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Global Gene Expression Differences in Joints of Mice with Divergent Post Traumatic Osteoarthritis Phenotypes

J. Kibui

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Disclaimer

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Julie Kibui

Summer Internship

Georgetown University

Lawrence Livermore National Laboratory

Final Report

Contents

THE LAB

PROJECT 1

Global Gene Expression Differences in Joints of Mice with Divergent Post Traumatic Osteoarthritis Phenotypes

ABSTRACT

Osteoarthritis (OA) is a debilitating joint disease characterized by cartilage degradation which prompts pain, stiffness and swelling. Contributing factors include age, genetics, obesity, injury and overuse of joints. OA is defined by an acute phase and a chronic phase whereby inflammation and degeneration of articular cartilage and other tissues is followed by joint pain and limited mobility. Patients remain asymptomatic until substantial joint damage has occurred and therefore rely on long term surgical joint replacement and pain management as their sole treatment options. For this reason, there is an increasing need to identify early stage osteoarthritis biomarkers. Our study aimed to identify and characterize gene expression variances in 3 different mouse strains (STR/ort, C57BL/6 and MRL/MpJ) with different susceptibility to post traumatic osteoarthritis (PTOA). Through RNA sequence analysis of whole knee joint RNA, we identified differentially expressed genes associated with the initial stages of PTOA in relation to mice with divergent phenotypes. These results will help elucidate potential mechanisms responsible for PTOA outcomes.

Introduction

Post traumatic osteoarthritis (PTOA) is a subsidiary of osteoarthritis defined by severe cartilage degradation months or years post injury. It is characterized by an acute phase and a chronic phase whereby inflammation and degeneration of articular cartilage and other tissues occurs, followed by joint pain and limited mobility. Patients remain asymptomatic until substantial joint damage has occurred. Currently, there are no known predictive biomarkers or disease modifying therapeutic agents therefore patients rely on long term surgical joint replacement and pain management as their sole treatment options. For this reason, there is an increasing need to identify early stage osteoarthritis biomarkers.

Our study aimed to identify and characterize gene expression variances in 3 different mouse strains (STR/ort, C57BL/6 and MRL/MpJ), we identified differentially expressed genes associated with the initial stages of PTOA in relation to mice with divergent phenotypes. These results will help elucidate potential mechanisms responsible for PTOA outcomes. In order to do this, I first had to analyze RNA sequence data generated from 3 different mouse strains as mentioned above. Below in figure one is a previously established histological assessment of the OA phenotypes of these 3 mice. (Chang, 2016)

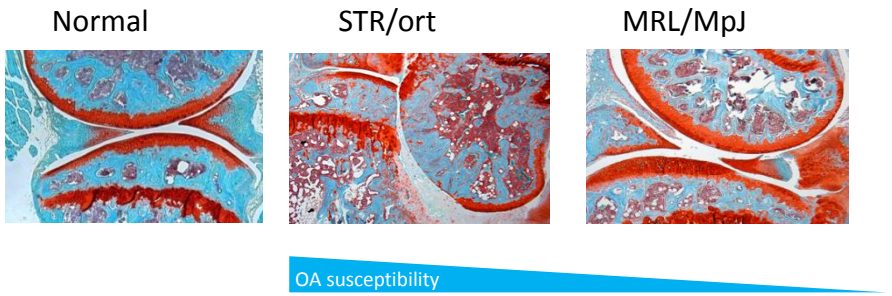

Figure 1. Histological assessment of out mouse cohort with various OA susceptibility. (Chang, 2016)

Methods:

Our methodology is summarized in figure 2 below.

