Spring 5-2020

Transcriptomic Analysis of Cytokine-Treated Tissue-Engineered Cartilage as An In Vitro Model of Osteoarthritis

Jiehan Li

Washington University in St. Louis

McKelvey School of Engineering

Department of Biomedical Engineering

Thesis Examination Committee:

Farshid Guilak, Chair

Bo Zhang

Kareem Azab

Transcriptomic Analysis of Cytokine-Treated Tissue-Engineered Cartilage as An *In Vitro* Model of Osteoarthritis

By

Rachel (Jiehan) Li

A thesis presented to the McKelvey School of Engineering of Washington University in St. Louis in partial fulfillment of the requirements for the degree of Master of Science

May 2020

St. Louis, Missouri

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my thesis advisor Dr. Farshid Guilak for his unwavering support and patient guidance during my master training. Dr. Farshid Guilak's ability to push the boundaries and investment into my professional development changed the way I address new challenge and has enable my growth as a scientist and an engineer. I would also like to thank Dr. Bo Zhang for his invaluable idea and suggestion on bioinformatics aspect. Dr. Zhang's methodical approach to research has instilled in me an utmost respect for scientific rigor.

My thanks are extended to the rest of the Guilak lab members during the tenure of my Master. Especially, I want to acknowledge Ali Ross and Sara Oswald for their encouragement and insightful discussion and comments for my thesis research.

I would also like to thank other members of my thesis committee, Dr. Kareem Azan. His insights and feedback have been invaluable to my thesis.

Lastly, I would like to acknowledge the funding sources that supported this research: the Nancy Taylor Foundation for Chronic Diseases, the Arthritis Foundation, NIH grants (AG46927, AG15768, AR76820, AR75899, P30 AR74992, P30 AR073752), and the Bioinformatics Research Core at the Washington University Center of Regenerative Medicine for Funding for this project and technical support.

Rachel (Jiehan) Li

Washington University in St. Louis

May 2020

Table of Contents

List of Tables iii

List of Figures iv

Abstract of Thesis vi

Chapter 1: 1

Introduction 1

1.1 Current Treatment of OA 1

1.2 Inflammation in OA 2

1.3 Current Models of OA 3

1.3 iPSC Model System in OA 3

Chapter 2: 5

Transcriptomic Analysis of Cytokine-Treated Tissue-Engineered Cartilage as an *In Vitro* Model of Osteoarthritis 5

2.1 Introduction 5

2.2 Materials and Methods 7

2.3 Results 9

2.4 Discussion 29

2.5 Limitations 33

2.6 Conclusion 34

References 35

Appendix Figure 1: Venn diagrams showing number of overlapping upregulated (+) or downregulated (-) DEGs between different models 40

Appendix Table 1: Common genes of gene ontology (GO) terms in response of IL-1β 41

Appendix Table 2: Common genes of gene ontology (GO) terms in response of TNF-α..... 42

List of Tables

Table 1. Top 30 dysregulated genes common to both IL-1β treated pellets (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL)..... 12
Table 2. Top 30 dysregulated genes common to both IL-1β treated pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL)..... 14
Table 3. Top 30 dysregulated genes common to both IL-1β treated tissue-engineered pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA). 16
Table 4. Top 30 dysregulated genes common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF)..... 17
Table 5. Top 30 dysregulated genes common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and untreated human osteoarthritic cartilage (cartilage.OA). 19

List of Figures

Figure 1. Flow chart and overview of 4 datasets used in transcriptomic comparison. (A) RNA sequencing data from IL-1β or TNF-*α* treated tissue engineered cartilage pellets, which were named as pellet.IL and pellet.TNF separately; (B) RNA sequencing data from mouse IL-1β treated primary chondrocytes, which was named as mchondro.IL; (C) RNA sequencing data from IL-1β or TNF-*α* human osteoarthritic cartilage, which were named as OAcartilage.IL and OAcartilage.TNF; (D) RNA sequencing data from human OA cartilage, which were named as cartilage.OA. 6
Figure 2. Venn diagrams showing shared differentially expressed genes (DEGs). (A) Shared DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL), murine primary chondrocytes (mchondro.IL) and human osteoarthritic cartilage (OAcartilage.IL), as well as non-treated human osteoarthritic cartilage (cartilage.OA). 23.9% (427 (=223+110+41+64) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are also dysregulated in mchondro.IL; 23.4% (417 (=188+110+41+78) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are differentially expressed in OAcartilage.IL; 22.0% (392 (=209+78+41+64) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are differentially expressed in cartilage.OA. (B) Shared DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (OAcartilage.TNF), as well as non-treated human osteoarthritic cartilage (cartilage.OA). 21.7% (255(=171+84) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF are shared between OAcartilage.TNF and pellet.TNF. 21.2% % (249(=165+84) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF are also detected as dysregulated genes in cartilage from OA patients. (C) Shared DEGs pellet.IL, pellet.TNF, OAcartilage.IL, and OAcartilage.TNF. 47.7% (560(=370+48+118+24) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF overlap with DEGs in pellet.IL, and 72.4% (1134(=788+78+118+150) overlapping DEGs / 1565 total DEGs in OAcartilage.TNF) of DEGs in OAcartilage.TNF overlap with DEGs in OAcartilage.IL. 10
Figure 3. Venn diagrams showing number of overlapping upregulated (+) or downregulated (-) differentially expressed genes (DEGs) between different models. (**A**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL). (**B**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL). (**C**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA). (**D**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-*α* treated human osteoarthritic cartilage (OAcartilage.TNF). (**E**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (cartilage.OA). 11
Figure 4. Top gene ontology (GO) terms and pathways identified by functional enrichment analysis through Enrichr and GO Biological Process 2018, (top 10 ranked by P value). (**A**) Upregulated (+) and downregulated (-) differentially expressed genes (DEGs) in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) (**B**) Upregulated (+) and downregulated (-) DEGs in IL-1β treated murine primary chondrocytes (mchondro.IL). (**C**) Upregulated (+) and downregulated (-) DEGs in IL-1β treated human osteoarthritic cartilage (OAcartilage.IL). (**D**) Upregulated (+) and downregulated (-) DEGs in human osteoarthritic cartilage (cartilage.OA).

(**E**) Upregulated (+) or downregulated (-) DEGs in TNF-α treated tissue-engineered cartilage pellets (pellet.TNF). (**F**) Upregulated (+) or downregulated (-) DEGs in TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF). 23
Figure 6. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING DEGs common to both IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL), interaction score ≥ 0.90, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure. 25
Figure 7. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING DEGs common to both IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA), interaction score ≥ 0.70, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure. 26
Figure 8. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING. DEGs common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF), interaction score ≥ 0.90, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure. 27
Figure 9. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING. DEGs common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (cartilage.OA), interaction score ≥ 0.70, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure. 28
Figure 10. OA pathway generated from the common genes and enriched pathways in OAcartilage.IL, pellet.IL, and cartilage.OA, together with the common genes and enriched pathways in OAcartilage.TNF, pellet.TNF, and cartilage.OA. **(A)** Common genes – GO:BioProcess network of OA. **(B)** log2 fold change of genes in the network. 29

Abstract of Thesis

Transcriptomic analysis of cytokine-treated tissue-engineered cartilage as an in vitro model of osteoarthritis

By

Rachel (Jiehan) Li

Master of Science in Biomedical Engineering

Washington University in St. Louis, 2020

Research Advisor: Professor Farshid Guilak

Osteoarthritis (OA), as the most common form of arthritis and a leading cause of disability worldwide, currently has no disease-modifying drugs. Inflammation plays an important role in cartilage degeneration in OA, and pro-inflammatory cytokines, IL-1β and TNF-α, have been shown to induce degradative changes along with aberrant gene expression in chondrocytes, the only resident cells in cartilage. The goal of this study was to further understand the transcriptomic regulation of tissue-engineered cartilage in response to inflammatory cytokines using an *in vitro* miPSC model system. We performed RNA sequencing for the IL-1β or TNF-α treated tissue-engineered cartilage derived from murine iPSCs, and analyzed transcriptomic profiles by comparing with those of two different osteoarthritis models and human OA cartilage samples. We investigated differentially expressed genes (DEGs) as well as gene set enrichment and protein-protein interaction network, showing a significant similarity between model systems and human OA cartilage. Our analysis revealed a significant number of overlapping DEGs, together with consistent pathway enrichment in inflammatory response, cytokine-mediated response and extracellular matrix organization, which support that the murine iPSC model system can replicate

many of the characteristics of OA cartilage at the transcriptomic level, specifically in the catabolic aspect of inflammation induce OA. The murine iPSC model system provides a method for studying the pro-inflammatory response and pathogenesis in OA cartilage, and will be a valuable dataset for identifying therapeutic targets of inflammation induced OA.

Chapter 1:

Introduction

Osteoarthritis (OA) is the most common form of arthritis and a leading cause of disability worldwide. OA is a chronic joint disease that is characterized not only by degeneration and calcification of cartilage, but also by subchondral bone sclerosis, osteophyte formation, and joint inflammation, which lead to symptoms of joint pain, stiffness, and loss of mobility and flexibility in OA patients (Goldring & Goldring, 2010). Over 10% of men and 13% of women aged 60 and over are diagnosed with knee OA (Murphy et al., 2008). In 2015, it was estimated that 30.8 million adults suffer from osteoarthritis in the United States (Cisternas et al., 2016). However, there are no cure of OA currently (W. Zhang, Robertson, Zhao, Chen, & Xu, 2019). Further insight in transcriptomic profile of osteoarthritic cartilage is critical to development of therapeutic treatment. This thesis value the capability of murine iPSCs derived tissue-engineered cartilage to replicate OA environment. In this thesis, total RNA of murine iPSCs derived tissue-engineered cartilage in response of pro-inflammation cytokines were extracted, sequenced and analyzed. Further transcriptomic comparison of iPSC OA model, IL-1β treated murine chondrocytes, IL-1β and TNF-α treated human OA cartilage, and untreated human OA cartilage were done with the RNA sequencing data from published online datasets and the RNA seq result of the iPSC OA model generated in our lab.

1.1 Current Treatment of OA

Current treatment of OA is still mainly limited to pain relievers and anti-inflammatory drugs, which may alleviate symptoms, but cannot restore the joint function (W. Zhang et al., 2019). Traditional medications for OA include paracetamol, nonsteroidal anti-inflammatory drugs, and

opiate analgesics; however, they are limited and only show moderate treatment effects with potentially adverse side effects and toxicities (Meek, Van de Laar, & H, 2010; W. Zhang et al., 2019). No disease-modifying osteoarthritis drugs (DMOADs) are currently available, which in part is due to the fact that the development of DMOADs are limited by the understanding of this complex chronic joint disease, as well as the lack of good *in vitro* disease models for drug screening.

1.2 Inflammation in OA

OA has many risk factors such as aging, joint injury or trauma, genetics, obesity, metabolic disorders and inflammation. Pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), have been shown to play an important role in OA (Goldring & Otero, 2011). These pro-inflammatory cytokines are elevated in synovium, chondrocytes and other surrounding tissue and induce aberrant expression of catabolic and inflammation-related genes in an OA joint, which also prevents articular cartilage repair. IL-1β and TNF-α induce cartilage degradation and inflammation by activating NF-*κ*B signaling, which increases matrix metalloproteinases and aggrecanase expression, nitric oxide (NO) and prostaglandin E synthase 2 (PGE2) synthesis, and reduces production of extracellular matrix (ECM) components(Goldring & Marcu, 2009; Studer, Jaffurs, Stefanovic-Racic, Robbins, & Evans, 1999; Wojdasiewicz, Poniatowski, & Szukiewicz, 2014). A number of anti-inflammatory agents have been developed as potential therapies for OA or as an approach to inhibit inflammatory effects on cartilage tissue (Alten et al., 2008; Cohen et al., 2011; Guler-Yuksel et al., 2010; Magnano et al., 2007; Verbruggen, Wittoek, Vander Cruyssen, & Elewaut, 2012). Although these anti-inflammatory mediators show promising effects in murine cartilage, most of them failed in clinical trials of human patients with OA due to limited efficacy and no significant treatment effects compared to

placebo. Thus, it is critical to further understand the molecular mechanisms in OA cartilage using disease models for the development of therapeutic interventions.

1.3 Current Models of OA

Common animal models used for OA are the knee joints. Models can be classified by primary OA (spontaneous models) or Secondary OA (post-trauma and other causes). Primary OA models closely simulate the natural progression of osteoarthritis development, while Secondary OA models are conditionally induced osteoarthritis to study specific causes or risk factors of OA. Secondary OA models can be further classified by surgical or chemical induced (Kuyinu, Narayanan, Nair, & Laurencin, 2016). Anterior Cruciate Ligament Transection (ACLT) and Destabilization of the Medial Meniscus (DMM) are two common methods widely used in OA research, as they are highly reproducible and progress rapidly. Toxic or inflammatory compounds are also used in OA research for generated chemically induced OA model (Kuyinu et al., 2016).

In vitro models are essential for drug development for their high tractability, convivence and rapid progression. Besides mechanical loading, cytokine induction has also been heavily studied in OA. Two pro-inflammatory cytokines, IL-1β and TNF-α, are the most common components used to generated OA-like process in cartilage tissue or chondrocytes (Johnson, Argyle, & Clements, 2016).

1.3 iPSC Model System in OA

Current *in vitro* models involve treating isolated cells or cartilage explants with cytokines such as IL-1β or TNF-α (Johnson et al., 2016; Kuyinu et al., 2016). These approaches have provided important information for the differences in OA compared to healthy tissues, however they lack

an abundant source of cells or tissues with a controlled genetic background, which is necessary for high-throughput DMOAD screening. Induced pluripotent stem cells (iPSCs) can aid high-throughput drug screening due to their capacity for extensive expansion as well as consistent differentiation and genetic tractability (Engle & Puppala, 2013). In previous work, our lab developed a rigorous protocol to generate tissue-engineered cartilage from murine iPSCs (miPSCs) (Diekman et al., 2012). Here, we treated tissue-engineered cartilage pellets differentiated from miPSCs with IL-1β or TNF-α to recapitulate OA environment and investigated transcriptomic profile of the cytokine treated pellets with RNA sequencing.

