Advances in Identifying Early OA

Organizers:

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Speakers:

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Significance and Purpose:

Joint injuries initiate changes in joint tissue homeostasis that often culminate in osteoarthritis. Currently, many groups focus their research on mechanistically connecting the initial injury event and the eventual osteoarthritis. The primary purpose of this workshop is to highlight recent advances in the understanding of acute responses to joint injury in different joint tissues. The three presenters have each approached the identification of early OA from unique perspectives in their research. This workshop will bring together these unique perspectives. The goal is to establish a more comprehensive understanding of how the interplay between different tissues, matrix gene expression, cytokine and growth factor production, and metabolomics responses during the acute post-injury phase affects the joint.

Educational Need:

Current clinical treatment of minor joint injuries is focused more on restoring joint stability that it is on preventing degradative osteoarthritic changes in the long term. Often minor joint injuries such as ACL or meniscal tears are not treated clinically for 2-3 weeks post-injury. During this time, many of the cellular and molecular changes can have long-lasting or even permanent damaging effects on joint structures. Researchers from diverse fields are helping identifying new therapeutic intervention strategies during the acute post-injury phase. There is a concurrent need to educate both patients and clinicians to take preventative measures during these critical early times, to help mitigate the downstream development of OA.

Learning Objectives:

Update attendees on the recent advances in understanding how cellular and molecular changes that occur during the acute post-injury timeframe can affect long-term joint health.

Abstract:

Established OA cannot be cured with existing medicine, and joint replacement is often the only solution. Joint injury, even a mild injury such as ACL or meniscal tear, can increase the lifelong risk for osteoarthritis of the joint by a factor of 10 or more. A strong focus of recent research has been to identify intervention strategies that can decrease the risk of OA after injury. It has become apparent that the acute post-injury phase is important in the downstream development of OA. During this acute phase, the activities at the cellular and molecular levels can affect the integrity of structures within the joint. While the original mechanical damage caused by the injury is unchangeable, the "secondary" cell-mediated injury provides multiple

opportunities for intervention. The goal of this workshop is to convey the recent advances in understanding the early events during the acute post-injury phase. Three speakers have approached this problem from unique and complementary research perspectives. Dr. Ron June has studied the injury-induced changes in the abundance of small molecule metabolites and in the metabolic flux. Dr. Christopher Little has studied the contribution of the different tissues to the initiation and progression of post-injury OA. Dr. Steven Olson has studied the contribution of gene expression and inflammatory cytokines to the initiation of OA. The workshop organizers, Drs. Haudenschild and Christiansen, have studied the contribution of primary response genes and bone turnover to OA initiation. Discoveries in each of these areas have advanced our understanding of the global changes in the joint that occur during the first hours to days after an injury.

Injury-Induced Changes in Small-Molecule Metabolites in Synovial Joints

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Introduction

Osteoarthritis is a debilitating joint disease affecting more than 100 million people worldwide. There are many causes of osteoarthritis (OA) including joint trauma and injury. Post-traumatic osteoarthritis (PTOA) occurs following joint trauma. The initiating trauma results in cell biological changes across multiple tissues within the synovial joint. These changes typically activate matrix catabolism resulting in degradation of articular cartilage. As the cartilage degrades, joint function deteriorates resulting in stiffness and pain.

Many groups have made great strides in elucidating many diverse mechanisms that drive normal joint physiology and injury-induced degradation. However, clinical strategies to prevent PTOA face many barriers including both a limited understanding of the interplay of biological signals between joint tissues and the absence of measurable biomarkers associated with disease onset and progression. Metabolomic profiling of joint tissues is a promising approach to address these challenges.