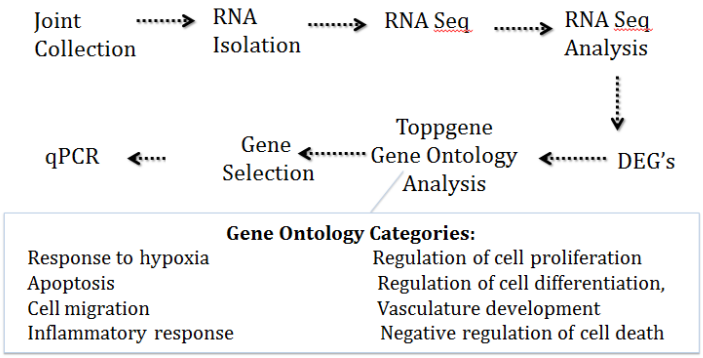

Figure 2. Methods. Injured and uninjured joints were collected at day 1 and week 1 post tibial compression(TC) injury followed by RNA isolation, sequencing and differential gene expression analysis via computation analysis and qPCR.

i) Joint Injury

To mimic ACL rupture in humans, our study used a non-invasive tibial compression method to induced knee injury in mice. The right leg of each mouse was injured via applying a single compressive load of (10-12N). This method caused ACL dislocation and displacement of the joint.

ii) RNA Isolation and Sequencing

Both injured and contralateral joints were collected over a range of time points: before injury and day 1 and week 1 post injury. RNA was then isolated from the knee joints using an RNeasy Qiagen kit. The isolated RNA was sequenced using an Illumina HiSeq 2000.

iii) DEG Analysis

Here specifically, we sought to identify upregulated genes in STR/ort mice in comparison to C57BL/6 and MRL/MpJ. To do so, we performed RNA sequence analysis and identified 167 genes significantly upregulated in STR mice. We then performed gene ontology analysis using ToppGene where we selected gene ontology categories pertaining to response to hypoxia, regulation of cell proliferation, apoptosis, regulation of cell differentiation, cell migration, vasculature development, inflammatory response, negative regulation of cell death. We then compared the list of Differentially Expressed Genes (DEG) to genes associated with known arthritis phenotypes, significantly narrowing our list of genes.

Of those, we selected 5 for quantification and validation via real time PCR. The fold change for each gene was then determined in both MRL and STR relative to C57BL/6. The results are shows on the following page.

Results:

Through various previously described analytical techniques, I was able to identify genes of interest that were highly expressed in STR in comparison to WT and MRL. Selected DEG's:

A) **CXCL1**-chemokine (C-X-C motif) ligand 1

B) **DUSP1**- Dual Specificity Phosphatase 1

C) **CCL7**- Chemokine (C-C motif) ligand 7

D) **PTGS2**- Prostaglandin- Endoperoxide Synthase 2

E) **ERRFI1**- ERBB Receptor Feedback Inhibitor 1

Fold changes for each of the genes are shown in figure 2 below.