Chapter 2:

Transcriptomic Analysis of Cytokine-Treated Tissue-Engineered Cartilage as an *In Vitro* Model of Osteoarthritis

2.1 Introduction

The goal of this study was to further understand the transcriptomic regulation of tissue engineered cartilage in response to inflammatory cytokines using an *in vitro* miPSC model system. Previous studies (Willard et al., 2014) have shown that some of the characteristics of OA are recapitulated in murine iPSCs, but the overall effects of IL-1β and TNF-α on the transcriptome are unknown in comparison to OA cartilage. Thus, we examined the transcriptomic profile of IL-1β or TNF-α stimulated miPSCs derived tissue-engineered cartilage (pellet.IL or pellet.TNF, **Figure 1A**), and compared it to publicly available data sets from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database and SkeletalVis: (1) IL-1β treated murine chondrocytes (mchondro.IL, **Figure 1B**), (2) IL-1β or TNF-α treated human OA cartilage (OAcartilage.IL or OAcartilage.TNF, **Figure 1C**), and (3) untreated human OA cartilage (cartilage.OA, **Figure 1D**). Differentially expressed genes and gene set enrichment analysis were performed and were used to evaluate the ability of this *in vitro* culture system to replicate the osteoarthritis cartilage. Furthermore, the transcriptomic analysis comparison among different OA models provided valuable information on target genes and pathways, as well as insights into the molecular and cellular mechanisms of inflammation in OA.

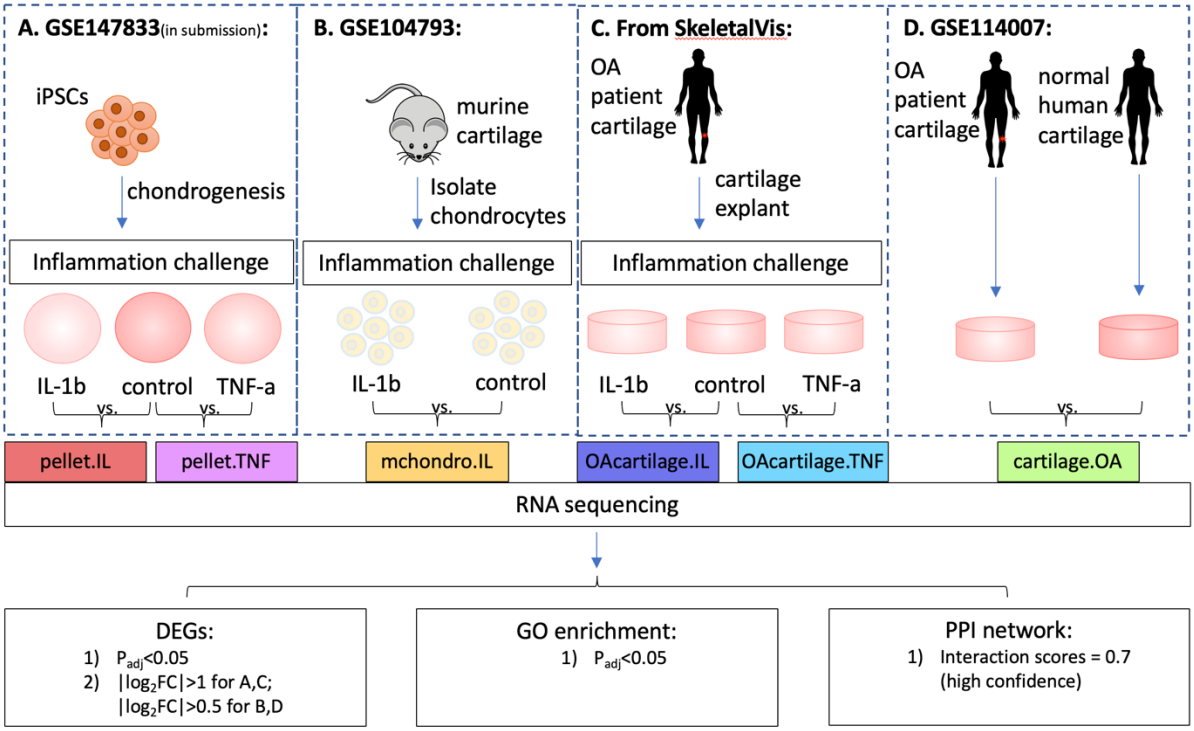


Figure 1. Flow chart and overview of 4 datasets used in transcriptomic comparison. (A) RNA sequencing data from IL-1β or TNF-α treated tissue engineered cartilage pellets, which were named as pellet.IL and pellet.TNF separately; (B) RNA sequencing data from mouse IL-1β treated primary chondrocytes, which was named as mchondro.IL; (C) RNA sequencing data from IL-1β or TNF-α human osteoarthritic cartilage, which were named as OAcartilage.IL and OAcartilage.TNF; (D) RNA sequencing data from human OA cartilage, which were named as cartilage.OA.

2.2 Materials and Methods

2.2.1 RNA-Sequencing of Tissue-Engineered Cartilage

Murine iPSCs were chondrogenically differentiated following an established protocol from our lab (Diekman et al., 2012) and were given 1ng/ml IL-1β, 20ng/ml TNF-α, or control media without cytokines for 72 hours (Figure 1A). Total cellular RNA was isolated with Total RNA Purification Plus Kit (Norgen) for mRNA sequencing. Three biological replicates for each condition were sequenced on Illumina HiSeq3000 (n=3) (Ross et al., 2020). The IL-1β treated iPSC derived cartilage was designated pellet.IL, while the TNF-α treated group was named pellet.TNF (Figure 1A).

2.2.2 RNA Sequencing Data Information

Gene expression datasets from IL-1β treated murine chondrocytes (GSE104793) and human OA patient cartilage (GSE114007) were obtained from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo). In the dataset GSE104793, primary mouse articular chondrocytes were isolated from mouse cartilage tissue and cultured with 1 ng/ml IL-1β or control media for 24 hours before cellular RNA isolation and mRNA sequencing (Son et al., 2017). This group was called mchondro.IL in **Figure 1B** and the following part of this paper.

Human OA cartilage mRNA expression profiles in response to cytokine treatment were obtained from SkeletalVis (http://phenome.manchester.ac.uk/)(Jamie Soul, Boot-Handford, Schwartz, & University of, 2017; J. Soul, Hardingham, Boot-Handford, & Schwartz, 2019). OA cartilage explants were harvested from 3 patients with OA at total knee replacement and cultured

with serum free Dulbecco's modified Eagle's medium (DMEM) for 3 days before treated with 0.1ng/ml IL-1β, 10ng/ml TNF-α, or control media for 72 hours before cellular RNA isolation and mRNA sequencing (Jamie Soul et al., 2017) **(Figure 1C)**. The IL-1β treated human OA cartilage was named OAcartilage.IL, and the TNF-α treated OA cartilage was named OAcartilage.TNF in this paper.

In dataset GSE114007, cartilage samples were harvested from the knee cartilage of 20 patients with OA and compared to 18 cartilage samples from normal patients without any joint disease or trauma (Fisch et al., 2018). This group of mRNA sequencing result was named cartilage.OA in **Figure 1D** and in the following comparisons.

2.2.3 Data Preprocessing and DEG Screening

The limma package in R was used for differential expression analysis. To find DEGs (differentially expressed genes), we performed Benjamini-Hochberg multiple testing correction and the cutoff values for DEG identification were adjusted such that the adjusted P-value was less than 0.05 ($P_{adj} < 0.05$) and the absolute value of $\log_2$(fold change) (Log2FC) was set as greater than 1 ($|Log2FC| > 1$) for cytokine treated pellets and untreated human OA cartilage. For cytokine treated human OA cartilage and IL-1β treated murine chondrocytes, genes were selected with $|Log2FC| > 0.5$. Murine gene IDs were mapped to homologous human genes with Mouse Genome Database (MGD) at the Mouse Genome Informatics website for comparisons between human and mouse transcriptomic profiles.

2.2.4 Functional and Pathway Analysis

Gene set enrichment analysis was performed through the Enrichr web-based tool with GO categories “Biological process 2018” (https://amp.pharm.mssm.edu/Enrichr/)(Chen et al., 2013; Kuleshov et al., 2016). This analysis was used to provide information about the biological processes that are significantly enriched in upregulated or downregulated DEGs. A standard FDR cutoff of 0.05 was used as the cut-off of GO term enrichment.

2.2.5 PPI Network Integration

To investigate the protein-protein interactions (PPI), the STRING database (version 11.0) was used to construct functional protein association networks. Interaction scores more than 0.70 (high confidence) were defined as significant. Both up-regulated overlapping DEGs and down-regulated overlapping DEGs were investigated through STRING, to analysis the major cellular function with the predicted functional protein-protein interactions.

2.3 Results

2.3.1 Differentially Expressed Genes in Cytokine Treated OA Models and OA Cartilage From Patients

The analysis of differentially expressed genes was performed with the 4 groups of data separately, and the mouse and human cartilage gene expression datasets shared 11613 orthologue gene groups. In pellet.IL, 1781 (849 up-regulated and 932 down-regulated) genes were detected as differentially expressed genes (DEGs) after treated with IL-1β; while in pellet.TNF, 1174 (635 up-regulated and 512 down-regulated) genes were detected as DEGs after treated with TNF-α. In mouse mchondro.IL, 1914 (857 up-regulated and 1057 down-regulated) gene were classified as DEGs. In OAcartilage.IL, 1974 (812 up-regulated and 1162 down-regulated) were detected as

DEGs, and in OAcartilage.TNF 1565 (780 up-regulated and 785 down-regulated) genes were detected as DEGs. In cartilage.OA, 1687 (1066 up-regulated and 621 down-regulated) DEGs are found. Gene names of DEGs for each model and OA cartilage are listed in **Supplemental File S1**.

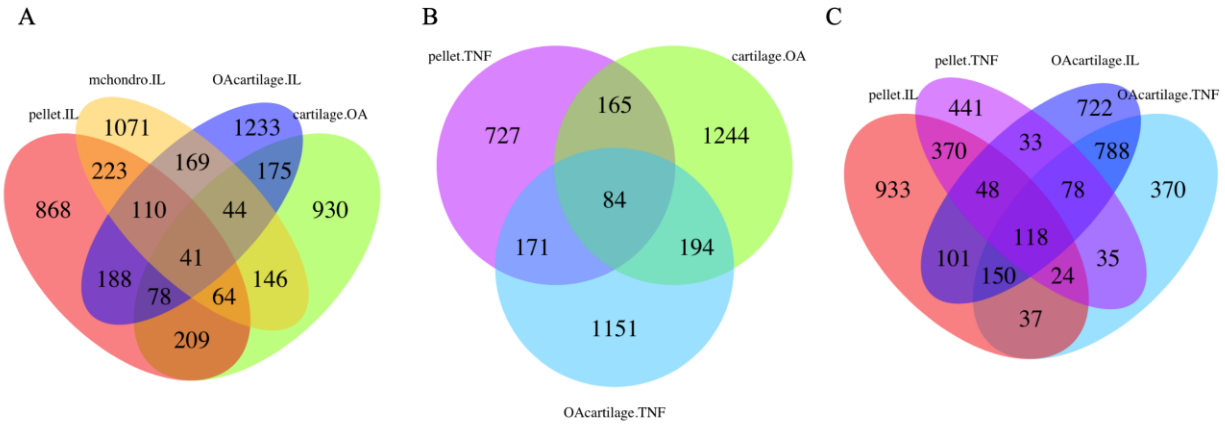


Figure 2. Venn diagrams showing shared differentially expressed genes (DEGs). (A) Shared DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL), murine primary chondrocytes (mchondro.IL) and human osteoarthritic cartilage (OAcartilage.IL), as well as non-treated human osteoarthritic cartilage (cartilage.OA). 23.9% (427 (=223+110+41+64) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are also dysregulated in mchondro.IL; 23.4% (417 (=188+110+41+78) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are differentially expressed in OAcartilage.IL; 22.0% (392 (=209+78+41+64) overlapping DEGs / 1781 total DEGs in pellet.IL) of DEGs in pellet.IL are differentially expressed in cartilage.OA. (B) Shared DEGs in TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (OAcartilage.TNF), as well as non-treated human osteoarthritic cartilage (cartilage.OA). 21.7% (255(=171+84) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF are shared between OAcartilage.TNF and pellet.TNF. 21.2% % (249(=165+84) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF are also detected as dysregulated genes in cartilage from OA patients. (C) Shared DEGs pellet.IL, pellet.TNF, OAcartilage.IL, and OAcartilage.TNF. 47.7% (560(=370+48+118+24) overlapping DEGs / 1174 total DEGs in pellet.TNF) of DEGs in pellet.TNF overlap with DEGs in pellet.IL, and 72.4% (1134(=788+78+118+150) overlapping DEGs / 1565 total DEGs in OAcartilage.TNF) of DEGs in OAcartilage.TNF overlap with DEGs in OAcartilage.IL.

Venn diagrams were used to show the number of shared and different DEGs in groups. Each model showed more than 20% of shared DEGs compared to other models and OA cartilage

samples **(Figure 2A, B)**, indicating that these models partially replicate the DEGs from human OA cartilage. Comparing between different cytokine treatments shows that 47.7% of genes in the murine iPSC model dysregulated after TNF-α treatment were also differentially expressed with IL-1β treatment, while 72.4% of DEGs in TNF-α treated human OA cartilage were shared with DEGs responding to IL-1β treatment **(Figure 2C)**.

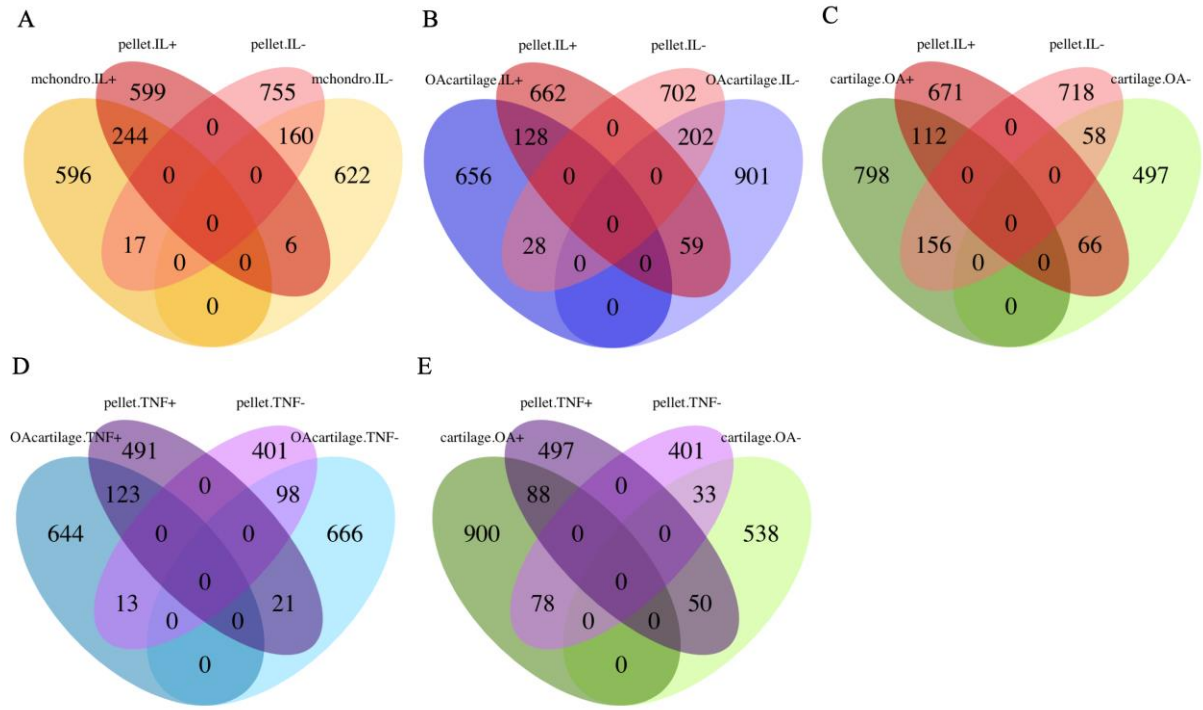


Figure 3. Venn diagrams showing number of overlapping upregulated (+) or downregulated (-) differentially expressed genes (DEGs) between different models. (**A**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL). (**B**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL). (**C**) Upregulated (+) or downregulated (-) DEGs in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA). (**D**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-*α* treated human osteoarthritic cartilage (OAcartilage.TNF). (**E**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (cartilage.OA).