Overview of Metabolomic Profiling

Metabolomics is an emerging technique for characterizing cell physiology and human disease. Metabolomic profiling has proven useful for many diseases from cancer to maternal health[1,2]. Importantly, several groups have begun applying

metabolomic profiling to osteoarthritis and joint disease[3-5]. The myriad processes that comprise cell function utilize and produce a diverse set of small molecules including substrates, reactants, products, and signaling molecules. These small molecule metabolites can be quantified using liquid chromatography coupled to mass spectrometry (LC-MS). There are two types of metabolomic profiles: (i) global metabolomic profiles describe an untargeted set of metabolites and (ii) targeted metabolomic profiles describe a smaller set of pre-defined molecules (*e.g.* glucose and its derivatives.) Metabolite flux measurements involve observing the change in abundance of a targeted metabolite following an experimental or clinical timecourse. Flux measurements provide useful information on the dynamics of pathway activation within a cell or tissue.

Methods

Mice were subjected to non-invasive injury by a single mechanical overload using femorotibial compression[6]. This overload results in a bony avulsion fracture at the ACL insertion in the posterior femur[7]. In this study, we used 2 groups of animals: (i) injured animals with the right knee subjected to the single mechanical overload at 1 mm/s and (ii) control animals subjected to an identical anesthesia protocol without injury. Animals were euthanized 60 minutes after injury, and joint metabolites were harvested from tissue extracts including the femoral condyles, ligaments, menisci, synovium, and tibial plateau. Metabolites were extracted in 70:30 methanol:acetone with 5 cycles of vortexing and freezing. Metabolites were resuspended in 50:50 acetonitrile:water for LC-MS. Hydrophobic Interaction Chromatography was performed with an aqueous normal-phase using an ANP/HILIC HPLC column (Cogent Diamond Hydride Type-C). The column was coupled to an Agilent 1290 HPLC system, and chromatography.

Results and Discussion

Injured joints had distinct metabolomic profiles from joints of uninjured mice (Figure 1A). When comparing the right knee joints, injured mice expressed 66 metabolites undetected in control mice, whereas control mice expressed 81 metabolites undetected in injured mice (Figure 1B). Unsupervised clustering identified two clusters of interest (Figure 1C). In injured joints, we found substantial upregulation of metabolites related to Vitamin D3 signaling (Figures 1D). We observed downregulation of deoxycytidine triphosphate consistent with injury-induced upregulation of primary response genes. Enrichment analysis revealed glutamine and glutamate, arginine and proline, and pyrimidine metabolism to be downregulated after injury. Enrichment analysis also revealed pathways upregulated after injury
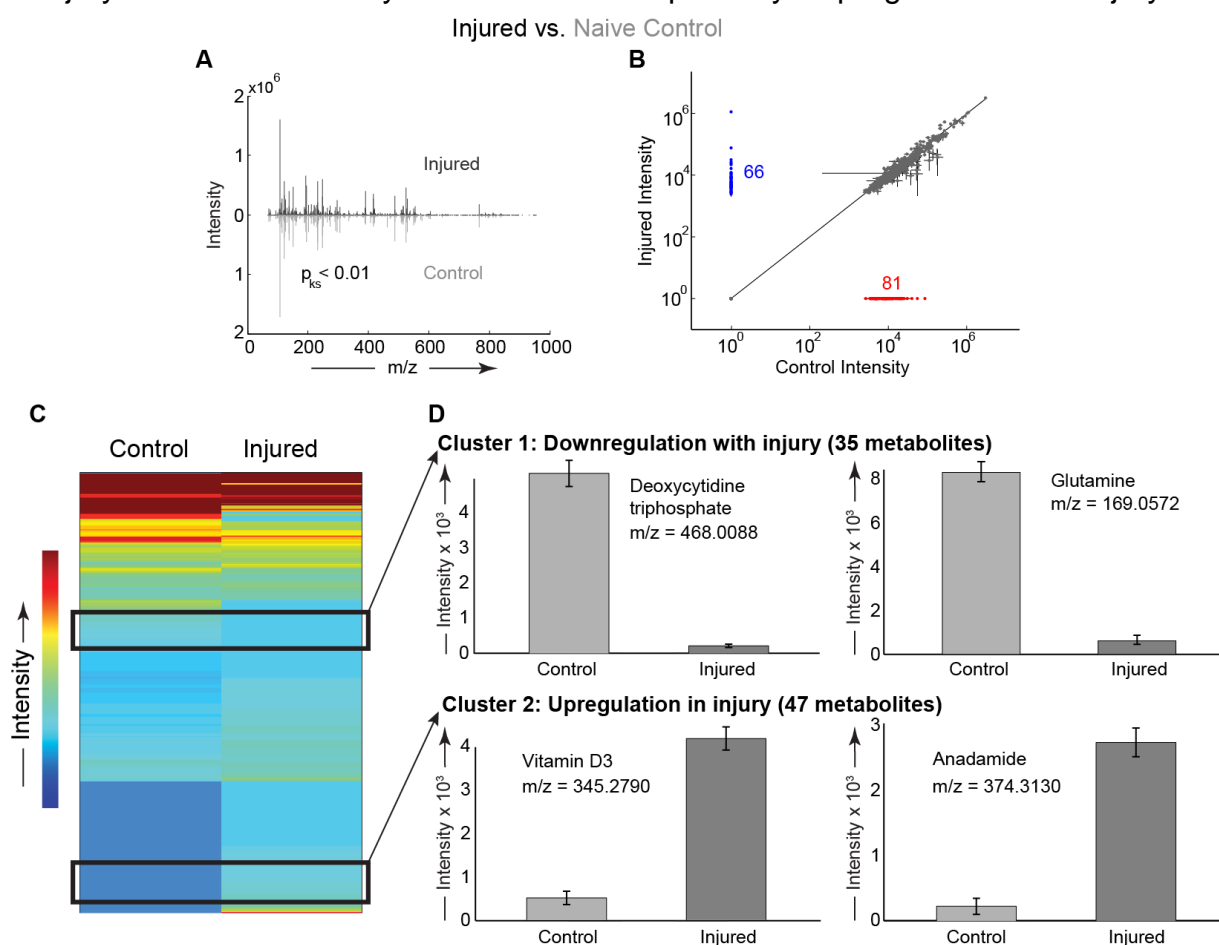