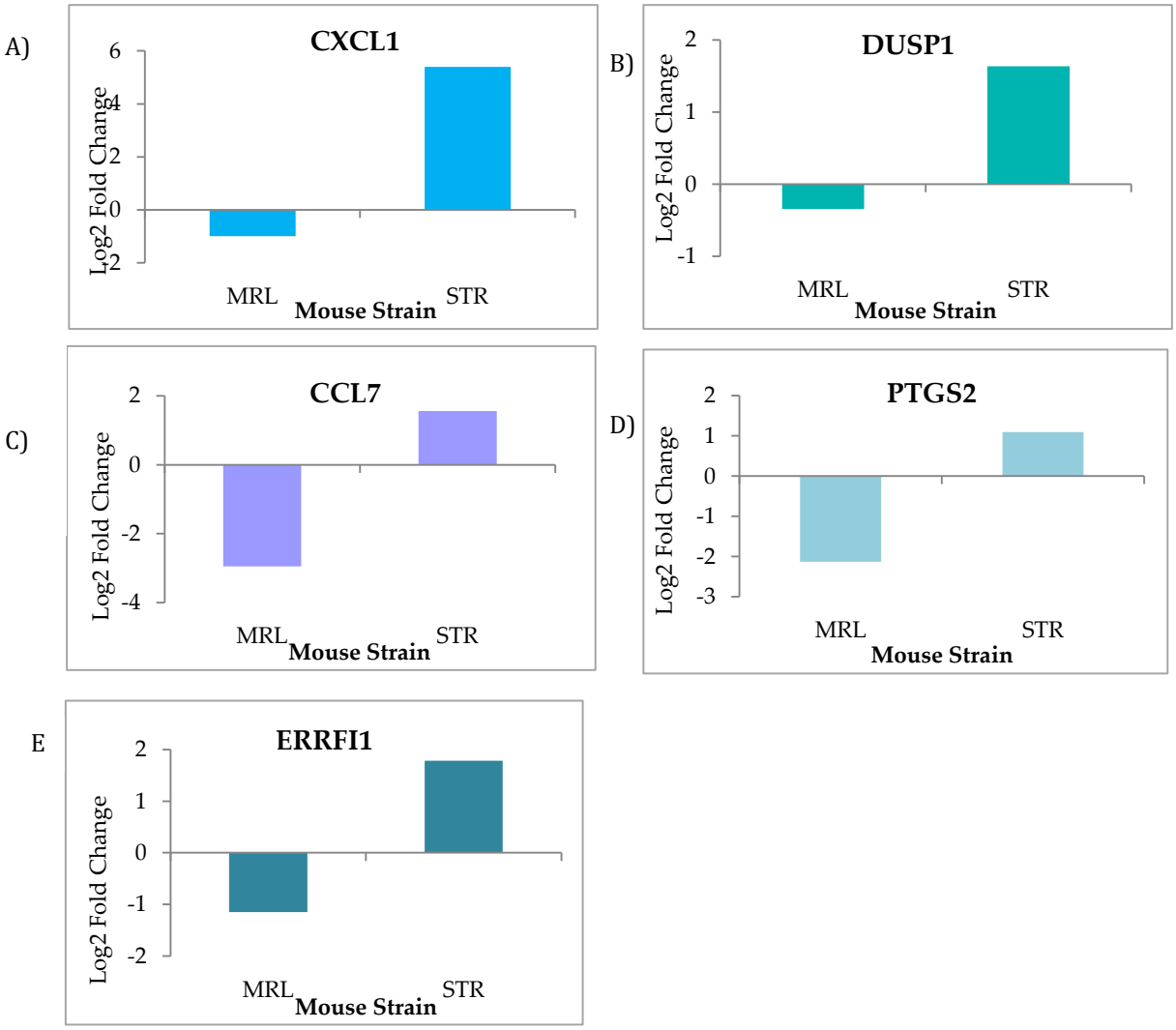

Figure2. Real-time PCR validation of gene expression differences elicited by the five genes. A) **CXCL1**- chemokine (C-X-C motif) ligand 1. B) **DUSP1**- Dual Specificity Phosphatase 1. C) **CCL7**- Chemokine (C-C motif) ligand 7. D) **PTGS2**- Prostaglandin- Endoperoxide Synthase 2 E) **ERRFI1**- ERBB Receptor Feedback Inhibitor 1

Gene expression of RNA generated from joints at day 1 and week one were established and plotted.

