AWARD NUMBER: W81XWH-19-1-0335

TITLE: Proteomics of Knee Osteoarthritis

PRINCIPAL INVESTIGATOR: Blaine Christiansen

CONTRACTING ORGANIZATION: University of California, Davis

REPORT DATE: October 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2020	Annual	9/1/2019 – 8/31/2020

4. TITLE AND SUBTITLE
Proteomics of Knee Osteoarthritis

5a. CONTRACT NUMBER
W81XWH-19-1-0335

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gabriela Loots (PI), Blaine Christiansen (Partner-PI)

E-Mail: bchristiansen@ucdavis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, Davis
4635 2nd Ave
Sacramento, CA 95817

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes; (3) MRL/MpJ (super healers); and (4) STR/ort (spontaneous OA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

INTRODUCTION:

MRL/MpJ (super healers) mice have a unique ability to repair wounds and are protected from cartilage degradation subsequent to joint trauma. The hypothesis is that in response to injury, MRL/MpJ mice synthesize proteins that (1) protect the joint from cartilage degradation and/or (2) promote cartilage regeneration. The PIs propose to generate an atlas of the injury-activated proteome in mouse models with varying susceptibility to posttraumatic osteoarthritis (PTOA): (1) C57BL/6; (2) C57BL/6 treated with streptozotocin (STZ), a model of type 1 diabetes; (3) MRL/MpJ (super healers); and (4) STR/ort (spontaneous OA). By conducting comparative proteomics of injured and uninjured joints, the PIs will identify novel protein candidates for further exploration as potential therapies for treating injured joints. The project's specific aims are (1) application of in vivo metabolic labeling to quantify and characterize de novo protein synthesis, cellular proliferation, and mineral apposition in injured joints of mice with varying susceptibility to PTOA and (2) identification of newly synthesized RNA and proteins in the articular cartilage and immune cells of injured knees using a liquid sample interface for the AMS instrument in combination with liquid chromatography-mass spectrometry (LC-MS).

KEYWORDS:

MRL/MpJ; STR/ort; osteoarthritis, post-traumatic osteoarthritis, diabetes, streptozotocin, MetRS, superhealer, chondrocytes, knee joint, anterior cruciate ligament, ACL, de novo protein synthesis, PTOA

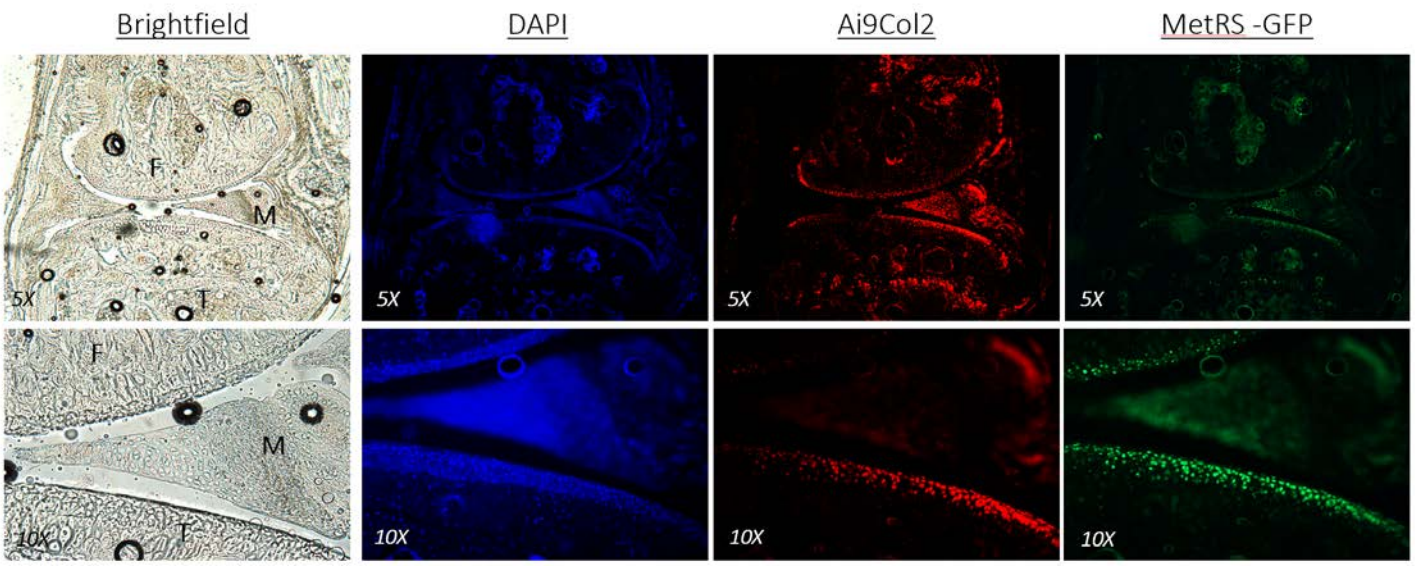
ACCOMPLISHMENTS:

For the 1st year of this grant, our may focus has been on conducting tasks associated with Aim 1/Major Task 1 (Sub Aim 1A) of the proposal, following the original tasks and timeline we are highlighting in 'green' subtasks that have been competed, in 'yellow' subtasks that have started and are in progress, and in 'blue' subtasks that have not yet started but will initiate in the next funding period.