Figure 1 Joint injury induces widespread changes in metabolomic profiles. (A) The median profiles were different (p < 0.01) between injured and control mice. (B) There were 66 metabolites found in injured joints that were not detected in injured joints. There were 81 metabolites detected in control joints that were not found in injured joints. (C) Clustering analysis identified two clusters of interest. (D) Select metabolites depleted (top row) or upregulated (bottom row) after injury

including metabolism of retinoids and anandamide, phospholipid biosynthesis, hydroxyproline degradation.

Future Directions

Metabolomic profiling is a powerful tool for analyzing joint injury and osteoarthritis. These initial results suggest widespread changes in synovial joint biology after injury. By extending these methods to include additional timepoints and focusing on particular tissues (*e.g* subchondral bone), we may improve our understanding of the pathophysiological mechanisms mediating injury. Such an understanding may pave the way for validation of new drug targets to prevent post-traumatic osteoarthritis.

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# Inflammatory Cytokines and Gene Expression in Acute PTOA
Steven Olson, MD, Duke University Medical Center

1) PTOA – important contribution to burden of OA, Leading cause of soldiers being declared unfit for duty after an extremity injury[1-3].

2) Articular Fractures are a common cause of PTOA. The quality of articular reduction does not explain the observed clinical variation in PTOA development after articular fracture[4, 5].