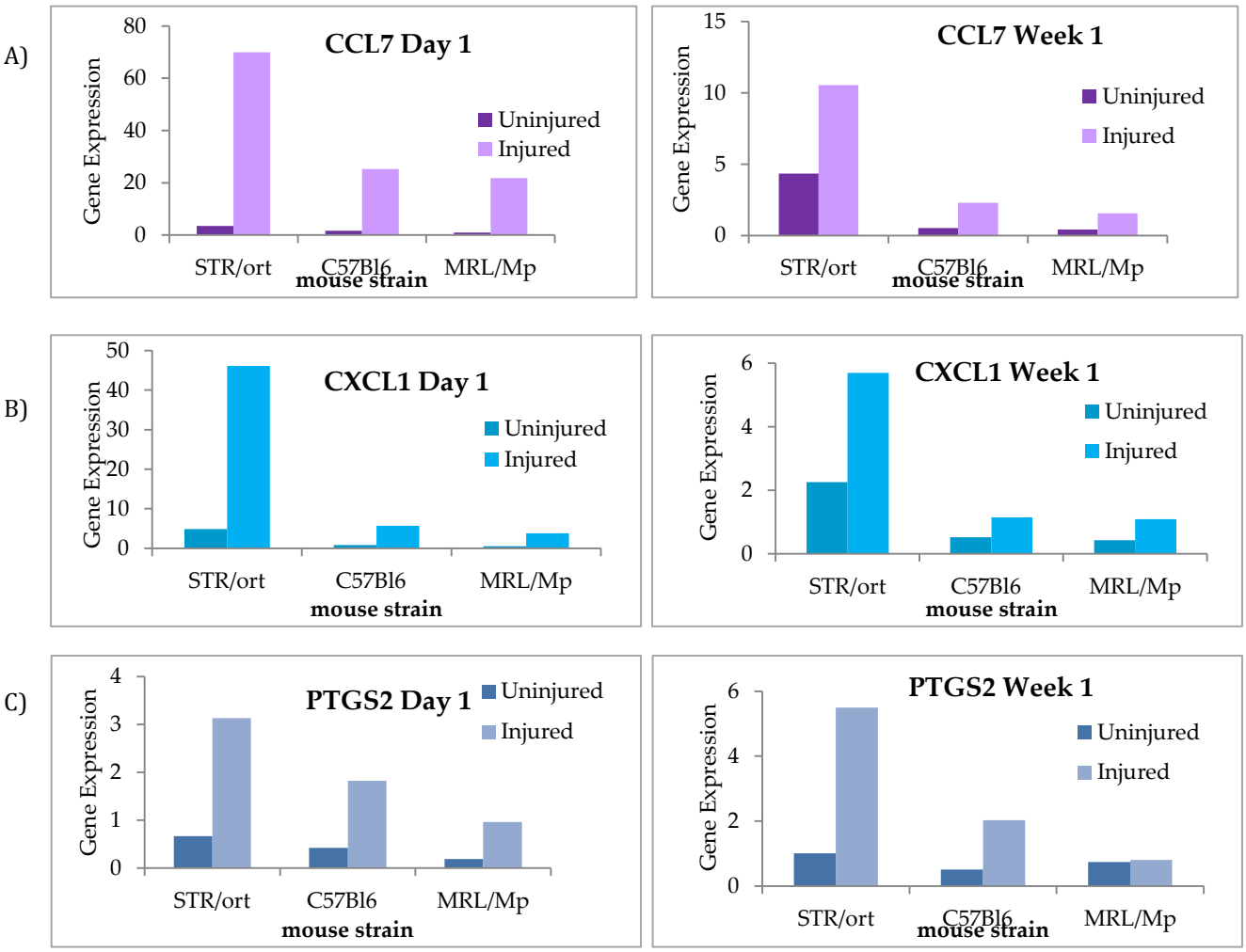

Figure 3. Gene expression of A)CCL7 B) CXCL1 C) PTGS2 following RNA sequence analysis of RNA generated from joints at day 1 and week one, respectively.

Discussion

Through RNA sequence analysis and qPCR validation of gene expression in our 3 different mouse strains, we identified 167 genes significantly upregulated in STR/ort mice in comparison to C57BL/6 and MRL/MpJ. Of those, 5 were selected via gene ontology analysis for quantification and were successfully validated via qPCR.

Assessment of fold change in all 5 genes showed expression patterns consistent with our hypothesis. Expression of these genes was significant in STR/ort and decreased with the PTOA phenotype gradient as we expected to see in our control animals. Furthermore, as seen in figure 3, expression of each gene was particularly high in injured joints relative to uninjured at both time points observed, suggesting that they are implicated early stages of the disease.

Although both ERRFI1 and DUSP1 were highly expressed in STR/ort as shown in Figure 2, little to no expression of these genes was observed at day 1 and week 1 as indicated by RNA sequence data analysis, hence no gene expression data for these 2 genes was plotted in figure 3. Lack of expression at these time points could indicate that these genes perhaps do not directly contribute to respective phenotypes at these time points, however further assessment should be performed to conclusively answer this.

Moving forward, our potential candidate genes CCL7, CXCL1 and PTGS2 can undergo further assessment via gene knockout for instance, to further determine phenotypic differences in the absence of these genes.

Our data provides insight into unravelling the molecular mechanisms underlying OA phenotypes in order to allow identification of potential biomarkers for early and accurate genetic prediction, detection and tracking of OA for the development of future treatment venues. Future directions

in our study will include further identifying both up and down regulated genes in both injured and uninjured joints relative to each other as well as across the different strains over various time points.

