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14. ABSTRACT

Osteoarthritis (OA), including post-traumatic osteoarthritis (PTOA), is the most common form of the arthritis affecting more than 27 million Americans. It is estimated that 12% of the current OA population suffers from post-traumatic injuries. Despite myriad research in the field, there are presently no known disease-modifying OA drugs (DMOADs) to treat the disease. Over the past few years, the data from several independent research groups indicate a novel and key, irreversible, step leading to joint destruction. This pivotal step occurs once the chondrocytes have degraded their pericellular matrix, exposing the surface of the chondrocytes to collagen type II. Collagen type II then binds to the chondrocyte cell surface via a tyrosine kinase receptor known as discoidin domain receptor 2 (DDR2). This induces the synthesis and release of matrix metalloproteinase 13 (MMP-13), an enzyme with the unique ability to degrade collagen type II and aggrecan. The end result is OA. Thus, targeting the induction of MMP-13, from the inhibition of DDR2 activity, is one of the key steps in preventing the irreversible joint destruction. In this investigation, we will determine whether or not a novel small-molecule inhibitor of DDR2 can inhibit the induction of MMP-13 in chondrocytes, thus retarding the progression of OA.

15. SUBJECT TERMS

Post-traumatic osteoarthritis (PTOA), small-molecule inhibitor, chondrocyte, DDR2, MMP-13, destabilization of the medical meniscus (DMM)

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INTRODUCTION Post-traumatic osteoarthritis (PTOA) is defined as the disease secondary to joint trauma. Because of their high risk of injury both on and off the battlefield, military populations are especially prone to PTOA. There are currently no effective ways to prevent joints from becoming OA after injuries. Results from many research groups demonstrate that matrix metalloproteinase 13 (MMP-13) may be one of the therapeutic targets for the development of disease-modifying OA drugs (DMOADs) to treat the disease. In fact, multiple pharmaceutical companies have attempted to inhibit the activity of MMP-13 as a means of delaying the progression of OA. However, the broad biological effects of MMP-13 restrain its application as a target enzyme of inhibitor drugs in the treatment of OA (4). However, this concern does not apply to targeting molecules that regulate the expression of MMP-13 in chondrocytes since those molecules may be more specific to chondrocytes. Data from our own investigations and others' studies suggest that a cell surface receptor tyrosine kinase (RTK), discoidin domain receptor 2 (DDR2) can induce expression of MMP-13 in chondrocytes in response to cartilage damage. This prompt us to determine whether or not the inhibition of DDR2 activity by a small-molecule inhibitor can significantly delay the progressive process of articular cartilage degeneration. In this proposal, we hypothesize that **inhibition of DDR2 activity by small-molecule inhibitors can inhibit the induction of MMP-13 and reduce articular cartilage destruction**. Thus, we plan to perform experiments to test our hypothesis. The novel small-molecule inhibitors that we plan to use in this project are specifically selected for the inhibition of DDRs.

KEYWORDS: Post Traumatic Osteoarthritis (PTOA), DDR2, MMP-13, Small-Molecule Inhibitor, Destabilization of Medial Meniscus (DMM)

ACCOMPLISHMENTS A) Major activities: This is an 18-month grant application. In the first 12 months of the grant, we preformed DMM surgery on mice to test the protective effect of the small-molecule inhibitor of DDR2 on the articular cartilage of knee joints. We have made good progress and are in the process of accomplishing the entire project by the end of this grant application. B) Specific objective: to assay the protective effect of the small-molecule inhibitor of DDR2 on the articular cartilage of knee joints, induced by DMM, by histology. C) Significant results: We have completed DMM surgery on mice and the treatment with the inhibitor by intra-articular injection. We also started to analyze knee joints by histology and immunohistostaining.

***Objective*:** To demonstrate that pharmacologic inhibition of DDR2 activity has a disease-modifying OA effect. We treat mice with a DDR2 inhibitor by intra-articular injection in mouse DMM model. Articular

cartilage and other joint tissues are characterized in animals treated with the inhibitor or vehicle alone as a negative control.