Specific Aim 1.	Timeline (months)	Status	Site 1 (LLNL)	Site 2 (UCD)
Major Task 1. (Sub Aim 1A): Quantify *de novo* protein synthesis in injured joints	**1-24**			
Subtask 1.1. Obtain IACUC/ACURO approval; breed MRL/MpJ, Str/Ort and C57BL/6 cohorts.	1-12	completed	Breed animals	
Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, STR/ort mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 genotypes x 24 mice per group x 7 time points).	3-15	In progress	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 1.3. Sample collection from 336 animals from Subtask 1.2	3-15	In progress	Dissect joints, extract proteins	microCT (168 scans)
Subtask 1.4. Induce type 1 diabetes in 6 week old C57BL/6 mice for 4 weeks.	7-9	completed	Administer STZ	
Subtask 1.5. Induce traumatic OA in 10 week old C57BL/6 and STZ mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 treatments x 24 mice per group x 7 time points).	10-18	In progress	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 1.6. Sample collection from 336 animals from Subtask 1.5	10-18	In progress	Dissect joints, extract proteins	microCT (168 scans)
Subtask 1.7. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	10-24	In progress	^{14}C measurements by AMS	
Subtask 1.8. BONCAT analysis	10-24	In progress	Click-chemistry; quantification	
Subtask 1.9. FUNCAT/Histological analysis	10-24	In progress	Embed, section, visualize proteins	Embed, section, visualize proteins
Milestone 1: IACUC/ACURO Approvals				
Milestone 2: Complete Sample Collection for STR and MRL strain				
Milestone 3: Complete Sample Collection for diabetic mice				
Milestone 4: Complete Proteomic Analysis for 1, 3, 5 and 7 days post injury				
Milestone 5: Complete Proteomic Analysis for 14, 21 and 42 days post injury				
Milestone 6.1: Manuscript #1 describing injury-induced phenotypic and molecular changed in T1D mice				

Milestone 6.2: Manuscript #2 describing injury-induced proteomic changed in mouse strains with varying susceptibility to PTOA

What was accomplished under these goals?



Subtask 1.1. Obtain IACUC/ACURO approval; breed MRL/MpJ, Str/Ort and C57BL/6 cohorts.

IACUC approval was obtained for both primary PI and the partner PI. Breedings of animals were initiated at LLNL, and as soon as animals reached 10 weeks of age, were transported to partner PI where the animals were injured and returned to LLNL. Unfortunately, mid-March the state of California order us to shelter in place due to Covid-19 pandemic, our institutions went to minimum critical operations and requested non-essential workers to shift their efforts to remote working, at LLNL only Covid-19 related research was deemed essential work for scientists working in the Biology and Biotechnology Division. However, our animal care facility at LLNL (ACF) was deemed essential, so most of our animals continued to be bred under the PI (and Ms. Deepa Murugesh') directions. While we continued to expand our colony, we did miss out on conducting injuries for some animals that became 10 weeks of age during this period, in which case we saved these animals and used them for additional breedings.

On 7/2/2020, LLNL authorized us to resume this project, on 7/8/2020 most of the staff returned to work, and on 9/15/2020 we were able to resume injuries in Dr. Blaine Christiansen laboratory. As of now, we are back to normal operation, and do not foresee any anticipated problems on meeting milestones and deliverables in the future.

Subtask 1.2. Induce traumatic OA in 10 week old MRL/MpJ, STR/ort mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 genotypes x 24 mice per group x 7 time points).

Because we couldn't conduct injuries, for the past 6 months we have focused on characterizing the proteome of uninjured joints. Using Ai9;Col2-ER-Cre; MetRS transgenics, we confirmed that the articular cartilage is robustly labeled (Figure 1), we dissected the articular cartilage, treated the chondrocytes with AHA, ex vivo and using FACS confirmed that AHA treated cells synthesize new protein that is AHA labeled (Figure 2).

Figure 1. Ai9;Col2-ER-Cre; MetRS mice received TMX at 2 weeks of age, and using histology we confirmed that Col2-ER-Cre highlights chondrocytes of the articular cartilage, with high specificity. The GFP allele also confirmed that the MetRS transgene robustly activates in the articular cartilage.

Subtask 1.3. Sample collection from 336 animals from Subtask 1.2

Injuries have resumed on 9/15/2020, we will continue to collect samples, stockpile them and analyze them in the next fiscal year.