PROJECT 2

Examine gut response to LPS induced intestinal epithelial cell shedding in mice with varying susceptibility to PTOA

Introduction

Osteoarthritis (OA) is a debilitating joint disease characterized by cartilage degradation which prompts pain, stiffness and swelling. Contributing factors include age, genetics, obesity, injury and overuse of joints. OA is defined by an acute phase and a chronic phase whereby inflammation and degeneration of articular cartilage and other tissues is followed by joint pain and limited mobility. Following post traumatic injury, individuals are at risk for facing unwarranted pathogenic exposure and are likely to die from sepsis. Our study aimed on using an established LPS sepsis animal model to mimic these clinical settings. The study would determine global gene expression in whole joints and determine gene expression differences that occur following an induced inflammatory response under traumatic conditions. Using a cohort of 4 mice strains (C57BL/6, C3H/HeJ, MRL/MpJ, STR/ort) with different susceptibility to OA, we intraperitoneally injected the bacterial endotoxin lipopolysaccharide (LPS) to mimic an immune response caused by pathogenic bacteria. We aimed on examining how the gut of these different strains of mice responded to LPS challenge and specifically how identifying gene expression changes and how these molecular changes correlated with the various PTOA phenotypes.
The small intestine (S.I) inner surface is covered by millions of villus projections forming a single cell thick epithelium. The small intestine functions in various ways. It is specialized to efficiently digest, transport and regulate the absorption of nutrients and water into the circulation component of the gut barrier (part of the innate immune system) which prevents the entry of harmful microbes, toxins, and antigens from the intestinal lumen. Tissue homeostasis of the S.I is

maintained by S.I turnover every (3-5 days in humans, 2-3 days in mice) equal number of lost cells and cells generated by cell division. New epithelial cells are generated in the crypt, and migrate up the villus until they are shed at the villus tip. Approximately 10^{11} and $2x10^{8}$ cells shed per day this amounts to ~1400 IECs shed from a single tip per day.

Loss of IEC from the villus exceeds the rate of cells being produced from the crypt- this has been observed in various inflammatory conditions. This cell loss has also been identified in the early events in the pathogenesis of various diseases. IEC shedding leads to increased gut permeability during inflammatory responses however this is not well understood. Our model uses induce IEC shedding via administering lipopolysaccharide (LPS) to further unravel gut responses in different mouse strains.

Goal:

Using a cohort of 4 mice strain with 5 mice each, we intraperitoneally injected the bacterial endotoxin lipopolysaccharide (LPS) would have on LPS-induced loss of intestinal epithelial cells and various gene expression patterns associated with this loss.

We suspect that STR/ort which are highly susceptible and spontaneously PTOA will display severe immune responses following LPS challenge. Further, MRL/MpJ which have very low susceptibility may likely be more tolerant to LPS challenge and show a milder immune response in comparison to STR/ort mice.

Methods:

Our experimental testing method uses chemically induced animal model testing whereby we mimic a bacterial infection via LPS induced intestinal epithelial cell shedding in mice with varying susceptibility to PTOA. To determine global gene expression in whole joints and determine gene expression differences that occur following an induced inflammatory response under traumatic conditions

Intraperitoneally inject PBS or LPS (1mg/kg) into our 20 animals consisting of the following strains C57Bl6, C3H/HeJ, MRL/MpJ, STR/ort. Small intestine tissues collected & processed for histology & RNAseq. This is followed by an analysis of RNAseq data and an assessment of gene expression changes for each mouse strain and further understand how these changes correlate with PTOA phenotypes.

Discussion:

The animals were treated with LPS and their intestinal and other tissues were dissected and stored and RNA was isolated. Due to time limitations, the RNA was not sequenced and analyzed. Future studies will include RNA library preparation, sequencing and data analysis to determine the molecular changes associated with LPS challenge animal models with varying susceptibility to PTOA.

Acknowledgements:

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Mentors Signature: Gabriela Loots Date: 7/28/2016