To better understand the advantages and limitations of tissue-engineered cartilage as a model system, DEGs were classified by up-regulated or down-regulated and comparisons were made between cytokine treated tissue-engineered cartilage and IL-1β treated murine chondrocytes, cytokine treated human OA cartilage or untreated cartilage from OA patients.

When pellet.IL and mchondro.IL were compared, 427 overlapping DEGs were detected with high significance (p= 4.070184e-19). Among them, 404 genes were regulated in the same direction, while 23 genes were regulated in opposite direction **(Figure 3A)**. *Vnn2*, *Nos2*, *Ccl5*, *Cfb*, *Ccl20*, *Serpina3f*, *Ch25h*, *Col10a1*, and *Matn1* show the highest absolute value of Log2FC in IL-1β treated pellet among all the overlapping DEGs **(Table 1)**.

Table 1. *Top 30 dysregulated genes common to both IL-1β treated pellets (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL).*

Gene Name	Functional Annotation	Log2 Fold Change	
		pellet.IL	mchondro.IL
Vnn3	Vascular non-inflammatory molecule 3; Amidohydrolase	8.20	4.30
Nos2	Nitric oxide synthase; Produces nitric oxide (NO) and mediates cysteine S- nitrosylation of PTGS2/COX2.	6.11	6.25
Ccl5	C-C motif chemokine 5	6.10	4.33
Cfb	Complement factor B	5.46	3.77
Ccl20	C-C motif chemokine 20; Chemotaxis of dendritic cells, T-cells and B-cells	5.45	2.80
Serpina3f	Serine (or cysteine) peptidase inhibitor, clade A, member 3F	5.45	0.52
Ch25h	Cholesterol 25-hydroxylase; Regulating lipid metabolism, cell positioning and movement in lymphoid tissues	5.14	4.94
C3	Complement C3; Activation of the complement system	4.97	5.39
Orm1	Alpha-1-acid glycoprotein 1; Modulates activition the immune system during the acute-phase reaction	4.89	1.41

Orm2	Alpha-1-acid glycoprotein 2; Modulates activation the immune system during the acute-phase reaction	4.86	1.04
Tnfsf15	Tumor necrosis factor ligand superfamily member 15; Mediates activation of NF-kappa-B	4.83	0.97
Lcn2	Neutrophil gelatinase-associated lipocalin; Regulation of apoptosis, innate immunity and renal development.	4.79	2.72
Gpr84	G-protein coupled receptor 84	4.72	2.97
Tnfrsf9	Tumor necrosis factor receptor superfamily member 9	4.52	1.61
Gdnf	Glial cell line-derived neurotrophic factor; Survival and morphological differentiation of dopaminergic neurons	4.42	0.71
Tubb2b	Tubulin beta-2B chain; Major constituent of microtubules	-2.90	-1.32
Kcnt2	Potassium channel, subfamily T, member 2	-2.92	-1.21
Inhbe	Inhibin beta E chain; TGF-beta family	-3.03	-0.88
Ucma	Unique cartilage matrix-associated protein; Negative control of osteogenic differentiation	-3.13	-1.05
Ptgis	Prostacyclin synthase	-3.25	-0.64
Chad	Chondroadherin; Promotes attachment of chondrocytes, fibroblasts, and osteoblasts.	-3.31	-1.52
Cmtm5	CKLF-like MARVEL transmembrane domain containing 5	-3.34	-2.18
Frzb	Secreted frizzled-related protein 3; Antagonist of Wnt8 signaling. Regulates chondrocyte maturation and long bone development	-3.34	-1.93
Zfp648	Zinc finger protein 648	-3.35	-1.11
C1qtnf3	C1q and tumor necrosis factor related protein 3	-3.47	-3.68
Mall	MAL-like protein; T cell differentiation protein-like	-3.70	-1.08
Matn3	Matrilin-3; Major component of the extracellular matrix of cartilage	-4.90	-2.5
Omd	Osteomodulin; Involved in biomineralization	-4.99	-1.50
Col10a1	Collagen alpha-1(X) chain; Product of hypertrophic chondrocytes	-5.27	-2.52
Matn1	Cartilage matrix protein; binds to collagen	-5.79	-3.30

Similarly, when pellet.IL and OAcartilage.IL were compared, 417 overlapping DEGs were detected with high significance (p= 1.933738e-10). Among them, 330 genes were regulated in the

same direction, while 87 genes were regulated in opposite direction **(Figure 3B)**. *Saa1*, *Nos2*, *Ccl5*, *Cxcl1*, *Ccl20*, *Ch25h*, *Csf3*, *Tnip3*, *Draxin6*, *Il6*, *Col10a1*, and *Omd* show the highest absolute value of Log2FC in IL-1β treated pellet among all the overlapping DEGs **(Table 2)**. However, in comparison of mchondro.IL and cartilage.OA, 276 overlapping DEGs were detected, which is non-significant (p= 0.5694874), Among these DEGs, 94 genes were regulated in the same direction and 182 genes were regulated in the opposite direction **(**Appendix Figure 1.A**)**.

Table 2. *Top 30 dysregulated genes common to both IL-1β treated pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL).*

Gene Name	Functional Annotation	Log2 Fold Change	
		pellet.IL	OAcartilage.IL
Saa1	Serum amyloid A-1 protein; Major acute phase protein	7.76	3.03
Nos2	Nitric oxide synthase; Produces nitric oxide (NO) and mediates cysteine S- nitrosylation of PTGS2/COX2.	6.11	1.71
Ccl5	C-C motif chemokine 5	6.10	2.50
Cxcl1	neutrophil activation during inflammation	5.90	3.24
Ccl20	C-C motif chemokine 20; Chemotaxis of dendritic cells, T-cells and B-cells	5.45	3.70
Ch25h	Cholesterol 25-hydroxylase; Regulating lipid metabolism, cell positioning and movement in lymphoid tissues	5.14	2.07
Csf3	Granulocyte colony-stimulating factor	5.11	3.51
Tnip3	TNFAIP3 interacting protein 3	5.11	2.09
Draxin	Chemorepulsive axon guidance protein; an antagonist of Wnt signaling pathway	5.09	2.68
Il6	Interleukin-6	5.07	4.53
Mmp13	Collagenase 3; Degradation of extracellular matrix proteins	4.91	3.41
Lcn2	Neutrophil gelatinase-associated lipocalin; Regulation of apoptosis, innate immunity and renal development.	4.79	2.31
Slc15a3	Solute carrier family 15 member 3; Proton oligopeptide cotransporter.	4.76	1.02
Gpr84	G-protein coupled receptor 84	4.72	2.56

Tnfrsf9	Tumor necrosis factor receptor superfamily member 9	4.52	2.38
Ptgis	Prostacyclin synthase	-3.25	-1.46
Clec3a	C-type lectin domain family 3 member A; Promotes cell adhesion to laminin and fibronectin	-3.26	-5.33
Fam43b	Family with sequence similarity 43, member B	-3.26	-1.95
Chad	Chondroadherin; Promotes attachment of chondrocytes, fibroblasts, and osteoblasts.	-3.31	-4.16
Cmtm5	CKLF-like MARVEL transmembrane domain containing 5	-3.34	-2.41
Frzb	Secreted frizzled-related protein 3; Antagonist of Wnt8 signaling. Regulates chondrocyte maturation and long bone development	-3.34	-3.02
C1qtnf3	C1q and tumor necrosis factor related protein 3	-3.47	-2.02
Ogn	Mimecan; Induces bone formation in conjunction with TGF-beta-1 or TGF-beta-2	-3.60	-4.17
Cytl1	Cytokine-like protein C17	-3.62	-2.66
Pthlh	Parathyroid hormone-related protein; Regulation of endochondral bone development	-3.64	-1.53
Adamtsl2	ADAMTS-like 2	-4.02	-1.68
Nxph3	Neurexophilin-3	-4.64	-1.88
Matn3	Matrilin-3; Major component of the extracellular matrix of cartilage	-4.90	-3.94
Omd	Osteomodulin; Involved in biomineralization	-4.99	-3.75
Col10a1	Collagen alpha-1(X) chain; Product of hypertrophic chondrocytes	-5.27	-2.90

When pellet.IL and cartilage.OA were compared, 392 overlapping DEGs were detected with high significance (p= 1.075255e-20). Among them, 170 genes were regulated in the same direction, and 222 genes were regulated in opposite direction **(Figure 3C)**. *Mmp9*, *Cybb*, *C1qtnf7*, *Tnip3*, *Mmp13*, *Tnfsf15*, *Tnfrsf9*, *Gdnf*, *Tnfsf11*, *Traf3ip3*, *Gm12695*, *Adamtsl2*, and *Peg10* show the highest absolute value of Log2FC in IL-1β treated pellet among all the overlapping DEGs between IL-1β treated tissue-engineered cartilage pellet and untreated human osteoarthritic cartilage **(Table 3)**. Comparison of overlapping DEGs in OAcartilage.IL and cartilage.OA revealed a

significant overlapping (p= 0.01509899) of 336 DEGs: 142 of them were regulated in the same direction and 194 DEGs responded differently to IL-1β **(**Appendix Figure 1.B**)**.

Table 3. *Top 30 dysregulated genes common to both IL-1β treated tissue-engineered pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA).*

Gene Name	Functional Annotation	Log2 Fold Change	
		pellet.IL	cartilage.OA
Mmp9	Matrix metalloproteinase-9; Bone osteoclastic resorption	7.97	5.13
Cybb	Cytochrome b-245 heavy chain; Membrane-bound oxidase of phagocytes	5.96	3.29
C1qtnf7	C1q and tumor necrosis factor related protein 7	5.59	1.01
Tnip3	TNFAIP3 interacting protein 3	5.11	2.59
Mmp13	Collagenase 3; Degradation of extracellular matrix proteins	4.91	3.69
Tnfsf15	Tumor necrosis factor ligand superfamily member 15; Mediates activation of NF-kappa-B	4.83	5.21
Tnfrsf9	Tumor necrosis factor receptor superfamily member 9	4.52	2.06
Gdnf	Glial cell line-derived neurotrophic factor; Survival and morphological differentiation of dopaminergic neurons	4.42	2.86
Tnfsf11	Tumor necrosis factor ligand superfamily member 11; Osteoclast differentiation and activation factor.	4.13	2.01
Traf3ip3	TRAF3-interacting JNK-activating modulator; TRAF3-mediated JNK activation	4.08	1.39
S100a8	Protein S100-A8; regulation of inflammatory processes and immune response	3.83	3.75
Tnfsf8	Tumor necrosis factor ligand superfamily member 8;Induces proliferation of T-cells	3.83	2.47
Msr1	Macrophage scavenger receptor types I	3.69	3.47
Atp8b3	Phospholipid-transporting ATPase IK	3.56	1.27
Mme	Neprilysin; Destruction of opioid peptides	3.48	3.44
Myzap	Myocardial zonula adherens protein	-1.94	-2.41
Tinagl1	Tubulointerstitial nephritis antigen-like	-1.95	-1.95
Stc2	Stanniocalcin-2	-2.00	-2.42
Kcnk5	Potassium channel, subfamily K, member 5	-2.16	-1.53

Slc37a2	Glucose-6-phosphate exchanger SLC37A2	-2.26	-1.59
Gprc5a	Retinoic acid-induced protein 3; A negative modulator of EGFR signaling	-2.38	-2.20
Ndnf	Protein NDNF; Promotes matrix assembly and cell adhesiveness	-2.41	-1.06
Kcnt2	Potassium channel, subfamily T, member 2	-2.92	-1.03
Myoc	Myocilin; Negatively regulates cell-matrix adhesion and stress fiber assembly through Rho protein signal transduction	-3.12	-2.85
Ucma	Unique cartilage matrix-associated protein; Negative control of osteogenic differentiation	-3.13	-2.50
Cytl1	Cytokine-like protein C17	-3.62	-1.66
Arhgef37	Rho guanine nucleotide exchange factor 37	-3.65	-1.69
Peg10	Retrotransposon-derived protein PEG10; Inhibits the TGF-beta signaling	-3.80	-1.24
Adamtsl2	ADAMTS-like 2	-4.02	-1.90
Gm12695	C1orf87 homolog; C1orf87, calcium ion binding	-4.37	-1.22

For the TNF-α treated model, when pellet.TNF and OAcartilage.TNF were compared, 255 overlapping DEGs were detected with a highly significant value (p= 3.079532e-12). Among them, 221 genes were regulated in the same direction, while 34 genes were regulated in opposite direction **(Figure 3D)**. *Ccl5*, *Il2rg*, *Cxcl2*, *Ccl20*, *Cfb*, *Tnip3*, *Gpr84*, *Il1f9*, and *Slc15a3* show the highest absolute value of Log2FC in TNF-α treated pellet among all the overlapping DEGs **(Table 4)**.