Subtask 1.4. Induce type 1 diabetes (T1D) 6 week old C57BL/6 mice for 4 weeks.

This task has been completed, sufficient T1D animals have been generated for injuries in subtask 1.5.

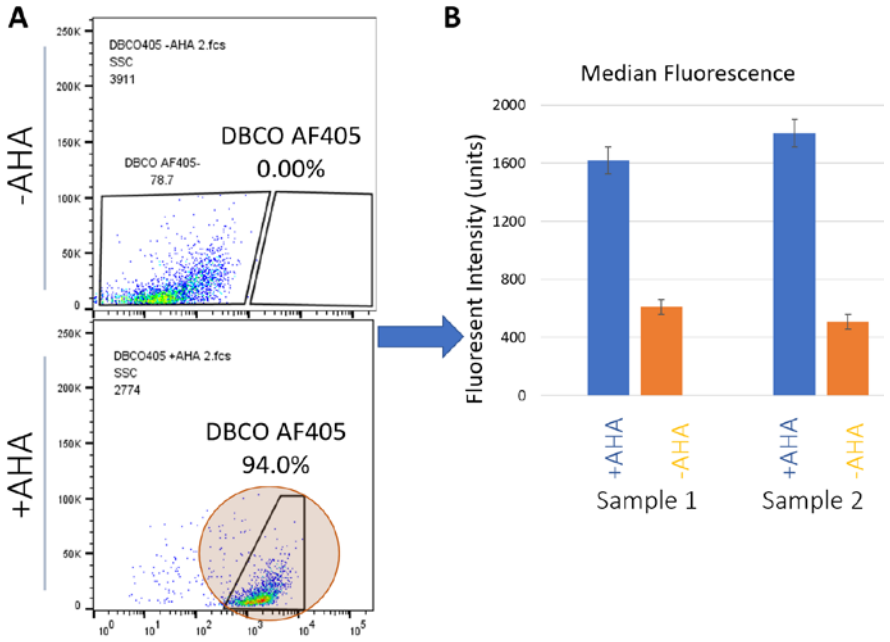


Figure 2. Chondrocytes purified from Ai9;Col2-ER-Cre were treated with AHA *in vitro*, cells were fixed and sorted. 94% of AHA treated chondrocytes were fluorescent (A), suggesting that AHA has been retained and incorporated into the newly synthesized proteins. The signal intensity in AHA+ cells was 4X above background of AHA- cells (B).

Subtask 1.5/1.6. Induce traumatic OA in 10 week old C57BL/6 and STZ mice. Mice with receive AHA/^{14}C-threonine; 336 mice will be used (2 treatments x 24 mice per group x 7 time points). Sample collection from 336 animals from Subtask 1.5

In this task we examined diabetes-related molecular changes in the mouse cartilage knee joints that could contribute to OA. Sixteen weeks old streptozotocin-induced diabetic male mice were assessed for OA and molecular changes in the knee cartilage. Cartilage degeneration measurements of the knee joint showed a mild OA phenotype in the diabetic group, characterized by proteoglycan loss, when compared to the control group, suggesting that diabetes can moderately promote OA. However, diabetes did not intensify post-traumatic osteoarthritis induced by a non-invasive tibial compression injury model and T1D injured joints exhibited PTOA with similar intensity as the non-diabetic control mice. Changes in cartilage knee joint gene expression were examined by RNA sequencing (RNAseq) and identified cohorts of genes differentially regulated between diabetic and non-diabetic animals. Consistent with the PTOA phenotypes, these molecular changes were similar in the injured joints. Our findings confirmed that diabetes can be a risk factor for OA, however, it did not provide strong evidence that T1D enhances cartilage degeneration, post injury. This work is not being prepared into a manuscript (Rios-Arce et al. 2020, *in preparation*). This manuscript has been added onto the SOW table as **Milestone 6.1: Manuscript #1 describing injury-induced phenotypic and molecular changed in T1D mice**

We will initiate the proteomic analysis, but given the nature of the mild phenotype produced by PTOA, we don't expect that the proteomic data will highlight any novel insights into proteins that contribute to a more severe PTOA than the phenotype achieved in C57Bl6 mice controls.

Subtask 1.7. AMS analysis to quantify 14C-threonine levels in injured and uninjured animals

This work has not initiated, samples are still being collected

Subtask 1.8. BONCAT analysis

This work has not initiated, samples are still being collected

Subtask 1.9. FUNCAT/Histological analysis

This work has not initiated, samples are still being collected

Major Tasks 2/3 work will initiate in the next fiscal years. One major change that is worth mentioning, is that we have had increasing difficulties breeding the STR/Ort strain of mice. These mice are giving us consistent small litters, of 1-2 pups and it has been unsustainable to have breeders and generate cohorts for experiments. As an alternative to this OA susceptible strain, we are therefore searching for alternative strains of mice that produce a spontaneous OA phenotype, in particular we are considering using the C3H/HeJ mouse strain in the future.