3) A model of closed articular fracture was that develops PTOA – in the knee of B6 mice[6]
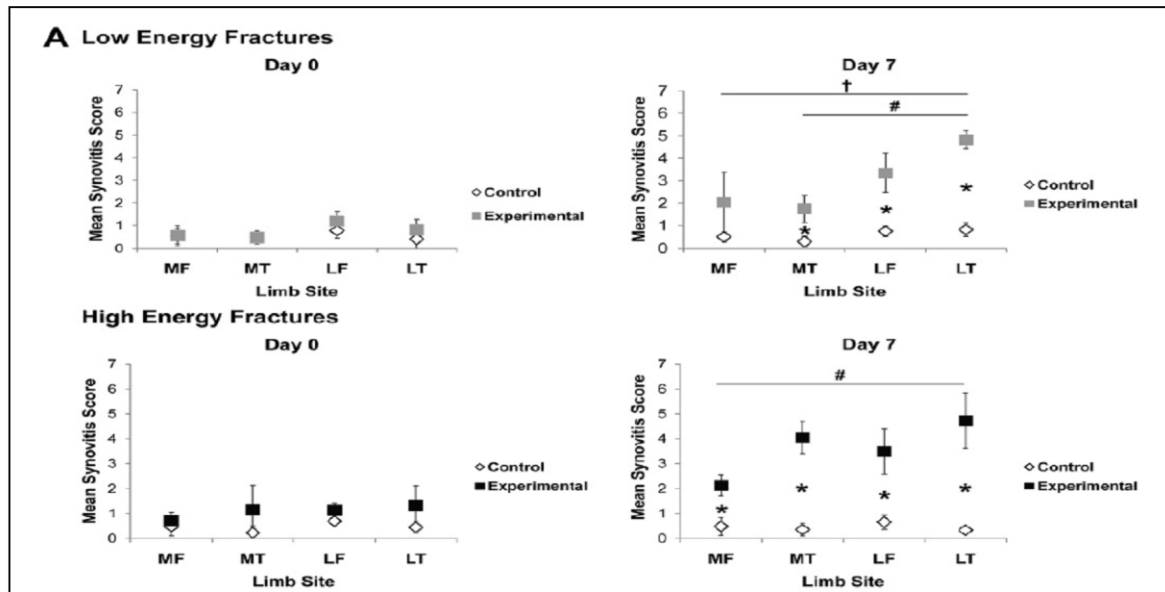

Reproduced from Furman BD, Strand J, Hembree WC, Ward BD, Guilak F, Olson SA. Joint degeneration following closed intraarticular fracture in the mouse knee: a model of posttraumatic arthritis. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2007 May;25(5):578-92. Epub 2007/02/03.

4) Inflammation, not chondrocyte death increases with increasing injury severity[7]