Table 4. *Top 30 dysregulated genes common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF).*

Gene Name	**Functional Annotation**	**Log2 Fold Change**	
		pellet.TNF	OAcartilage.TNF
Ccl5	C-C motif chemokine 5	8.15	5.00
Il2rg	Cytokine receptor common subunit gamma	7.30	1.70
Cxcl2	C-X-C motif chemokine 2; Chemotactic for human polymorphonuclear leukocytes	7.03	2.25
Ccl20	C-C motif chemokine 20; Chemotaxis of dendritic cells, T-cells and B-cells	6.75	4.34

Cfb	Complement factor B	6.11	1.08
Tnip3	TNFAIP3 interacting protein 3	6.09	2.75
Gpr84	G-protein coupled receptor 84	6.07	2.30
Il1f9	Interleukin-36 gamma; an agonist of NF-kappa B activation	5.92	2.70
Slc15a3	Solute carrier family 15 member 3; Proton oligopeptide cotransporter.	5.05	1.57
Traf1	TNF receptor-associated factor 1; Adapter molecule that regulates the activation of NF-kappa-B and JNK.	4.99	2.55
Cxcl10	C-X-C motif chemokine 10; a proinflammatory cytokine	4.85	4.79
Nos2	Nitric oxide synthase; Produces nitric oxide (NO) and mediates cysteine S- nitrosylation of PTGS2/COX2.	4.78	2.06
Mmp10	Stromelysin-2; Can degrade fibronectin, gelatins and collagens. Activates procollagenase	4.74	1.94
Slc7a11	Cystine/glutamate transporter	4.63	2.21
Slc2a6	Solute carrier family 2 (facilitated glucose transporter)	4.42	2.3012152
C1qtnf3	C1q and tumor necrosis factor related protein 3	-1.88	-1.2424457
Gdf10	Growth/differentiation factor 10; Inhibits osteoblast differentiation via SMAD2/3 pathway.	-1.89	-2.5585607
Thbs2	Thrombospondin-2; Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions	-1.91	-1.62
Aqp3	Aquaporin-3; Water channel	-2.03	-0.71
Adamtsl2	ADAMTS-like 2	-2.15	-1.42
Fxyd3	FXYD domain-containing ion transport regulator 3	-2.20	-1.46
Smim5	Small integral membrane protein 5	-2.25	-1.42
Col1a1	Collagen alpha-1(I) chain; Type I collagen	-2.34	-1.37
Sv2b	Synaptic vesicle glycoprotein 2B	-2.34	-1.94
Nxph4	Neurexophilin	-2.55	-1.34
Tub	Tubby protein; Functions in signal transduction from heterotrimeric G protein-coupled receptors.	-2.82	-1.05
Mfap4	Microfibril-associated glycoprotein 4; Elastic fiber assembly andmaintenance	-3.37	-3.08
Ndufa4l2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2	-3.39	-1.62

Chrdl2	Chordin-like protein 2; negatively regulates cartilage formation/regeneration	-3.65	-2.47
Car9	Carbonic anhydrase 9; Reversible hydration of carbon dioxide. Participates in pH regulation	-4.79	-1.52

When pellet.TNF and cartilage.OA were compared, 249 overlapping DEGs were detected with a highly significant value (p= 6.401139e-15). Among them, 121 genes were regulated in the same direction, while 128 genes were regulated in opposite direction **(Figure 3E)**. In comparison of overlapping DEGs in TNF-α treated human osteoarthritic cartilage and untreated human osteoarthritic cartilage, 227 overlapping DEGs were detected with a significant value of 0.003 (p=0.003104403). Among them, 123 genes are regulated in the same direction and 154 genes respond differently to TNF-α **(**Appendix Figure 1.C**)**. *Mmp9*, *Cybb*, *Tnip3*, *Nlrp3*, *Tmem132e*, *Aldh1a2*, and *Msr1* showed the highest absolute value of Log2FC in TNF-α treated pellet among all the overlapping DEGs between pellet.TNF and cartilage.OA **(Table 5)**. The rest of the overlapping DEGs are regulated in same direction and listed in **Supplemental File S1**.

Table 5. *Top 30 dysregulated genes common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and untreated human osteoarthritic cartilage (cartilage.OA).*

Gene Name	**Functional Annotation**	**Log2 Fold Change**	
		pellet.TNF	cartilage.OA
Mmp9	Matrix metalloproteinase-9; Bone osteoclastic resorption	7.03	5.13
Cybb	Cytochrome b-245 heavy chain; Membrane-bound oxidase of phagocytes	6.90	3.29
Tnip3	TNFAIP3 interacting protein 3	6.09	2.59
Nlrp3	NACHT, LRR and PYD domains-containing protein; Innate immunity and inflammation	5.32	1.86
Tmem132e	Transmembrane protein 132E	5.04	1.41
Aldh1a2	Retinal dehydrogenase 2	4.51	1.13

Msr1	Macrophage scavenger receptor types I	4.28	3.47
Gdnf	Glial cell line-derived neurotrophic factor; Survival and morphological differentiation of dopaminergic neurons	3.71	2.86
Vegfc	Vascular endothelial growth factor C; Angiogenesis and endothelial cell growth	3.65	1.98
Ptges	Prostaglandin E synthase; Catalyzes the oxidoreduction of PGH2 to PGE2	3.31	1.77
Adtrp	Androgen-dependent TFPI-regulating protein	3.20	1.97
Tnfrsf9	Tumor necrosis factor receptor superfamily member 9	3.16	2.06
S100a8	Protein S100-A8; regulation of inflammatory processes and immune response	3.09	3.75
AA467197	Normal mucosa of esophagus-specific gene 1 protein	3.08	2.73
C1qtnf7	C1q and tumor necrosis factor related protein 7	3.00	1.01
Pfkfb3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	-1.51	-2.38
Pdk1	Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1; regulation of glucose and fatty acid metabolism	-1.51	-1.53
Vit	Vitrin; Promotes matrix assembly and cell adhesiveness	-1.54	-2.24
Necab3	N-terminal EF-hand calcium-binding protein 3	-1.54	-1.25
Ramp1	Receptor activity-modifying protein 1; receptor for calcitonin-gene-related peptide (CGRP)	-1.54	-1.50
Egln3	Egl nine homolog 3; DNA damage response	-1.67	-1.74
Stc2	Stanniocalcin-2	-1.69	-2.42
Peg10	Retrotransposon-derived protein PEG10; Inhibits the TGF-beta signaling	-1.74	-1.24
Pck1	Phosphoenolpyruvate carboxykinase, cytosolic [GTP]	-1.95	-2.62
Irf4	Interferon regulatory factor 4; Transcriptional activator	-1.99	-2.20
Bnip3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; Apoptosis-inducing protein that can overcome BCL2 suppression.	-2.13	-1.91
Adamtsl2	ADAMTS-like 2	-2.15	-1.90
Slc16a3	Monocarboxylate transporter 4	-2.50	-1.20
Apln	Apelin; modulate immune responses in neonates	-2.74	-1.08
Ankrd37	Ankyrin repeat domain 37	-3.02	-2.24

We also compared DEGs in response of different cytokines treatment, IL-1β or TNF-α. In iPSC model system, 560 overlapping genes were detected with a highly significant value (p= 7.52312e-181). Among them, 487 genes were regulated in the same direction while 73 genes were regulated in the opposite direction. In IL-1β and TNF-α treated human osteoarthritic cartilage, there were 1105 overlapping DEGs, and all of them were in the same direction **(**Appendix Figure 1.D, E).

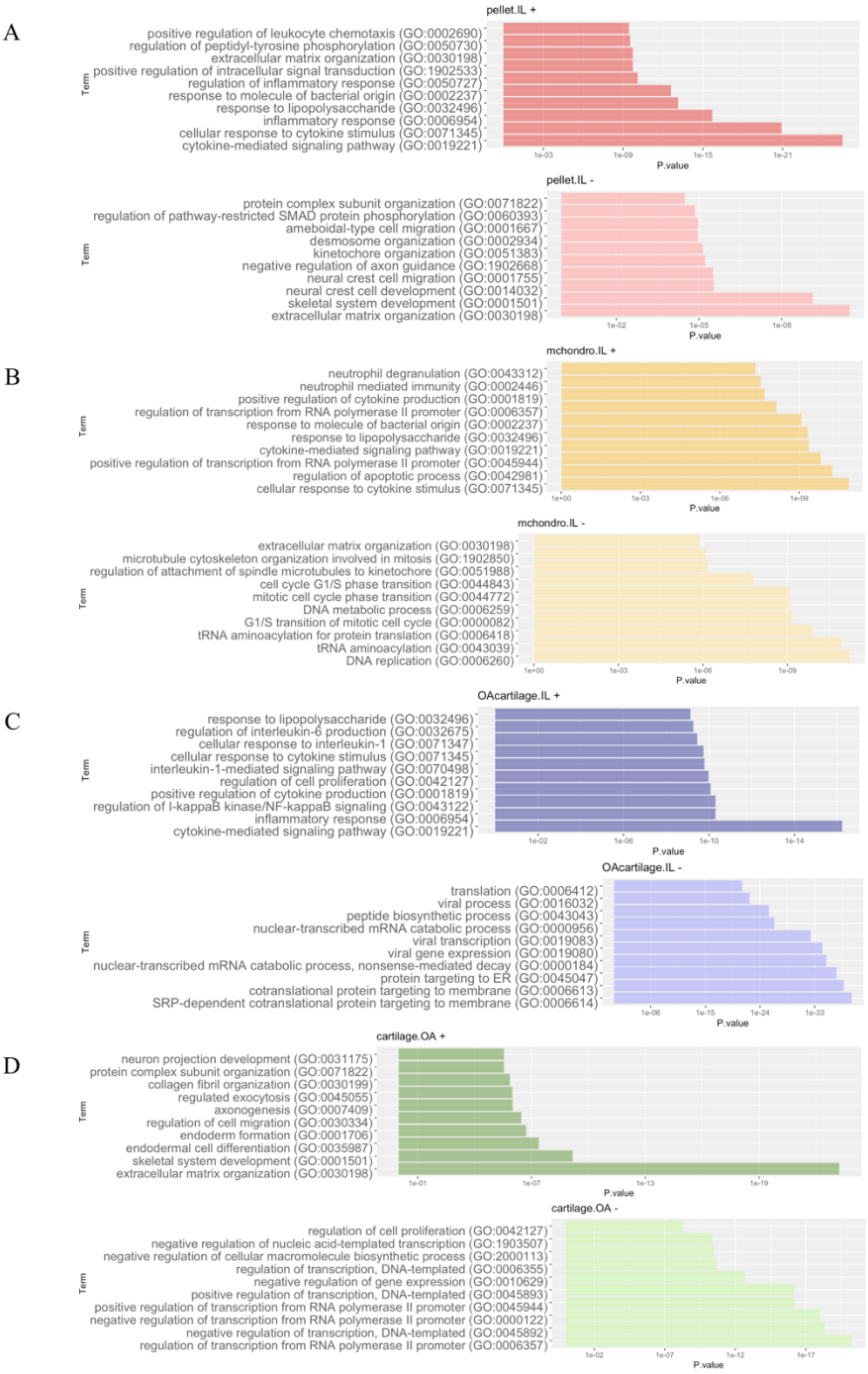
2.3.2 Pathway Analysis in Cytokine-Treated OA Model and OA Cartilage From Patients

To gain further insight into the shared and differentially expressed biological processes and mechanisms, gene set enrichment analysis was performed using the up-regulated and down-regulated DEGs. In total, each group of DEGs generated more than 20 significant GO terms, most of which were involved in biological processes related to extracellular matrix organization, cytokines stimuli, and inflammatory response. Annotated GO terms were ranked by P value, and terms with lowest P value in each group of DEGs were shown in **Figure 4**. The whole table of annotated GO term for each model and OA cartilage are provided in **Supplemental File 2**.

Pathways related to cellular response to various stimuli, such as "GO: 0019221 Cytokine-mediated signaling pathway", "GO: 0071345 Inflammatory response", and "GO: 0032496 Response to lipopolysaccharide" were generally annotated in up-regulated DEGs of all cytokine treated models and untreated OA cartilage, while they were only highly ranked in cytokine treated models **(Figure 4 and Supplemental File 2)**. Overlapping genes in these terms included *Ccl5, Ccl20, Chad, Lif, Nfkbia, Ripk2, Sod2, Tnfaip3,* and *Tnfrsf9* et al **(Appendix Table 1, 2)**.

Cartilage related biological processes, such as "GO: 0030198 Extracellular matrix organization" and "GO:0001501 Skeletal system development" were generally annotated in most groups.

However, “GO: 0030198 Extracellular matrix organization” was only annotated in up-regulated DEGs rather than down-regulated DEGs of OA cartilage **(Figure 2 and Supplemental File 2)**. Overlapping genes in these terms included collagen family, Hapln1, matrilin family, and Sox9, et al (Appendix Table 1, 2).



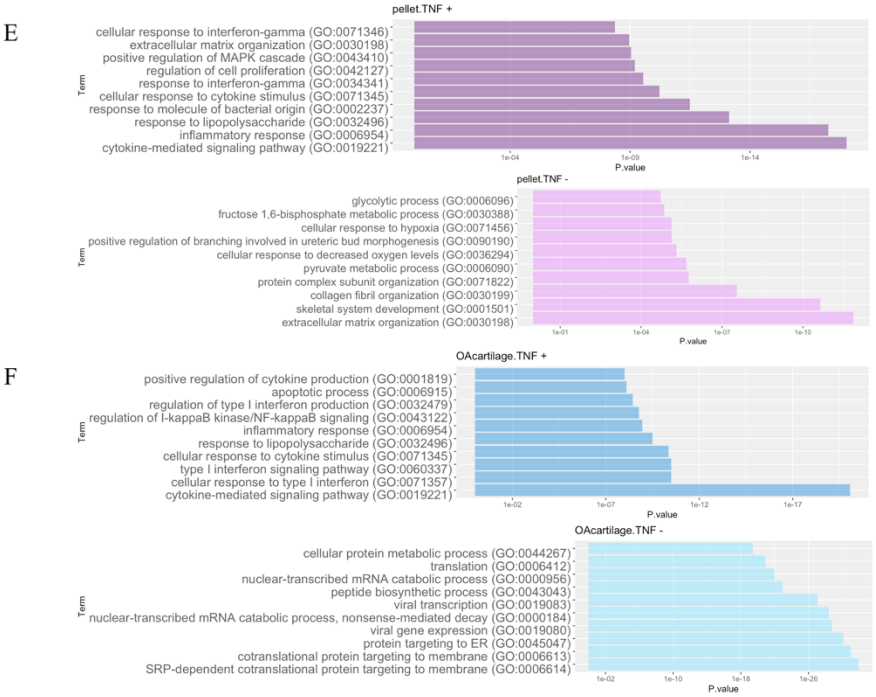


Figure 4. Top gene ontology (GO) terms and pathways identified by functional enrichment analysis through Enrichr and GO Biological Process 2018, (top 10 ranked by P value). (**A**) Upregulated (+) and downregulated (-) differentially expressed genes (DEGs) in IL-1β treated tissue-engineered cartilage pellets (pellet.IL) (**B**) Upregulated (+) and downregulated (-) DEGs in IL-1β treated murine primary chondrocytes (mchondro.IL). (**C**) Upregulated (+) and downregulated (-) DEGs in IL-1β treated human osteoarthritic cartilage (OAcartilage.IL). (**D**) Upregulated (+) and downregulated (-) DEGs in human osteoarthritic cartilage (cartilage.OA). (**E**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated tissue-engineered cartilage pellets (pellet.TNF). (**F**) Upregulated (+) or downregulated (-) DEGs in TNF-*α* treated human osteoarthritic cartilage (OAcartilage.TNF).