Major Task 2. (Sub Aim 1B) Quantify cell proliferation in injured joints	**13-27**		
Subtask 2.1. Breed necessary cohorts of MRL/MpJ, STR/Ort and C57BL/6.	13-18	Breed animals	
Subtask 2.2 Induce traumatic OA in 10 week old MRL/MpJ, STR/ort, STZ and C57BL/6 mice. Mice with receive ^{14}C-thymidine; 336 mice used (4 genotypes x 12 mice per group x 7 time points).	16-24	Prepare cohorts of animals, transport to UCD for injury	336 animals will be injured, return animals to LLNL post injury
Subtask 2.3. Sample collection from 336 animals from Subtask 2.2	16-24	Dissect joints, extract DNA	
Subtask 2.4. AMS analysis to quantify ^{14}C-thymidine levels	19-27	^{14}C measurements by AMS	
Milestone 7: Complete Sample Collection for MRL, STR, STZ and B6 mouse strain			
Milestone 8: Complete AMS analysis			
Major Task 3. (Sub Aim 1C): Quantify mineral apposition in injured joints:	**19-33**		
Subtask 3.1. Breed necessary cohorts of MRL/MpJ, STR/Ort and C57BL/6 cohorts.	19-24	Breed animals	
Subtask 3.2 Induce traumatic OA in 10 week old MRL/MpJ, STR/ort mice, STZ and C57BL/6 mice. Mice with receive ^{45}Calcium. 192 mice will be used (4 genotypes x 12 mice per group x 4 time points).	22-30	Prepare cohorts of animals, transport to UCD for injury	192 animals will be injured, return animals to LLNL post injury
Subtask 3.3. Sample collection from 192 animals from Subtask 3.2.	22-30	Dissect joints, extract DNA	microCT (192 scans)
Subtask 3.4. LC analysis to quantify ^{45}Calcium- levels in injured and uninjured animals	25-33	Measure ^{45}Ca by liquid scintillation	
Milestone 9: Complete Sample Collection for MRL, STR, STZ and BL6 mouse strain			
Milestone 10: Complete microCT/LC analysis			
Milestone 11: Manuscript #2 describing injury induced cellular proliferation and osteophyte formation in mouse strains with varying susceptibility to PTOA.			

Specific Aim 2 and Major Task 4 work was supposed to initiate in the next fiscal year, however we have already initiated some work on these tasks. One change to the original plan that is worth mentioning, is that we have had increasing difficulties breeding the STR/Ort strain of mice. These mice are giving us consistent small litters, of 1-2 pups and it has been unsustainable to have breeders and generate cohorts for experiments. As an alternative to this OA susceptible strain, we are therefore searching for alternative strains of mice that produce a spontaneous OA phenotype, in particular we are considering using the C3H/HeJ mouse strain in the future.

Specific Aim 2.	Timeline	Status	Site 1 (LLNL)	Site 2 (UCD)
Major Task 4. (Sub Aim 2A): Characterize the injury-induced transcriptome and proteome in the articular cartilage.	**10-33**			
Subtask 4.1. Breed necessary cohorts of ***Ai9; Col2-ER-Cre; ~~UPRT;~~ MetRS*** animals. These mice will now be ***Ai9; Col2-ER-Cre; MetRS***	10-21	in progress	Genotype and breed animals	
Subtask 4.2 Induce traumatic OA in 10 week old ***Ai9; Col2-ER-Cre; ~~UPRT;~~ MetRS*** mice. Mice with receive 4TU, ^{14}C-threoninine and ANL. 432 mice will be used (2 genotypes x 24 mice per group x 9 time points).	16-24	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, return animals to LLNL post injury