The equation shown in panel B:
$$y = 0.044x - 0.65$$
$$R^2 = 0.70$$
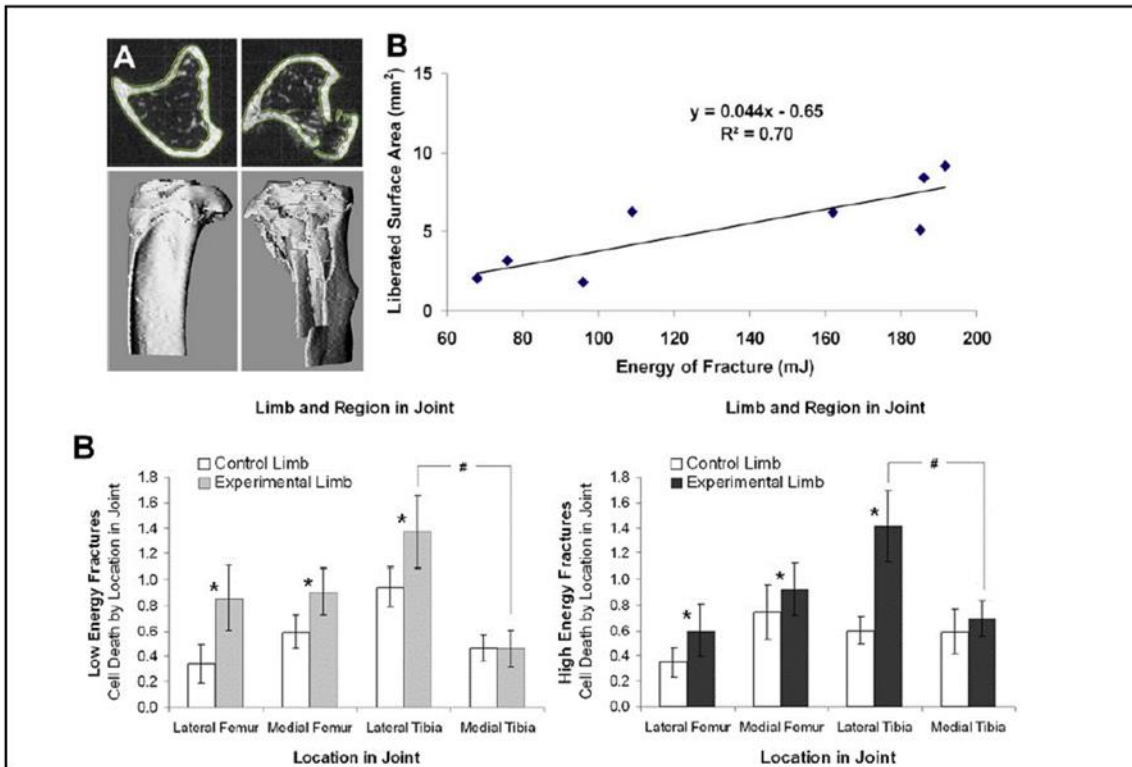
Reproduced from Lewis JS, Hembree WC, Furman BD, Tippets L, Cattel D, Huebner JL, et al. Acute joint pathology and synovial inflammation is associated with increased intra-articular fracture severity in the mouse knee. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2011 Jul;19(7):864-73. Epub 2011/05/31.

5) MRL/ MpJ Mice are protected from PTOA development 8 weeks following articular fracture[8]

6) Synovial gene expression in B6 and MRL/MpJ mice following closed articular fracture. MRL/MpJ have an initial inflammatory response to injury that is rapidly attenuated compared to B6 mice[9]
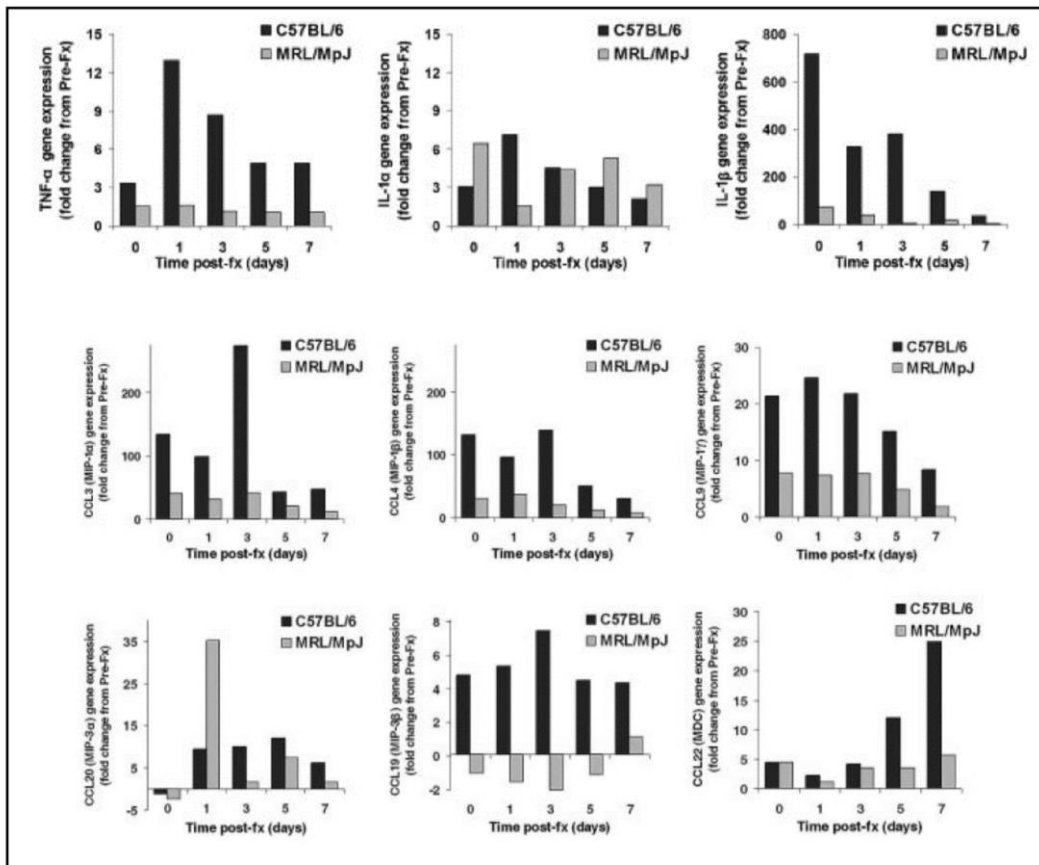
Reproduced from Lewis JS, Jr., Furman BD, Zeitler E, Huebner JL, Kraus VB, Guilak F, et al. Genetic and cellular evidence of decreased inflammation associated with reduced incidence of posttraumatic arthritis in MRL/MpJ mice. Arthritis and rheumatism. 2013 Mar;65(3):660-70. Epub 2012/12/04.