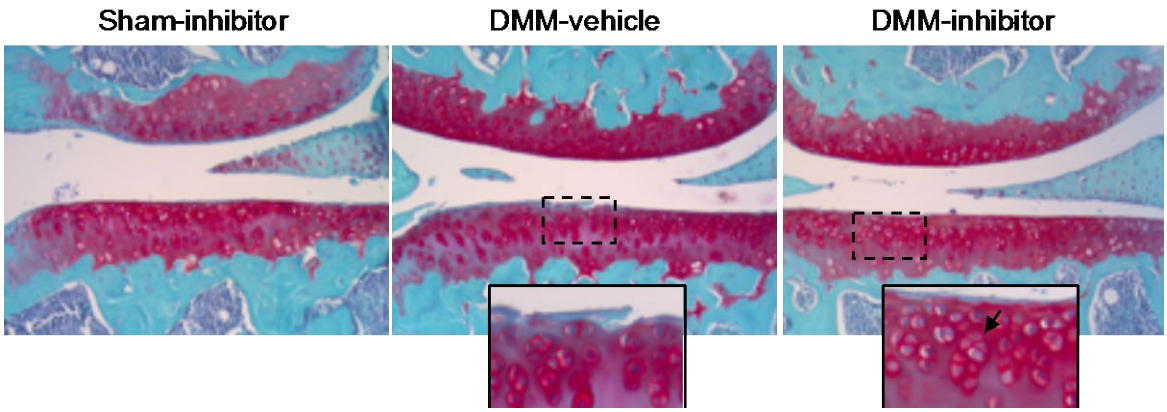
Methods and results

Experiment 1. To perform DMM surgery on mouse knee joints to induce OA. Briefly, mice (BL57/6) at the age of two months were anesthetized and the joint capsule immediately medial to the patellar tendon were incised and opened. The medial meniscotibial ligament (MMTL) was sectioned. The joint capsule was then closed. There were four different groups, Sham-PBS(vehicle), DMM-PBS(vehicle), Sham-inhibitor and DMM-inhibitor, at two time points, 8 and 12 weeks after DMM surgery in this experiment, see the following below. In a pilot experiment, the sample size of minimum 6 is required by the power analysis to achieve the specified confidence interval (95%) with at least 50% reduction of the score in the treatment group.

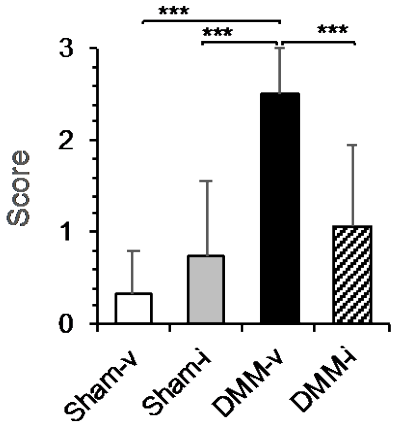
Weeks after DMM	Sham-PBS	DMM-PBS	Sham-inhibitor	DMM-inhibitor
8	n=6	n=8	n=8	n=12
12	n=6	n=8	n=7	n=14

Experient 2. Mice were received the inhibitor or PBS at 4 weeks after DMM. The inhibitor or PBS was administered by intra-articular injection, twice weekly, at dose of 50 nM/20 μl solution. The mice were then euthanized at 8 and 12 weeks after DMM for the collection of knee joints. The joints will be characterized by histology and immunohistochemistry analyses.

Experiment 3. All of knee joints were collected, fixed, decalcified and embedded in paraffin. The joints from the 8-week groups were cut by a 6-μm thickness of serial sections from anterior to posterior. Every 15th section was collected and stained by Safranin O/Fast Green. The morphology of the sections was scored by the modified Mankin's score system. In this score system, 0=Normal. 0.5=Loss of Safranin-O without structural changes. 1=Small fibrillations without loss of cartilage. 2=Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina. 3=Verical clefts/erosion to the calcified cartilage extending to <25% of the articular surface. 4=Vertical clefts/erosion to the calcified cartilage extending to 25 to 50% of the articular surface. 5=Vertical clefts/erosion to the calcified cartilage extending to 50 to 75% of the articular surface. 6=Vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface. The synovial membrane, subchondral bone, menisci and ligaments will also be examined.



This picture shows the morphology of the representative sections from sham-inhibitor group, DMM-vehicle group and DMM-inhibitor group at 8 weeks after DMM surgery. The fibrillation was evident in DMM-vehicle group, whereas, only chondrocyte clusters were seen in DMM-inhibitor group. The cartilage condition was evaluated, see the histogram below. The score of DMM-inhibitor group was significantly lower, compared with the score of DMM-vehicle group. This indicates that the progression of articular cartilage degeneration was delayed in DMM-inhibitor group. There was no significant difference in the scores between Sham-inhibitor and DMM-inhibitor groups.



TRAINING AND PROFESSIONAL DEVELOPMENT:

None

DISSEMINATION OF RESULTS TO CONMMUNITIES OF INTEREST?

Nothing to report.

PLANS FOR THE NEXT REPORTING PERIOND TO ACCOMPLISH THE GOALS: We will complete analysis of all samples that we have collected. If the inhibitor shows the protective effect on the articular

cartilage of knee joints in DMM model, we will examine molecular markers that may be regulated by the inhibitor, including DDR2 and MMP-13. We plan to prepare a manuscript to report our finding by the end of this grant funding.

IMPACT: Results from the partial histology analysis indicate that the inhibitor may be able to significantly delay the progressive process of articular cartilage degeneration in DMM model. We will continue to analyze all samples and finish experiments we plan to carry out.

CHANGES/PROBLES.

None

PRODUCTS. Results from the first 12 months of this grant reflect product from our work, which will be included in our future publication.

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Nearest person month worked: 12
Contribution to Project: DMM surgery, drug treatment, participation in analysis of mice and in the preparation of a manuscript.
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OTHER ORGANIZATIONS

None

SPECIAL REPORTING REQUIREMENTS

None

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None