This aim has been modified—UPRT/4TU will not be used and the RNA-seq task will be replaced with single cell sequencing				
Subtask 4.3. Sample collection from 432 animals from Subtask 4.2. ScRNA-seq has been conducted on uninjured joints	16-24	In progress	extract proteins, isolate RNA	
Subtask 4.4. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	19-30	not started yet	^{14}C measurements by AMS	
Subtask 4.5. BONCAT analysis	19-30	not started yet	Click-chemistry; quantification	
Subtask 4.6. FUNCAT/Histological analysis	19-30	not started yet	Embed, section, visualize proteins	Embed, section, visualize proteins
Subtask 4.7. LC-MS/MS analysis	22-33	not started yet	Protein identification	
Milestone 12: Complete Sample Collection for *Col2-ER-Cre; ~~UPRT;~~ MetRS*				
Milestone 13: Complete Chondrocyte-Specific RNA-seq analysis (scRNA-seq now)				
Milestone 14: Complete Chondrocyte-Specific Proteomic Analysis				
Milestone 15: Manuscript #3 describing injury-mediated chondrocyte specific protein and gene expression				
Major Task 5. (Sub Aim 2B): Characterize the injury-induced transcriptome and proteome in the immune system.	**13-36**			
Subtask 5.1. Breed necessary cohorts of ***Ai9; Csf1r-Cre; ~~UPRT;~~ MetRS*** animals. These mice will now be ***Csf1r; Ai9; MetRS***	13-25	In progress	Genotype and breed animals	
Subtask 5.2 Induce traumatic OA in 10 week old ***Ai9; Csf1r-Cre; ~~UPRT;~~ MetRS*** mice. Mice will receive 4TU, ^{14}C-threoninine and ANL. 432 mice (2 genotypes x 24 mice per group x 9 time points). This aim has been modified—UPRT/4TU will not be used and the RNA-seq task will be replaced with single cell sequencing	19-27	In progress	Prepare cohorts of animals, transport to UCD for injury	432 animals will be injured, return animals to LLNL post injury
Subtask 5.3. Sample collection from 432 animals from Subtask 4.2. ScRNA-seq has been conducted on uninjured joints	19-27	In progress	Extract proteins, isolate RNA	
Subtask 5.4. Breed MRL/MpJ animals.	19-25	not started yet	Breeding	
Subtask 5.5. Injure MRL/MpJ animals, administer AHA+^{14}C-threonine. 104 mice (12 mice x 9 time points)	19-25	not started yet	Transport cohorts to UCD for injury	104 animals will be injured, return to LLNL
Subtask 5.6. Sort macrophages and T-cells from joints, isolate proteins	21-27	not started yet	FACs, protein extraction	
Subtask 5.7. AMS analysis to quantify ^{14}C-threonine levels in injured and uninjured animals	22-33	not started yet	^{14}C measurements by AMS	
Subtask 5.8. BONCAT analysis	22-33	not started yet	Click-chemistry; quantification	
Subtask 5.9. FUNCAT/Histological analysis	22-33	not started yet	Embed, section, visualize proteins	Embed, section, visualize proteins

Subtask 5.10. LC-MS/MS analysis	25-36	not started yet	Protein identification	
Subtask 5.11. IHC validation	31-36	not started yet	Visualization of C57Bl/6 and MRL/MpJ protein expression	
Milestone 16: Complete Sample Collection for *Ai9; Csf1r-Cre; ~~UPRT;~~ MetRS*				
Milestone 17: Complete Immune-Specific RNA-seq analysis (scRNA-seq now)				
Milestone 18: Complete Immune-Specific Proteomic Analysis				
Milestone 19: Manuscript #4 describing injury-mediated immune specific protein and gene expression, highlighting MRL/MpJ specific proteins that may contribute to PTOA resistance				

For Major Task 4. (Sub Aim 2A): Characterize the injury-induced transcriptome and proteome in the articular cartilage. We originally proposed to carry out cell type specific RNA-seq analysis using the UPRT allele bred with the MetRS transgene to generate the ***Ai9; Csf1r-Cre; UPRT; MetRS*.** However, the advent of single cell-sequencing technology has proved to a more reliable and informative approach to generating cell-type specific RNA-seq data. We therefore we shifted our focus on scRNA-seq.

While we originally didn't plan to carry out these experiments until months 19-27 of the funding period, we took the opportunity to push some of this work into the 1st year to replace the injury work associated with **Major Task 1** we couldn't carry out due to shelter in place. Therefore, we initiated scRNA-seq characterization of uninjured joints. C57B6L/6J (B6) and MRL/MpJ (MRL) joints were euthanized at weeks of age (n=5), and both hindlimbs were collected by removing the leg at the hip joint. The synovial capsule and long bones were cleaned of muscle, tendon and ligaments. The cleaned long bones and joints were then separated from each other in an enzymatic cocktail to also account for cells within the synovial fluid of the joint, then digested using Collagenase 2, to a single cell suspension. Flow cytometry was completed for isolation of chondrocytes by negatively selecting for CD45 (immune) and Ter119 (erythroid) cells. Libraries were prepared for the double negative population using Chromium Single Cell 3' GEM, Library & Gel Bead Kit v3 following manufacturers protocol and then sequenced using Illumina NextSeq 500. Following sequencing, data was demultiplexed, quality checked, and aligned to the mouse genome (mm10) using Cell Ranger (10X Genomics, CA, USA). Data analysis was completed using Seurat R package and Loupe Cell Browser (10X Genomics, CA, USA).