Observed timeline of gene expression in the response to injury of B6 and MRL/MpJ mice
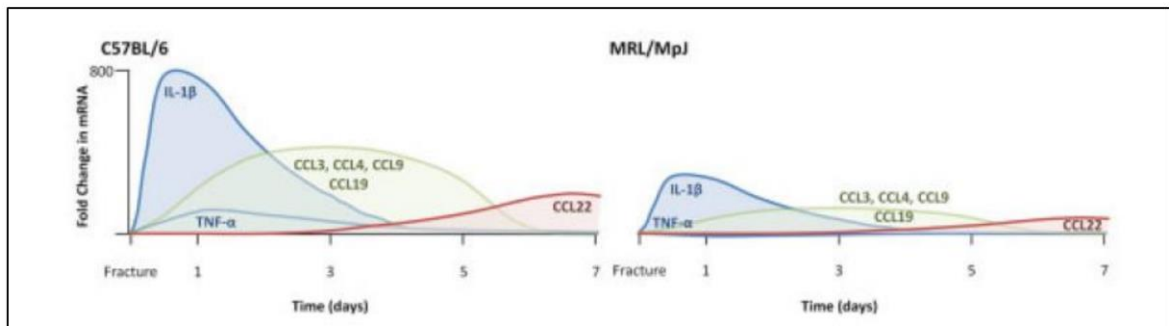
Reproduced from Lewis JS, Jr., Furman BD, Zeitler E, Huebner JL, Kraus VB, Guilak F, et al. Genetic and cellular evidence of decreased inflammation associated with reduced incidence of posttraumatic arthritis in MRL/MpJ mice. Arthritis and rheumatism. 2013 Mar;65(3):660-70. Epub 2012/12/04.

7) A single dose of IL-1 Ra given intra-articularly immediately following closed fracture of the tibial plateau in B6 mice prevent development of PTOA changes at 8 weeks[10, 11].

8) Preliminary data in humans demonstrates elevation in inflammatory cytokines after articular fracture[12-16].