2.3.3 PPI Network in Cytokine Treated OA Model and OA Cartilage From Patients

To further examine the common DEGs that characterize OA models and OA cartilage samples, protein-protein interaction (PPI) networks for common genes shared by different models and OA cartilage were established through STRING with interaction score ≥ 0.70 (high confidence), with all disconnected nodes are hidden in the network. Reactome pathway assessment was also done in STRING to reveal the significant biological pathways in overlapping DEGs.

For common DEGs that were co-directionally regulated in IL-1β treated tissue-engineered cartilage pellet (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL), there were mainly 4 clusters of genes in the PPI network **(Figure 5)**. These included clusters of genes that were related to the extracellular matrix (Collagen family, Itga10 et al.), cell cycle and mitosis (*Ccna2*, *Ube2c*, *Nuf2*, *Spc25* et al.), the immune system (H-2 class I histocompatibility complex, Orosomucoid, *Cd14*, *Ikbke*, et al.), and signal transduction (C-C Motif Chemokine Ligands, *Kng1*, *C3*, *Gng2*, *Ptger4* et al.).

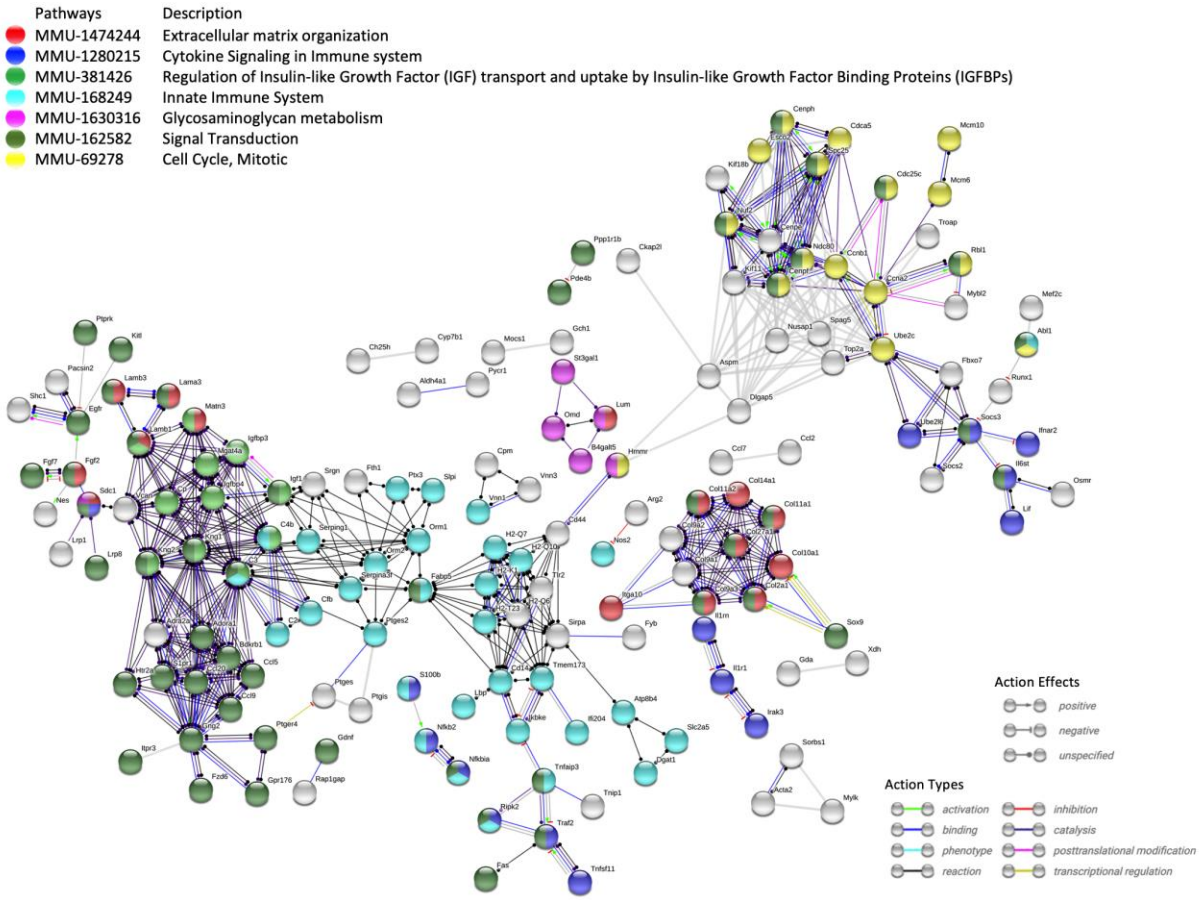


Figure 5. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING. DEGs common to both IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated murine primary chondrocytes (mchondro.IL), interaction score ≥ 0.90, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure.

For co-directionally regulated overlapping genes in IL-1β treated tissue-engineered cartilage pellet (pellet.IL) and IL-1β treated human OA cartilage (OAcartilage.IL), the cluster of extracellular matrix related genes was maintained, while the scale of signal transduction related genes cluster was reduced. Groups of NF-κB signal related genes and glycosaminoglycan metabolism proteins were shown with more interconnections **(Figure 6)**.

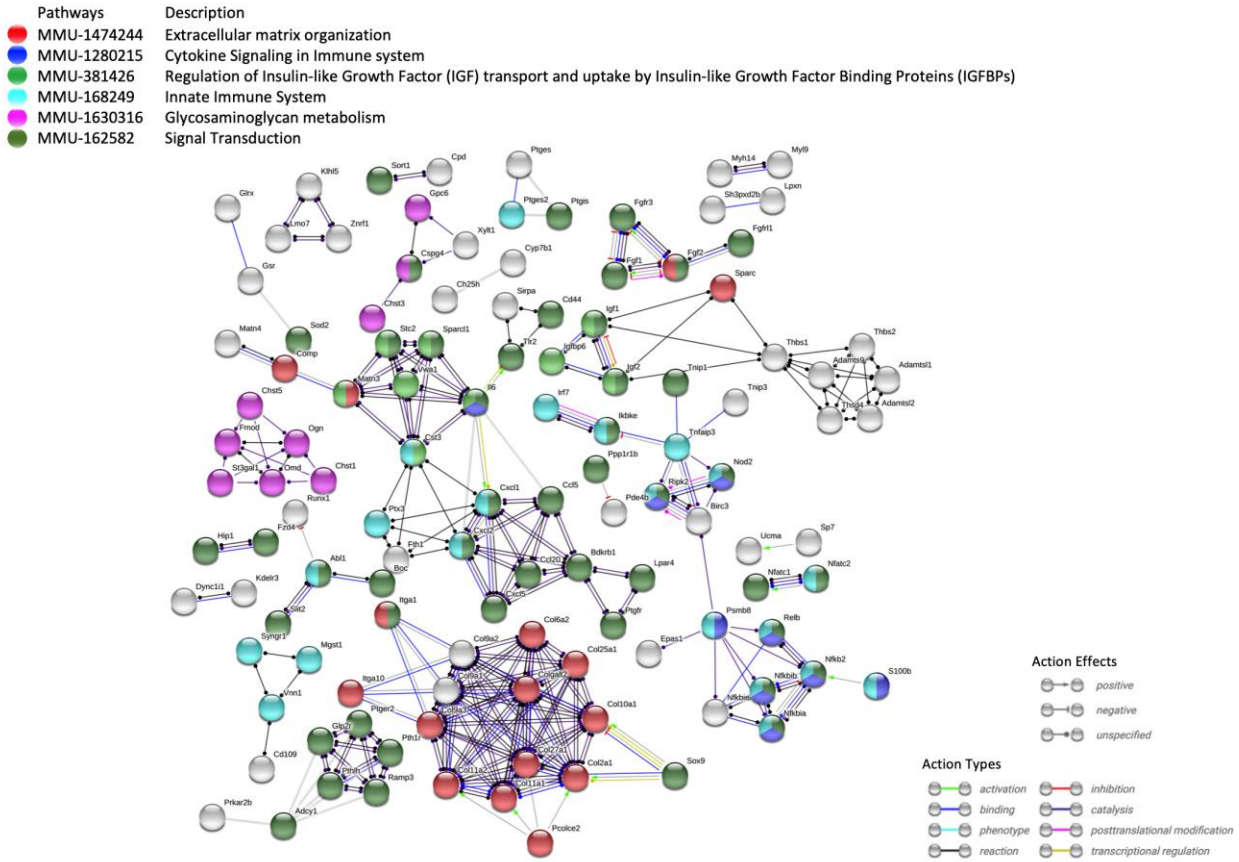


Figure 6. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING DEGs common to both IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and IL-1β treated human osteoarthritic cartilage (OAcartilage.IL), interaction score ≥ 0.90, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure.

There were fewer overlapping genes in IL-1β treated tissue-engineered cartilage pellet (pellet.IL) and untreated human OA cartilage (cartilage.OA), and the PPI network lost the extracellular matrix related cluster as the collagen family genes were regulated in different directions **(Figure 7)**. However, groups of ADAMTS and matrix metalloproteinase were shown in the network.

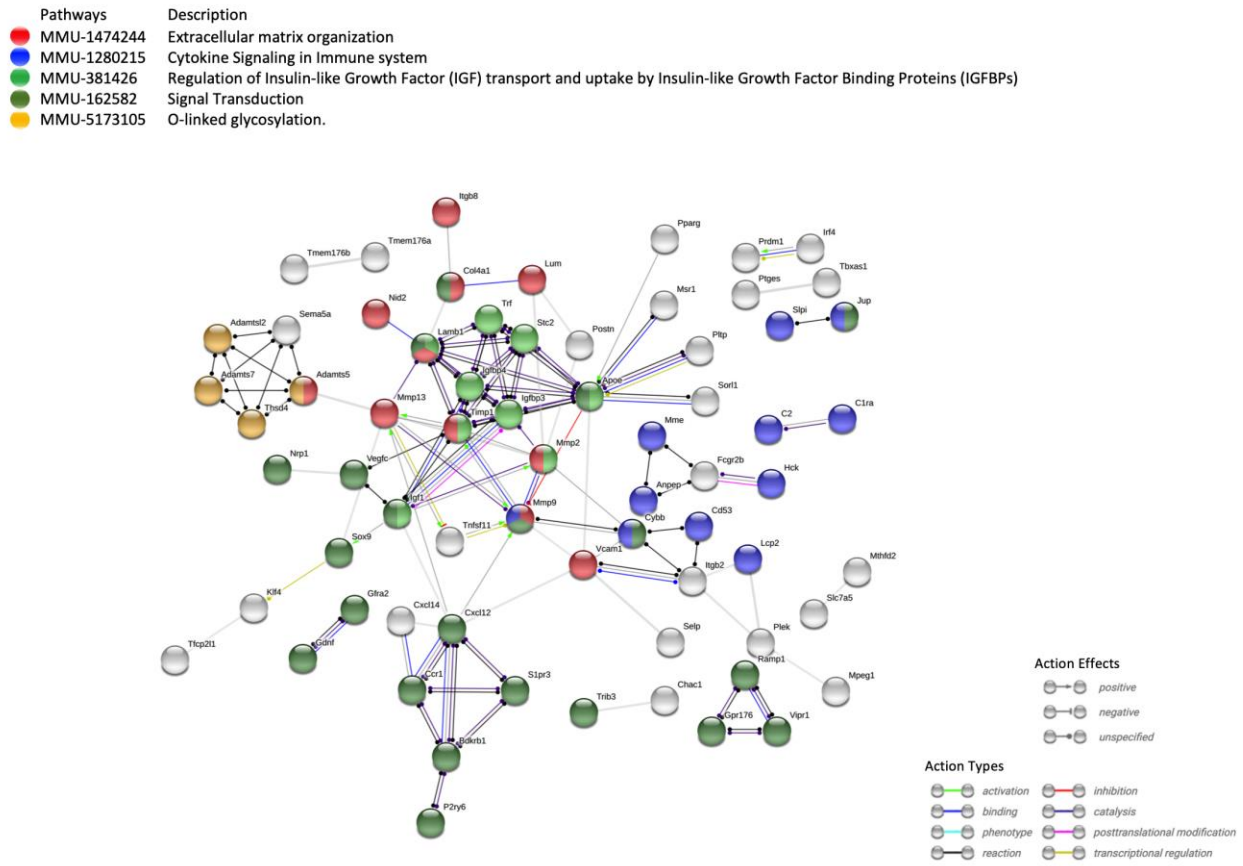


Figure 7. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING DEGs common to both IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and human osteoarthritic cartilage (cartilage.OA), interaction score ≥ 0.70, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure.

Similarly, in the PPI network for the TNF-α treated tissue-engineered cartilage pellet (pellet.TNF) and TNF-α treated human OA cartilage (OAcartilage.TNF), there was a compact

cluster of extracellular matrix related genes and small clusters of signal transduction related genes, mainly C-C Motif Chemokine Ligands and NF-κB signal related genes **(Figure 8)**.

