severe OA at 3 months after OA induction, this observation suggest that Piezo inhibition might delay OA progression, if not strongly inhibit it.

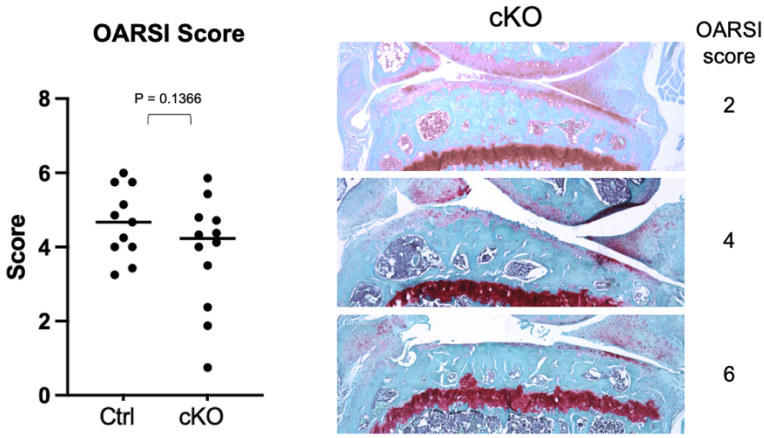

Figure 4. OA was evaluated on the tibial plateau of Piezo1 and Piezo2 doubly conditional knockout (cKO, Gdf5-Cre:Piezo1$^{fl/fl}$:Piezo2$^{fl/fl}$) mice. Left. OARSI OA score. Both control and cKO mice develop moderate to severe OA 12 weeks after DMM. The cKO group contains a few samples with mild OA legions. N = 11 for control (Ctrl) and 12 for cKO. Two-tailed Mann-Whitney U test. Right. Representative images of cKO knees with indicated OARSI scores. Brackets indicate the legion that reaches the calcified cartilage.

Impact on other deciplines: Nothing to report.

Impact on technology transfer: Nothing to report.

Impact on society byond science and technology: Nothing to report

5. Changes/Problems

Actual or anticipated problems and plans to resolve them:

1) We completed the Aim2, the investigation to test whether genetic ablation of Piezo channels show beneficial results. The results were not very encouraging but we found some Piezo-deficient joint samples show milder OA compared with the control group. Although the difference was not statistically proven, this observation suggests that Piezo inhibition may delay OA progression, if not inhibit it. Therefore, by optimizing the experimental system to focus on earler stages of OA development, this possible effect could be detected.

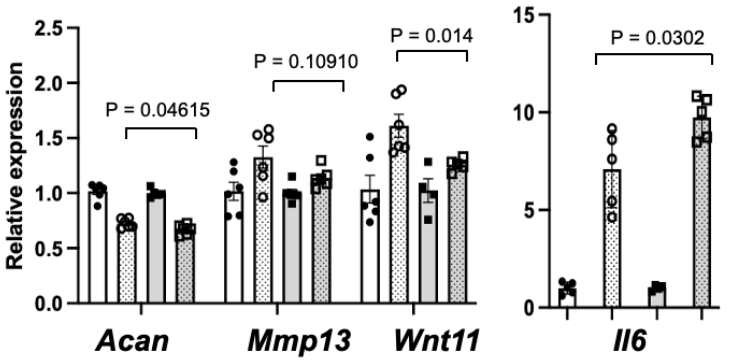

Figure 5. Gene expression responses to FFSS in cKO and control primary articular chondrocytes. FFSS was applied to cells for 4 hours and the gene expression was analyzed by qRT-PCR. Both cells respond similarly to FFSS.

2) For Aim 1. we were not able to obtain conclusive results due to the technical issue with the incapacitance meter. The preliminary data, nevertheless support that partial Piezo2 inhibition can mitigate OA-related pain. We found a relatively large variability of the static weight-bearing test, and therefore, to ensure a sufficient statistical power, the sample size will need to be increased.

6. Products

Journal and other publications, meeting presentision, Web sites

A manuscript summarizing the findings is now available at BioRxiv with the DOI #:
doi: https://doi.org/10.1101/2022.10.07.511314
The manuscript has been also submitted to a journal for peer review.

Technologies technique

Nothing to report

Inventions, patent applications, licenses

Nothing to report

Other products

Nothing to report

7. Participants & Other Collaborating Organizations

Name:	*Tatsuya Kobayashi*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-4264-5117
Nearest person month worked:	*4*
Contribution to Project:	*Mouse management and analysis*
Funding Support:	*NIH and current project*

Name:	*Cameron Young*
Project Role:	*Research technician*

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9
Contribution to Project:	*Mouse management, surgery, data analysis*
Funding Support:	*NIH and current project*

Changes in the active other support of the PD/PI(s) or senior/key personnel

Nothing to report

What other organizations

Nothing to report

8. Special Reporting Requirements

Nothing to report

9. Appendices

Nothing to report