ScRNA-seq analysis identified several common cell types including chondrocytes (*Sox9, Acan, Col2a1, Col10a1, Col9a1*), osteoblasts (*Alpl, Runx2, Bglap, Col1a1*), osteochondral precursors and fibroblasts (*Thy1, Pdgfra, Dpt*) in the joints of both B6 and MRLs. Strain-specific analysis highlighted distinct distributions of sub-populations (Figure 3). These populations were broken down into eleven sub-populations in MRL (Figure 3C) and eight sub-populations in B6 mice (Figure 3B). Several of these sub-populations in both strains exhibited robust *Col2a1, Col1a1*, or both, identifying them as chondrocytes, osteoblasts, or fibroblasts (Figure 4A). In addition, four fibroblast-like sub-populations were found in MRL samples compared to only 1 in the B6 samples. These sub-populations robustly expressed fibroblast markers *Thy1, Pdgfra, Dpt*, as well as some markers associated with stemness *CD34, CD44*, and *Ly6a/e* (Figure 4B). Additionally, a stem cell associated transcript, *Thsb4*, which was previously shown to promote regeneration of soft tissues was found at elevated levels in the MRL cells when compared to B6 cells, highlighting this gene as a potential candidate for future studies examining the regenerative potential of MRL cartilage (Figure 4C). MRLs also had a more distinct sub-population of *Col2a1^{low}/-Prg4^{high}* chondrocyte-like cells that also express chondrocyte markers *Sox9^{low}, Cilp*, and *Bmp2*. These cells were present but at a much lower level in the B6 clusters. Prg4 has a significant role in the lubrication of the joint, and has been shown to have a regenerative affect on the joint in non-inflammatory arthritis (Rhee et al 2005, J. Clin Invest.) *Prg4*-expressing cells may also serve as chondrocyte progenitors (Kozhemyakina et al, Arthritis Rheumatol. 2015)

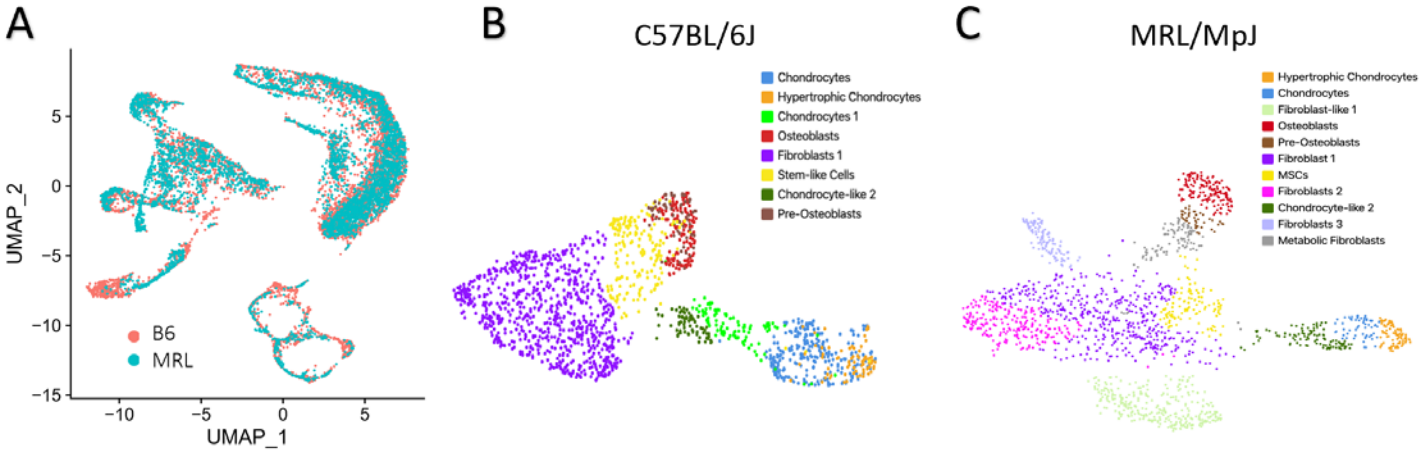


Figure 3. ScRNA-seq analysis of the murine knee joint of C57BL/6J **and** MRL/MpJ **mouse strains.** (A) UMAP (Uniform Manifold Approximation and Projection) plots showing total joint populations of MRL (blue) compared to C57BL/6J (red). C57BL/6J (B) and MRL/MpJ (C) and annotated cellular sub-populations (colored clusters)