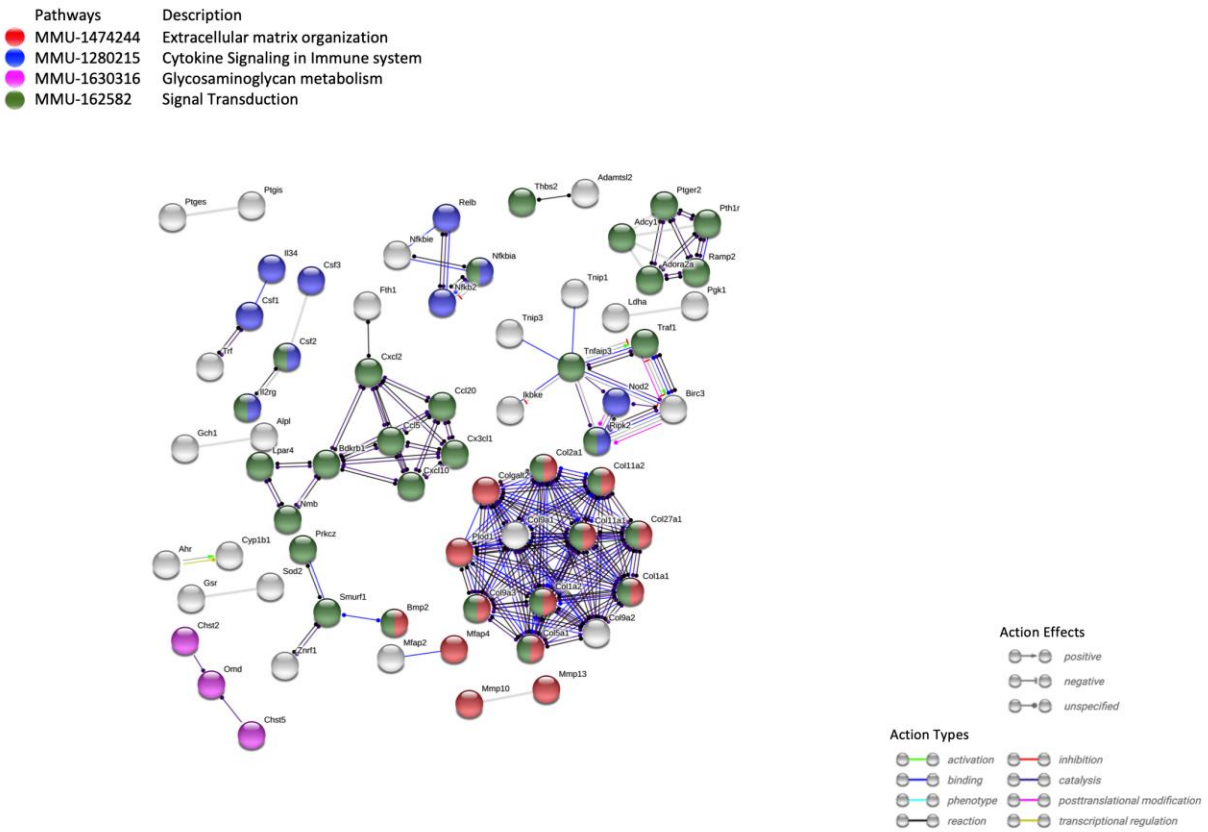


Figure 8. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING. DEGs common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF), interaction score ≥ 0.90, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure.

The reactome pathway assessment of co-directionally regulated overlapping genes network in TNF-α tissue-engineered cartilage pellet (pellet.TNF) and untreated human OA cartilage (cartilage.OA) revealed that matrix metalloproteinase and vascular endothelial growth factor were the central of the network, while no collagen family or NF-κB signaling pathway were found with reactome pathway assessment **(Figure 9)**.

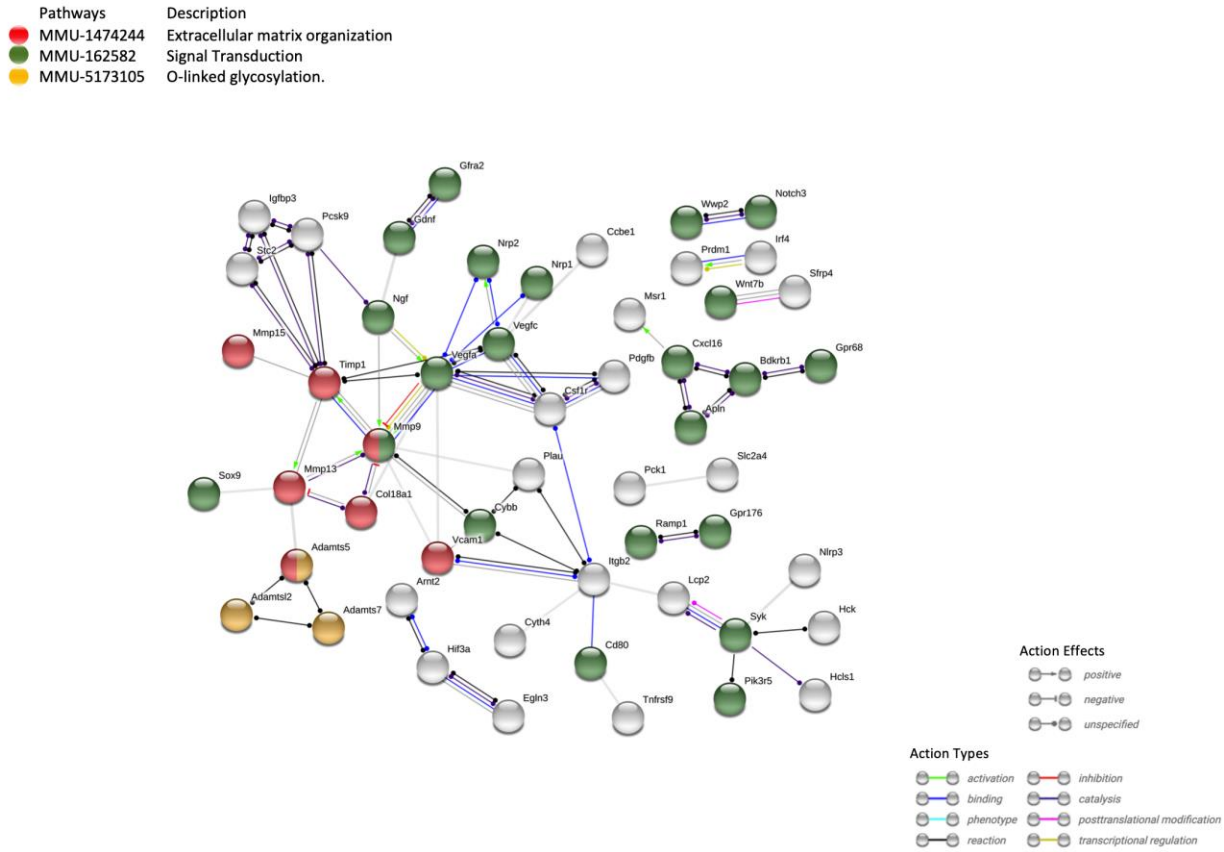


Figure 9. PPI network between differentially expressed genes (DEGs) that regulated in the same direction in two different groups, as established in STRING. DEGs common to both TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) and human osteoarthritic cartilage (cartilage.OA), interaction score ≥ 0.70, disconnected nodes were hidden in the network. Reactome pathway assessment of these genes were down in STRING, and nodes were colored with the Reactome pathway. Color labels of pathways were shown in legend table at left-top of the figure.

2.3.4 Osteoarthritis Pathway

An inflammation-induced OA signaling pathway was generated with the overlapping genes comparing transcriptomic profiles of all the OA groups, both in response to IL-1β and TNF-α stimuli and untreated OA cartilage samples **(Supplemental File 3)**. Furthermore, biological processing annotation of the list was done to create a network of the genes within the inflammation-induced OA signaling pathway **(Figure 10)**. The extracellular matrix related pathway

(Extracellular matrix organization, collagen fibril organization), skeletal system development, cell proliferation related signaling (regulation of cell proliferation, MAPK signaling pathways, activation of protein kinase activity), cellular response to cytokines and response to lipopolysaccharide were each identified as predominant signaling pathways in inflammation induced OA.

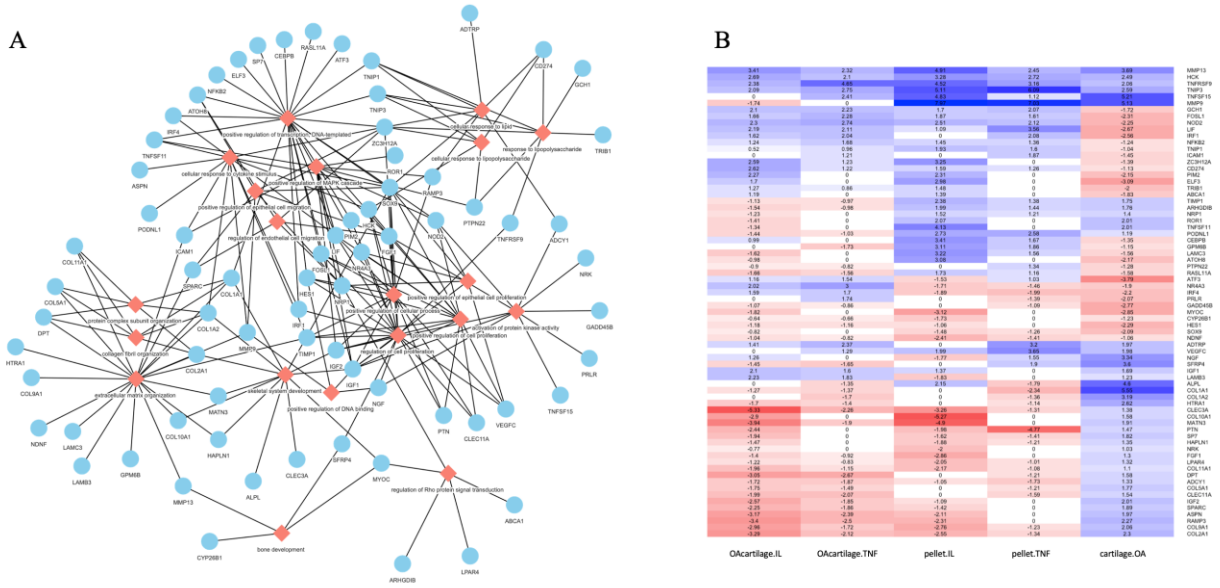


Figure 10. OA pathway generated from the common genes and enriched pathways in OAcartilage.IL, pellet.IL, and cartilage.OA, together with the common genes and enriched pathways in OAcartilage.TNF, pellet.TNF, and cartilage.OA. **(A)** Common genes – GO:BioProcess network of OA. **(B)** log2 fold change of genes in the network.

2.4 Discussion

In this study, we compared the transcriptomic profiles of IL-1β and TNF-α treated tissue-engineered cartilage pellets differentiated from murine iPSCs, IL-1β treated murine primary chondrocytes, IL-1β and TNF-α treated human OA cartilage, and cartilage from osteoarthritis patients to identify the abilities and limitations of *in vitro* OA models in replicating OA. Our findings show that the miPSC-based *in vitro* model replicates many of the transcriptomic characteristics of native cartilage treated with cytokines, as well as those of cartilage from OA

patients. The comparison of differentially expressed genes and pathway enrichment of multiple OA models and OA cartilage from patients also provides further insight into the effects of inflammation in osteoarthritis and creates opportunities to develop new therapeutic approaches for osteoarthritis treatment.

Through quantitative comparisons of overlapping DEGs and pathways, we valued the overall similarities and differences between cytokine treated *in vitro* OA models and untreated cartilage from OA patients. There have been a number of previous studies investigating DNA methylation(Jeffries et al., 2014; Moazedi-Fuerst et al., 2014), transcriptomics (Dunn et al., 2016; Fisch et al., 2018; Lv et al., 2019; Ren et al., 2018; Steinberg et al., 2017), and protein expression(Liao et al., 2018) in OA models or in cartilage from OA patients. However, most of these studies only characterized their OA disease model in terms of the expression of specific genes (Zhong, Huang, Karperien, & Post, 2016). A few studies integrated -omics datasets (DNA CpG methylation, RNA sequencing, and quantitative proteomics) (Steinberg et al., 2017) or conducted transcriptomics comparisons between different OA animal models and OA cartilage (J. Soul et al., 2019). Here, we found the differentially expressed genes in various cytokine-induced OA models and untreated OA cartilage and quantified the overlapping DEGs **(Figure 2)**. Both comparisons between IL-1β and TNF-α and various OA models showed a relatively large amount of overlapping DEGs, especially in comparison between response to different cytokines in same model system **(Figure 2,3)**. A core set of dysregulated genes among all datasets predominantly involved *Col2a1*, *col11a1*, *col9a1* (collagen family), *Nfkb2*, *Nfkbie*, *Tnfrsf9*, *Tnip1*, *Tnip3* (NF-κB signaling), *Mmp13*, and *Timp1*(Matrix Metallopeptidase). These results support previous findings that IL-1β and TNF-α treated tissue-engineered cartilage can replicate the inflammation-induced loss of homeostasis and dysregulation of extracellular matrix proteins.

Gene set enrichment analysis of up-regulated and down-regulated DEGs revealed the most significant terms in each OA model and OA cartilage sample. In total, 34 GO biological processing terms were annotated in all datasets, which mainly involved cellular response to cytokine stimulus, inflammatory response, extracellular matrix organization and disassembly, immunity, MAPK cascade, cell proliferation, and apoptotic process **(Supplemental file 2)**. Furthermore, the PPI networks established with overlapping genes further indicated that three major cluster of genes were common in most comparisons: NF-κB family (inflammation signaling), collagenase family and matrix metallopeptidase (Extracellular matrix organization) and cemokine ligands **(Figure 5-9)**. These results further supported the finding that the response of murine iPSC differentiated tissue-engineered cartilage pellets to pro-inflammatory cytokines replicated the transcriptomic expression changes in OA cartilage in inflammation related pathways, and thus provide a model system for finding potential targets for future therapies and approaches to overcome the inflammatory environment in OA.

By comparing gene expression changes in response to IL-1β and TNF-α treatment in different OA models, we were able to generate a list of common genes that are highly related to an inflammation-induced OA signaling pathway **(Figure 10, Supplemental File 3)**. These genes revealed the core signaling mediators in inflammation-induced OA. The cytokine stimulus and inflammatory related genes play a crucial role in the OA inflammation pathway, which is consistent with a number of previous cartilage pathway studies, e.g. *Tnfsf11* (Li et al., 2012), *Nfkb2* (Boyce, Yao, & Xing, 2010), *Fosl1* (Dunn et al., 2016), *Cebpb* (Hirata et al., 2012).