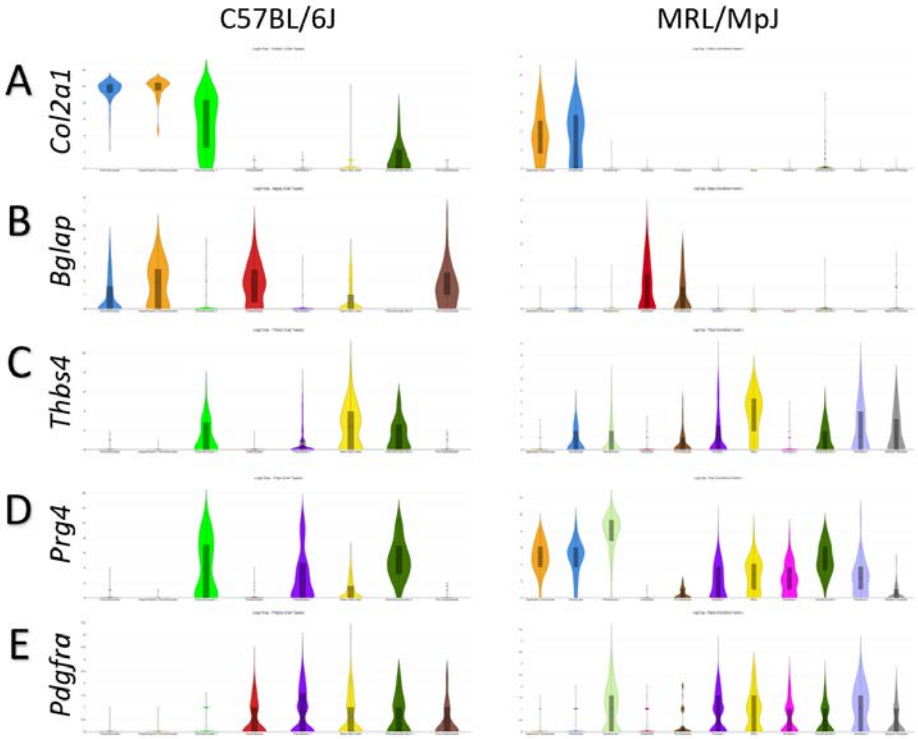


Figure 4: Violin plots showing differential gene expression between MRL/MpJ and C57BL/6J. (A) Chondrocytes (B) Osteoblasts (C) Stem-like (D) Prg4high Chondrocyte-like (E) Fibroblasts.

This study identified several sub-populations exhibiting stem, fibroblast and chondrocyte markers that may be responsible for the enhanced healing associated with the MRL regenerative phenotype. In particular, this data shows that MRL joint have several fibroblast-like sub-populations that are underrepresented in the B6 joint. We also determined that MRL joints harbored chondrocyte-like populations within the joint that may have regenerative potential and may drive the phenotype associated with MRL. This study demonstrates the varying sub-populations of fibroblast and chondrocyte-like cells suggesting they may have a significant role in healing, stem cell infiltration and decreased inflammation. This work was submitted as an abstract to the Orthopedic Research Society Annual Meeting to be held virtually Feb 13-16, 2021:

Jillian L. McCool[1,2], Aimy Sebastian[1], Nicholas R. Hum[1,2], Deepa K. Murugesh[1], Blaine A. Christiansen[3] and Gabriela G. Loots[1,2] ***Single Cell RNA Sequencing Highlights Unique Sub-populations of Cells in the Knee Joints of MRL/MpJ Superhealers*** [1]Lawrence Livermore National Laboratories, Physical and Life Sciences Directorate, Livermore, CA.; [2]UC Merced, School of Natural Sciences, Merced, CA.; [3]UC Davis Medical Center, Department of Orthopedic Surgery, Sacramento, CA.

What opportunities for training and professional development has the project provided?

At UC Davis, one postdoctoral fellow (Benjamin Osipov) is being trained on research related to this project. Dr. Osipov is developing technical skills such as non-invasive knee injury, survival surgery, and micro-computed tomography under the direct supervision and mentorship of Dr. Christiansen. Dr. Osipov also recently attended a week-long intensive course on flow cytometry. He will continue to attend the two premier bone and cartilage national meetings, ORS and ASBMR, every year during his fellowship, and he regularly attends seminars through the UC Davis Department of Orthopaedic Surgery and the UC Davis Veterinary Orthopaedic Research Laboratory. Dr. Osipov's training goals for his postdoctoral fellowship are: 1.) Didactic training in molecular and cell biology; 2.) Attaining proficiency in analytical techniques and experimental design; 3.) Professional development that leads to employment as an independent researcher at a leading research institution.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Our main focus for the next period will be to complete **Subtask 1.2-1.10**.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Change 1*:* Originally, we proposed to characterize cell-type specific protein expression in parallel with RNA expression using a genetic trick. The advent of single cell RNA sequencing is proving that this new approach is much more robust, sensitive and reliable to generate the same data more efficiently, therefore we will not be using the UPRT allele in combination with 4TU administration to purify and sequence bulk RNA from tissues. Instead we will conduct single cell RNA-seq for all the RNA-characterization. This change has actually allowed us to move our timeline forward, and some of these experiments have already been initiated ahead of time (see figures 3 and 4).