Our data also suggested that dysregulation of ECM components (*Col1a1*, *Col1a2*, *Col2a1*, *Col5a1*, *Col9a1*) and proteases (*Mmp9*, *Mmp13*) involved in ECM remodeling may be active as part of the inflammatory response in OA pathogenesis. Previous studies have shown that the

dysregulation of collagens plays a crucial role in the chondron-remodeling and cartilage degeneration in osteoarthritis (Luo et al., 2017). Type II collagen (*Col2a1*) is the major component that forms the highly dense collagen fibril network in cartilage, while collagen type IX and XI, as minor components, fill in this proteoglycan matrix (Xia et al., 2014). Type X collagen (*Col10a1*) has been shown as a marker of chondrocyte hypertrophy (Zheng et al., 2003) and observed in hypertrophic zone and calcified zone in cartilage and also in arthritis cartilage (Gannon et al., 1991; Shen, 2005). *Mmp9* (gelatinase B) is upregulated in hypertrophic chondrocytes, which degrades gelatin (Rose & Kooyman, 2016; Vu et al., 1998). *Mmp13* (Collagenase-3) is highly over-expressed at early stage of OA cartilage and induced extracellular matrix degradation in cartilage (Burrage, Mix, & Brinckerhoff, 2006). *Timp3*, an inhibitor of *Mmp13*, reduces the activity of Collagenase-3(Knauper, Lopez-Otin, Smith, Knight, & Murphy, 1996). Wnt and Rho GTPase signaling has been implicated in chondrocyte hypertrophy and final maturation in previous studies (Day, Guo, Garrett-Beal, & Yang, 2005; Dell'accio, De Bari, Eltawil, Vanhummelen, & Pitzalis, 2008; Wang & Beier, 2005), which has been shown dysregulated in our study. *Sox9*, as an important transcription factor in chondrocytes differentiation, has been shown to repress the expression of ADAMTS at the early stage of OA (Q. Zhang et al., 2015). Regulation of cell proliferation and apoptosis is also responsible for cartilage degeneration in OA (Sun et al., 2015). Igf1 actives phosphorylation states of ERK1/2 or Akt signaling (McMahon, Prendergast, & Campbell, 2008; Montaseri et al., 2011). *Igf2* has been shown to reduce loss of chondrocytes, osteophyte formation and subchondral bone thickening in vivo injury induced mouse OA (Uchimura, Foote, Smith, Matzkin, & Zeng, 2015). *Clec3a* encodes tetranectin and is also detected as a DEG in multi-omics OA study (Steinberg et al., 2017), in addition to being implicated in osteogenesis and bone mineralization in a previous study (Wewer et al., 1994).

2.5 Limitations

A limitation in our findings is that this iPSC OA model only partially replicates the gene expression changes in real OA cartilage. There were hundreds of unshared DEGs and DEGs regulated in different orientations in models and in OA cartilage. Specifically, we found that the cluster of collagen family genes were up-regulated in cartilage from OA patients while down-regulated in all *in vitro* models. These differences could be due to a number of factors. First, OA is a complex whole-joint disease, which can be caused by various risk factors, such as trauma or injury (Thomas, Hubbard-Turner, Wikstrom, & Palmieri-Smith, 2017), aging (Loeser, 2009), obesity (Courties, Gualillo, Berenbaum, & Sellam, 2015), and inflammation (Courties et al., 2015). Although most of them lead to similar symptoms or cartilage phenotype, different etiologies are involved in the OA development. Nonetheless, we found that these *in vitro* models replicated the catabolic aspects of pro-inflammatory environment in OA cartilage, and thus can serve as a research tool to future development of therapy and drug screening. While an inflammation-induced OA model will not completely replicate the multiple factors that lead to clinical OA, such models still serve as the primary *in vitro* system for research and OA drug screening. Second, there are different stages of OA, and many studies have shown that specific genes can be up- or down-regulated at earlier or later stages of the disease (Loeser et al., 2013; Zhong et al., 2016). Third, in the joint of OA patients, there are multiple cell types and tissues interacting with each other *in vivo*, which may affect gene expression changes in chondrocytes in response to stimuli. For example, synovial mesenchymal stem cells have been shown to alleviate osteoarthritis by promoting proliferation when co-cultured with meniscus chondrocytes(Qiong, Xia, Jing, & Haibin, 2020). Finally, the concentrations of cytokines also affect gene expression changes in models with variations over time. Studies of cytokine treated tissue-engineered cartilage with multiple time

points showed that increasing numbers of DEGs (772 to 1781 genes with IL-1β and 612 to 1147 genes with TNF-α) and that there were groups of genes with different temporal patterns of expression from 4 hour to 72 hour post-treatment (Ross et al., 2020).

2.6 Conclusion

Overall, our data support that cytokine treated tissue-engineered cartilage can replicate many of the characteristics of OA cartilage at the transcriptomic level. As this model represents the cytokine related regulation in OA cartilage degeneration, it can be used to identify targets in the inflammatory response, cellular response to cytokines, and NF-κB signaling pathways. Thus, it provides a model for studying the pro-inflammatory response and pathogenesis in OA cartilage, and will be a valuable dataset for identifying therapeutic targets for inflammation induced OA treatment. The ability of self-renewal and unlimited proliferation of iPSCs offers this model the possibility of high-throughput drug screening with a defined genotype. Also, this transcriptomic analysis comparison of OA disease models and human OA cartilage provides us with a further understanding of the systematic mechanism and molecular and cellular basis of inflammation in OA.

References

Alten, R., Gram, H., Joosten, L. A., van den Berg, W. B., Sieper, J., Wassenberg, S., . . . Jung, T. (2008). The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. *Arthritis Res Ther, 10*(3), R67. doi:10.1186/ar2438

Boyce, B. F., Yao, Z., & Xing, L. (2010). Functions of nuclear factor kappaB in bone. *Ann N Y Acad Sci, 1192*, 367-375. doi:10.1111/j.1749-6632.2009.05315.x

Burrage, P. S., Mix, K. S., & Brinckerhoff, C. E. (2006). Matrix metalloproteinases: role in arthritis. *Front Biosci, 11*, 529-543. doi:10.2741/1817

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., . . . Ma'ayan, A. (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics, 14*, 128. doi:10.1186/1471-2105-14-128

Cisternas, M. G., Murphy, L., Sacks, J. J., Solomon, D. H., Pasta, D. J., & Helmick, C. G. (2016). Alternative Methods for Defining Osteoarthritis and the Impact on Estimating Prevalence in a US Population-Based Survey. *Arthritis Care Res (Hoboken), 68*(5), 574-580. doi:10.1002/acr.22721

Cohen, S. B., Proudman, S., Kivitz, A. J., Burch, F. X., Donohue, J. P., Burstein, D., . . . Zack, D. J. (2011). A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee. *Arthritis Res Ther, 13*(4), R125. doi:10.1186/ar3430

Courties, A., Gualillo, O., Berenbaum, F., & Sellam, J. (2015). Metabolic stress-induced joint inflammation and osteoarthritis. *Osteoarthritis Cartilage, 23*(11), 1955-1965. doi:10.1016/j.joca.2015.05.016

Day, T. F., Guo, X., Garrett-Beal, L., & Yang, Y. (2005). Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell, 8*(5), 739-750. doi:10.1016/j.devcel.2005.03.016

Dell'accio, F., De Bari, C., Eltawil, N. M., Vanhummelen, P., & Pitzalis, C. (2008). Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis. *Arthritis Rheum, 58*(5), 1410-1421. doi:10.1002/art.23444

Diekman, B. O., Christoforou, N., Willard, V. P., Sun, H., Sanchez-Adams, J., Leong, K. W., & Guilak, F. (2012). Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. *Proc Natl Acad Sci U S A, 109*(47), 19172-19177. doi:10.1073/pnas.1210422109

Dunn, S. L., Soul, J., Anand, S., Schwartz, J. M., Boot-Handford, R. P., & Hardingham, T. E. (2016). Gene expression changes in damaged osteoarthritic cartilage identify a signature of non-chondrogenic and mechanical responses. *Osteoarthritis Cartilage, 24*(8), 1431-1440. doi:10.1016/j.joca.2016.03.007

Engle, S. J., & Puppala, D. (2013). Integrating human pluripotent stem cells into drug development. *Cell Stem Cell, 12*(6), 669-677. doi:10.1016/j.stem.2013.05.011

Fisch, K. M., Gamini, R., Alvarez-Garcia, O., Akagi, R., Saito, M., Muramatsu, Y., . . . Lotz, M. K. (2018). Identification of transcription factors responsible for dysregulated networks in human osteoarthritis cartilage by global gene expression analysis. *Osteoarthritis Cartilage, 26*(11), 1531-1538. doi:10.1016/j.joca.2018.07.012

Gannon, J. M., Walker, G., Fischer, M., Carpenter, R., Thompson, R. C., Jr., & Oegema, T. R., Jr. (1991). Localization of type X collagen in canine growth plate and adult canine articular cartilage. *J Orthop Res, 9*(4), 485-494. doi:10.1002/jor.1100090404

Goldring, M. B., & Goldring, S. R. (2010). Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann N Y Acad Sci, 1192*, 230-237. doi:10.1111/j.1749-6632.2009.05240.x

Goldring, M. B., & Marcu, K. B. (2009). Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther, 11*(3), 224. doi:10.1186/ar2592

Goldring, M. B., & Otero, M. (2011). Inflammation in osteoarthritis. *Curr Opin Rheumatol, 23*(5), 471-478. doi:10.1097/BOR.0b013e328349c2b1

Guler-Yuksel, M., Allaart, C. F., Watt, I., Goekoop-Ruiterman, Y. P., de Vries-Bouwstra, J. K., van Schaardenburg, D., . . . Kloppenburg, M. (2010). Treatment with TNF-alpha inhibitor infliximab might reduce hand osteoarthritis in patients with rheumatoid arthritis. *Osteoarthritis Cartilage, 18*(10), 1256-1262. doi:10.1016/j.joca.2010.07.011

Hirata, M., Kugimiya, F., Fukai, A., Saito, T., Yano, F., Ikeda, T., . . . Kawaguchi, H. (2012). C/EBPbeta and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2alpha as the inducer in chondrocytes. *Hum Mol Genet, 21*(5), 1111-1123. doi:10.1093/hmg/ddr540

Jeffries, M. A., Donica, M., Baker, L. W., Stevenson, M. E., Annan, A. C., Humphrey, M. B., . . . Sawalha, A. H. (2014). Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. *Arthritis Rheumatol, 66*(10), 2804-2815. doi:10.1002/art.38762

Johnson, C. I., Argyle, D. J., & Clements, D. N. (2016). In vitro models for the study of osteoarthritis. *Vet J, 209*, 40-49. doi:10.1016/j.tvjl.2015.07.011

Knauper, V., Lopez-Otin, C., Smith, B., Knight, G., & Murphy, G. (1996). Biochemical characterization of human collagenase-3. *J Biol Chem, 271*(3), 1544-1550. doi:10.1074/jbc.271.3.1544

Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., . . . Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res, 44*(W1), W90-97. doi:10.1093/nar/gkw377

Kuyinu, E. L., Narayanan, G., Nair, L. S., & Laurencin, C. T. (2016). Animal models of osteoarthritis: classification, update, and measurement of outcomes. *J Orthop Surg Res, 11*, 19. doi:10.1186/s13018-016-0346-5

Li, H., Li, L., Min, J., Yang, H., Xu, X., Yuan, Y., & Wang, D. (2012). Levels of metalloproteinase (MMP-3, MMP-9), NF-kappaB ligand (RANKL), and nitric oxide (NO) in peripheral blood of osteoarthritis (OA) patients. *Clin Lab, 58*(7-8), 755-762. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22997976

Liao, W., Li, Z., Li, T., Zhang, Q., Zhang, H., & Wang, X. (2018). Proteomic analysis of synovial fluid in osteoarthritis using SWATHmass spectrometry. *Mol Med Rep, 17*(2), 2827-2836. doi:10.3892/mmr.2017.8250

Loeser, R. F. (2009). Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. *Osteoarthritis Cartilage, 17*(8), 971-979. doi:10.1016/j.joca.2009.03.002

Loeser, R. F., Olex, A. L., McNulty, M. A., Carlson, C. S., Callahan, M., Ferguson, C., & Fetrow, J. S. (2013). Disease progression and phasic changes in gene expression in a mouse model of osteoarthritis. *PLoS One, 8*(1), e54633. doi:10.1371/journal.pone.0054633

Luo, Y., Sinkeviciute, D., He, Y., Karsdal, M., Henrotin, Y., Mobasheri, A., . . . Bay-Jensen, A. (2017). The minor collagens in articular cartilage. *Protein Cell, 8*(8), 560-572. doi:10.1007/s13238-017-0377-7

Lv, M., Zhou, Y., Polson, S. W., Wan, L. Q., Wang, M., Han, L., . . . Lu, X. L. (2019). Identification of Chondrocyte Genes and Signaling Pathways in Response to Acute Joint Inflammation. *Sci Rep, 9*(1), 93. doi:10.1038/s41598-018-36500-2

Magnano, M. D., Chakravarty, E. F., Broudy, C., Chung, L., Kelman, A., Hillygus, J., & Genovese, M. C. (2007). A pilot study of tumor necrosis factor inhibition in erosive/inflammatory osteoarthritis of the hands. *J Rheumatol, 34*(6), 1323-1327. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17516620

McMahon, L. A., Prendergast, P. J., & Campbell, V. A. (2008). A comparison of the involvement of p38, ERK1/2 and PI3K in growth factor-induced chondrogenic differentiation of mesenchymal stem cells. *Biochem Biophys Res Commun, 368*(4), 990-995. doi:10.1016/j.bbrc.2008.01.160

Meek, I. L., Van de Laar, M. A., & H, E. V. (2010). Non-Steroidal Anti-Inflammatory Drugs: An Overview of Cardiovascular Risks. *Pharmaceuticals (Basel), 3*(7), 2146-2162. doi:10.3390/ph3072146

Moazedi-Fuerst, F. C., Hofner, M., Gruber, G., Weinhaeusel, A., Stradner, M. H., Angerer, H., . . . Graninger, W. B. (2014). Epigenetic differences in human cartilage between mild and severe OA. *J Orthop Res, 32*(12), 1636-1645. doi:10.1002/jor.22722

Montaseri, A., Busch, F., Mobasheri, A., Buhrmann, C., Aldinger, C., Rad, J. S., & Shakibaei, M. (2011). IGF-1 and PDGF-bb suppress IL-1beta-induced cartilage degradation through down-regulation of NF-kappaB signaling: involvement of Src/PI-3K/AKT pathway. *PLoS One, 6*(12), e28663. doi:10.1371/journal.pone.0028663

Murphy, L., Schwartz, T. A., Helmick, C. G., Renner, J. B., Tudor, G., Koch, G., . . . Jordan, J. M. (2008). Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Rheum, 59*(9), 1207-1213. doi:10.1002/art.24021

Qiong, J., Xia, Z., Jing, L., & Haibin, W. (2020). Synovial mesenchymal stem cells effectively alleviate osteoarthritis through promoting the proliferation and differentiation of meniscus chondrocytes. *Eur Rev Med Pharmacol Sci, 24*(4), 1645-1655. doi:10.26355/eurrev_202002_20338

Ren, Y. M., Zhao, X., Yang, T., Duan, Y. H., Sun, Y. B., Zhao, W. J., & Tian, M. Q. (2018). Exploring the Key Genes and Pathways of Osteoarthritis in Knee Cartilage in a Rat Model Using Gene Expression Profiling. *Yonsei Med J, 59*(6), 760-768. doi:10.3349/ymj.2018.59.6.760

Rose, B. J., & Kooyman, D. L. (2016). A Tale of Two Joints: The Role of Matrix Metalloproteases in Cartilage Biology. *Dis Markers, 2016*, 4895050. doi:10.1155/2016/4895050

Shen, G. (2005). The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. *Orthod Craniofac Res, 8*(1), 11-17. doi:10.1111/j.1601-6343.2004.00308.x

Son, Y. O., Park, S., Kwak, J. S., Won, Y., Choi, W. S., Rhee, J., . . . Chun, J. S. (2017). Estrogen-related receptor gamma causes osteoarthritis by upregulating extracellular matrix-degrading enzymes. *Nat Commun, 8*(1), 2133. doi:10.1038/s41467-017-01868-8

Soul, J., Boot-Handford, R., Schwartz, J.-M., & University of, M. (2017). *A systems biology approach to knee osteoarthritis*.

Soul, J., Hardingham, T. E., Boot-Handford, R. P., & Schwartz, J. M. (2019). SkeletalVis: an exploration and meta-analysis data portal of cross-species skeletal transcriptomics data. *Bioinformatics, 35*(13), 2283-2290. doi:10.1093/bioinformatics/bty947

Steinberg, J., Ritchie, G. R. S., Roumeliotis, T. I., Jayasuriya, R. L., Clark, M. J., Brooks, R. A., . . . Zeggini, E. (2017). Integrative epigenomics, transcriptomics and proteomics of patient chondrocytes reveal genes and pathways involved in osteoarthritis. *Sci Rep, 7*(1), 8935. doi:10.1038/s41598-017-09335-6

Studer, R., Jaffurs, D., Stefanovic-Racic, M., Robbins, P. D., & Evans, C. H. (1999). Nitric oxide in osteoarthritis. *Osteoarthritis Cartilage, 7*(4), 377-379. doi:10.1053/joca.1998.0216

Sun, Y., Liu, W. Z., Liu, T., Feng, X., Yang, N., & Zhou, H. F. (2015). Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. *J Recept Signal Transduct Res, 35*(6), 600-604. doi:10.3109/10799893.2015.1030412

Thomas, A. C., Hubbard-Turner, T., Wikstrom, E. A., & Palmieri-Smith, R. M. (2017). Epidemiology of Posttraumatic Osteoarthritis. *J Athl Train, 52*(6), 491-496. doi:10.4085/1062-6050-51.5.08

Uchimura, T., Foote, A. T., Smith, E. L., Matzkin, E. G., & Zeng, L. (2015). Insulin-Like Growth Factor II (IGF-II) Inhibits IL-1beta-Induced Cartilage Matrix Loss and Promotes Cartilage Integrity in Experimental Osteoarthritis. *J Cell Biochem, 116*(12), 2858-2869. doi:10.1002/jcb.25232

Verbruggen, G., Wittoek, R., Vander Cruyssen, B., & Elewaut, D. (2012). Tumour necrosis factor blockade for the treatment of erosive osteoarthritis of the interphalangeal finger joints: a double blind, randomised trial on structure modification. *Ann Rheum Dis, 71*(6), 891-898. doi:10.1136/ard.2011.149849

Vu, T. H., Shipley, J. M., Bergers, G., Berger, J. E., Helms, J. A., Hanahan, D., . . . Werb, Z. (1998). MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell, 93*(3), 411-422. doi:10.1016/s0092-8674(00)81169-1

Wang, G., & Beier, F. (2005). Rac1/Cdc42 and RhoA GTPases antagonistically regulate chondrocyte proliferation, hypertrophy, and apoptosis. *J Bone Miner Res, 20*(6), 1022-1031. doi:10.1359/JBMR.050113

Wewer, U. M., Ibaraki, K., Schjorring, P., Durkin, M. E., Young, M. F., & Albrechtsen, R. (1994). A potential role for tetranectin in mineralization during osteogenesis. *J Cell Biol, 127*(6 Pt 1), 1767-1775. doi:10.1083/jcb.127.6.1767

Willard, V. P., Diekman, B. O., Sanchez-Adams, J., Christoforou, N., Leong, K. W., & Guilak, F. (2014). Use of cartilage derived from murine induced pluripotent stem cells for osteoarthritis drug screening. *Arthritis Rheumatol, 66*(11), 3062-3072. doi:10.1002/art.38780

Wojdasiewicz, P., Poniatowski, L. A., & Szukiewicz, D. (2014). The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm, 2014*, 561459. doi:10.1155/2014/561459

Xia, B., Di, C., Zhang, J., Hu, S., Jin, H., & Tong, P. (2014). Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif Tissue Int, 95*(6), 495-505. doi:10.1007/s00223-014-9917-9

Zhang, Q., Ji, Q., Wang, X., Kang, L., Fu, Y., Yin, Y., . . . Wang, Y. (2015). SOX9 is a regulator of ADAMTSs-induced cartilage degeneration at the early stage of human osteoarthritis. *Osteoarthritis Cartilage, 23*(12), 2259-2268. doi:10.1016/j.joca.2015.06.014

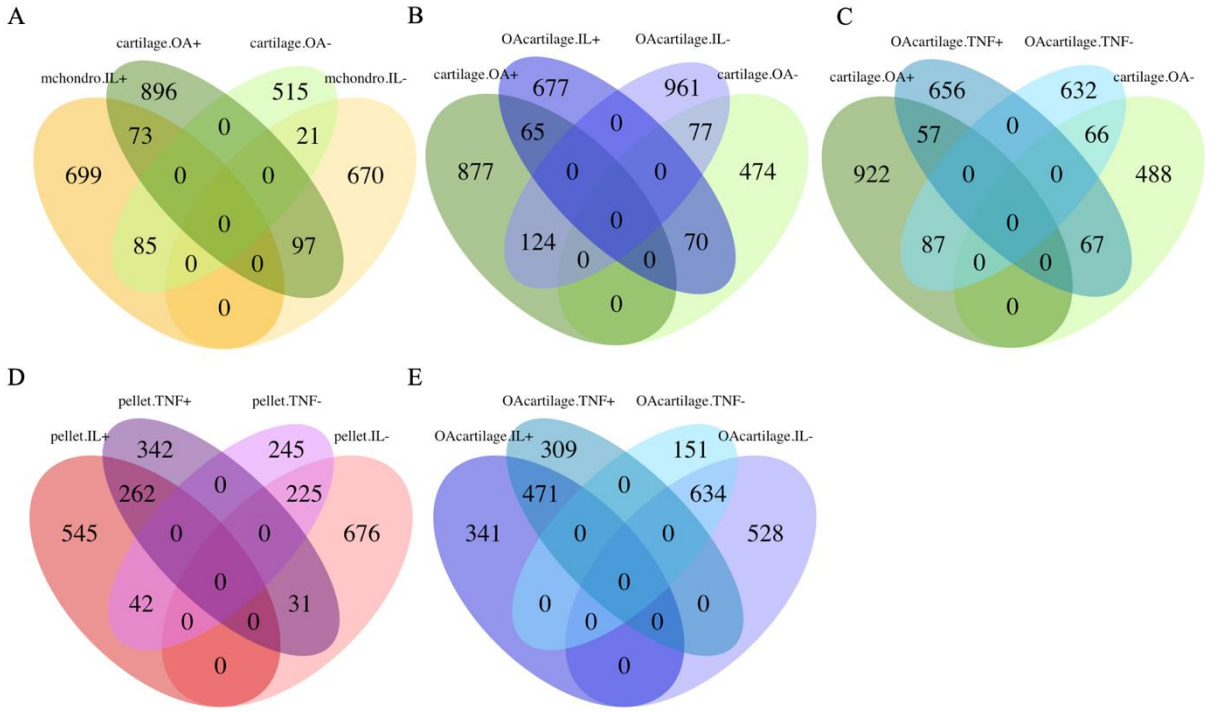
Zhang, W., Robertson, W. B., Zhao, J., Chen, W., & Xu, J. (2019). Emerging Trend in the Pharmacotherapy of Osteoarthritis. *Front Endocrinol (Lausanne), 10*, 431. doi:10.3389/fendo.2019.00431

Zheng, Q., Zhou, G., Morello, R., Chen, Y., Garcia-Rojas, X., & Lee, B. (2003). Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. *J Cell Biol, 162*(5), 833-842. doi:10.1083/jcb.200211089

Zhong, L., Huang, X., Karperien, M., & Post, J. N. (2016). Correlation between Gene Expression and Osteoarthritis Progression in Human. *Int J Mol Sci, 17*(7). doi:10.3390/ijms17071126

Ross, A. K., Coutinho de Almeida, R., Ramos, Y.F.M., Li, J., Meulenbelt, I., & Guilak, F. (2020). The miRNA-mRNA Interactome of murine iPSC-derived Chondrocytes in Response to Inflammatory Cytokines. Manuscript submitted for publication.

Appendix Figure 1: Venn diagrams showing number of overlapping upregulated (+) or downregulated (-) DEGs between different models



(A) Comparison of IL-1β treated murine chondrocytes (mchondro.IL) and untreated human osteoarthritic cartilage (cartilage.OA) showed 276 overlapping DEGs: 94 are regulated in the same direction, while 182 genes respond differently. (**B**) Comparison of IL-1β treated human osteoarthritic cartilage (OAcartilage.IL) and untreated human osteoarthritic cartilage (cartilage.OA) showed 336 overlapping DEGs: 142 are regulated in the same direction, while 194 genes respond differently. (**C**) Comparison of TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF) and untreated human osteoarthritic cartilage (cartilage.OA) showed 277 overlapping DEGs: 123 are regulated in the same direction, while 154 genes respond differently. (**D**) Comparison of IL-1β treated tissue-engineered cartilage pellets (pellet.IL) and TNF-α treated tissue-engineered cartilage pellets (pellet.TNF) showed 560 overlapping genes: 478 are regulated

in the same direction, while 73 genes respond differently. (**E**) Comparison of IL-1β treated human osteoarthritic cartilage (OAcartilage.IL) and TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF) showed 1105 overlapping genes with co-directionally regulation.

Appendix Table 1: Common genes of gene ontology (GO) terms in response of IL-1β

Term	DEGs in four study groups
extracellular matrix organization (GO:0030198)	FGF2; HAPLN1; SH3PXD2B; CYP1B1; COL10A1; GREM1; COL2A1; MATN4; CD44; MATN3; COL11A1; COL11A2; LAMB3; ITGA10; COL9A1; COL9A3; COL9A2
cellular response to cytokine stimulus (GO:0071345)	IL1RN; FGF2; TNFSF11; SOX9; IFNAR2; PTGIS; IL1R1; IRAK3; ASPN; CEBPD; CCL5; CHAD; CCL20; LIF; LCN2; SDC1; PTPN2
cytokine-mediated signaling pathway (GO:0019221)	IL1RN; HFE; FGF2; TNFSF11; IFNAR2; IL1R1; CHAD; TNFRSF9; LIF; NFKBIA; LCN2; SDC1; RIPK2; IRAK3; ASPN; CD44; CEBPD; CCL5; CCL20; SOD2; FAS
skeletal system development (GO:0001501)	COL11A2; HAPLN1; PAPSS2; FRZB; SH3PXD2B; COL10A1; SOX9; GDF10; IGF1; COL2A1; COL9A2; MATN3; CD44
regulation of cell proliferation (GO:0042127)	FGF2; FTH1; CYP1B1; SOX9; SCIN; ABL1; TNFRSF9; LIF; IGF1; NGF; GREM1; FRZB; SOD2; FAS; PTPN2
positive regulation of cell proliferation (GO:0008284)	FGF2; SOX8; SOX9; GREM1; CCL5; LIF; IGF1
response to lipopolysaccharide (GO:0032496)	TNFAIP3; PDE4B; CD14; GCH1; TNFRSF9; IRAK3; TNIP1; FAS; TRIB1
negative regulation of cell proliferation (GO:0008285)	TNFAIP3; FTH1; CYP1B1; SOX9; NGF; GREM1; TRIB1; FBLN1; SCIN; FRZB; SOD2; GDF5; PTPN2
regulation of cell migration (GO:0030334)	RND3; CYP1B1; CCL5; IGF1; SOD2
regulation of epithelial cell proliferation (GO:0050678)	IGF1; GDF5; SOX9

Common genes of gene ontology (GO) terms from functional enrichment analysis from GO Biological Process 2018 with DEGs in all 4 group of studies, IL-1β treated tissue-engineered cartilage pellets (pellet.IL), IL-1β treated murine primary chondrocytes (mchondro.IL), IL-1β treated human osteoarthritic cartilage (OAcartilage.IL) and untreated human osteoarthritic

cartilage (cartilage.OA).

Appendix Table 2: Common genes of gene ontology (GO) terms in response of TNF-α

Term	DEGs in three study groups
extracellular matrix organization (GO:0030198)	CYP1B1; COL2A1; COL11A1; COL11A2; COL9A1; COL9A3; COL9A2
cytokine-mediated signaling pathway (GO:0019221)	RIPK2; IFNGR2; CCL5; CHAD; CCL20; TNFSF15; TNFRSF9; LIF; SOD2; NFKBIA; FAS
response to lipopolysaccharide (GO:0032496)	TNFAIP3; PDE4B; MEF2C; GCH1; TNFRSF9; TNIP1; FAS
response to molecule of bacterial origin (GO:0002237)	GCH1; TNFRSF9; TNFAIP3; FAS
cellular response to cytokine stimulus (GO:0071345)	PTGIS; IFNGR2; CCL5; CHAD; CCL20; LIF
skeletal system development (GO:0001501)	COL11A2; PAPSS2; FRZB; GDF10; COL2A1; COL9A2
regulation of cell proliferation (GO:0042127)	FTH1; CYP1B1; SCIN; FRZB; TNFRSF9; LIF; SOD2; MDM2; FAS
negative regulation of apoptotic process (GO:0043066)	SOX8; MEF2C; RIPK2; SOD2; NFKBIA; FAS
positive regulation of cell proliferation (GO:0008284)	SOX8; MEF2C; CCL5; LIF; MDM2
collagen fibril organization (GO:0030199)	COL2A1; COL11A1; COL11A2; CYP1B1

Common genes of gene ontology (GO) terms from functional enrichment analysis from GO Biological Process 2018 with DEGs in all 3 group of studies, TNF-α treated tissue-engineered cartilage pellets (pellet.TNF), TNF-α treated human osteoarthritic cartilage (OAcartilage.TNF) and untreated human osteoarthritic cartilage (cartilage.OA).