Change 2*:* In the original proposal we aimed to include STR/ort mice as one strain of mice to analyzed because these mice develop spontaneous OA and upon injury they are highly susceptible to rapid cartilage degradation, however, we had had significant challenges breeding these mice, they produce only 1-2 pups per little and we cannot reliably generate cohorts for experiments. Since thse mice are not commercially available for purchase, we are now considering replacing this strain with a different strain, C3H/HeJ which we have used before and have some preliminary evidence that they may be more susceptible to PTOA than B6, in the next fiscal year we will explore the use of this strain.

Actual or anticipated problems or delays and actions or plans to resolve them

Challenges due to Covid-19 Pandemic*:* Mid-March the state of California order us to shelter in place due to the Covid-19 pandemic, and our institutions went to minimum critical operations and requested non-essential workers to shift their efforts to remote working. All research operations in the Department of Orthopaedic Surgery at UC Davis were paused for several months. On 8/26/2020, The Christiansen lab was authorized to resume some time-sensitive research activities under Phase 2X approval, and on 9/15/2020 we were able to resume mouse injuries related to this project. As of now, we are operating at approximately 33% capacity under Phase 2 guidelines, but we do not foresee any anticipated problems on meeting milestones and deliverables in the future.

Changes that had a significant impact on expenditures

The shelter in place order and pause of research operations has created a slight surplus of funds. However, now that we are resuming research activities and ramping up effort, we anticipate to fully spend the allocated funds, and to be able to complete the tasks in Aim1 that were delayed. We anticipate increasing our efforts on this project during the upcoming year, and we will be back on track soon.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not Applicable.

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Published Manuscripts acknowledging federal support by Grants PR180268PR180268P1

Melanie E Mendez, Deepa K Murugesh, Aimy Sebastian, Nicholas R Hum, Summer A McCloy, Edward A Kuhn, Blaine A Christiansen, Gabriela G Loots. Antibiotic Treatment Prior to Injury Improves Post-Traumatic Osteoarthritis Outcomes in Mice ***Int J Mol Sci***. 2020 Sep 3;21(17):E6424. PMID: 32899361 DOI: 10.3390/ijms21176424

Melanie E Mendez, Aimy Sebastian, Deepa K Murugesh, Nicholas R Hum, Jillian L McCool, Allison W Hsia, Blaine A Christiansen, Gabriela G Loots. LPS-Induced Inflammation Prior to Injury Exacerbates the Development of Post-Traumatic Osteoarthritis in Mice. ***J Bone Miner Res***. 2020 Jun 21.PMID: 32564401 DOI: 10.1002/jbmr.4117

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers, and presentations.

Several abstracts related to this research have been submitted to the 2021 ORS Annual Meeting.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Blaine Christiansen*
Project Role:	*Partnering PI*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID: 0000-0002-0105-6458*
Nearest person month worked:	2
Contribution to Project:	*Dr. Christiansen has performed non-invasive knee injuries in mice, and has performed micro-computed tomography imaging and analysis, and advised Dr. Loots's lab personnel on microCT methods*
Funding Support:	*In addition to this award, Dr. Christiansen receives funding support from the NIH – National Institute for Arthritis and Musculoskeletal and Skin Diseases, under award numbers R01 AR071459, R01 AR073772, and R01 AR075013*

Name:	*Benjamin Osipov*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID: 0000-0001-9456-3311*
Nearest person month worked:	10
Contribution to Project:	*Dr. Osipov has performed non-invasive knee injuries in mice, micro-computed tomography imaging and analysis, and statistical analysis*
Funding Support:	*In addition to this award, Dr. Osipov receives funding support from the NIH – National Center for Advancing Translational Sciences, under award number TL1 TR001861*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

New Active Support:

Modification of Post-Traumatic Osteoarthritis Progression with Joint Unloading
NIH / NIAMS R01 AR075013 03/01/2020 – 12/31/2024
25% effort $1,644,526 Total Direct Costs
National Institutes of Health, NIAMS
31 Center Drive, MSC 2350, Bethesda MD 20892
These studies will determine effective rehabilitation strategies for improving long-term joint health after injury. We

hypothesize that unloading during the early phase following injury will reduce early inflammation and protease activity in the joint, and will ultimately diminish articular cartilage degeneration and osteophyte formation relative to normally loaded mice. We further hypothesize that muscle and bone atrophy associated with unloading will be ameliorated with intermittent reloading and inhibition of bone resorption without leading to joint degeneration. We will first determine the effect of mechanical unloading during the early phase on inflammatory and catabolic processes and long-term joint degeneration. Next, we will determine the effect of intermittent reloading on muscle mass and strength and long-term joint degeneration. Finally, we will determine the effect of knee restabilization following injury on early inflammatory and catabolic processes and long-term joint degeneration. Role: PI
Role: Principal Investigator
Grant Status: Active
Overlap: None

What other organizations were involved as partners?

Nothing to Report.

SPECIAL REPORTING REQUIREMENTS

Nothing to Report.