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Acute Tissue-Specific Responses to Joint Injury

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Osteoarthritis (OA) at its end stage is ultimately a diagnosis based on a collection of pathological changes in multiple joint tissues, detected in many different ways (imaging, histology, biomarkers etc), and with associated clinical symptoms. There may be many initiating causes and many pathways to reach this final point of "joint failure", the emerging paradigm being that OA rather than being a single entity is

actually a collection of disease sub-types with similar end-stage pathology.[1-8] The challenge is to identify patterns in this heterogeneity that will allow us to better sub-categorize the disease in question, and then define the associated pathophysiology of these different phenotypes. In addition to clinically defined phenotypes (e.g. post-traumatic, metabolic), it is increasingly recognized that OA is a disease of the entire joint organ, with varying pathology in all tissues. Thus some OA phenotypes may be categorized by the relative involvement or disease in the different tissues – e.g. degree of synovitis, bone erosion versus bone formation, greater or lesser cartilage loss. Pre-clinical research in OA, and particularly that using *in vivo* models, is increasingly embracing the study of the joint as an organ and interpreting data in the context of the different OA phenotype that is being modeled.[9-11] However there remain unanswered questions that have important implications for understanding OA pathophysiology and improved translation of pre-clinical findings to patinets.[12]

This presentation will discuss the findings from our research that is aimed to address a number of these issues (outlined below), using animal models of joint injury to induce post-traumatic OA.

1.   The different tissues in the joint function together and communicate via mechanical and soluble signals to maintain homeostasis. *Does the joint wide pathology in end-stage OA therefore represent an inextricable consequence of this, where primary pathology in one tissue will lead to derangement in the remainder (Fig 1)?* We have investigated the joint-wide pathology associations in two different mouse models over time, and shown distinctly different relationships not only between tissues in the two disease phenotypes, but also between structural damage, measures of pain and its molecular regulation.
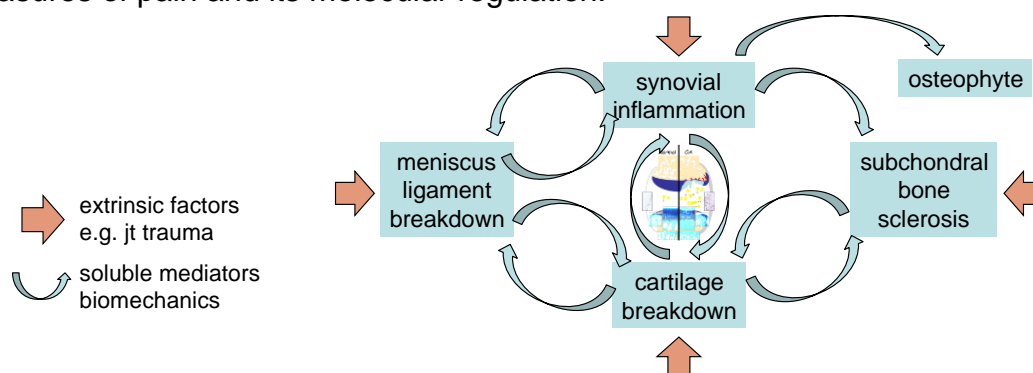
Figure 1. Pathogenesis of the joint-wide pathology in OA involves cross-talk between different tissues.

2. Current clinically defined OA phenotypes are quite broad delineating those that clearly have differing molecular pathophysiology and therefore likely distinct therapeutic targets and approaches e.g. post-traumatic (mechanical) OA, metabolic OA, inflammatory OA. *Is this level of stratification sufficient or is there evidence that further subdivision within one of these existing phenotypes is necessary?* We have examined different models of joint injury and subsequent post-traumatic OA development in mice and sheep, to investigate the contribution of biomechanics versus biological response of joint tissues to injury, to the onset and progression of OA pathology in different knee joint tissues and joint compartments. Together these studies have demonstrated that the injury mechanism and response of different joint tissues to the initial trauma, likely has a much greater impact on the development and progression of OA than the joint instability.

3. Joint injury results in inflammation, and the severity and resolution of this has been implicated in the long-term risk of OA. *Is there a difference in the inflammation that occurs with OA-inducing trauma/injury versus that which does not result in long-term OA?* We have examined the inflammatory cellular profile of synovial inflammation in mice and shown a distinct profile in OA-inducing injuries, and that targeting specific cell types can modulate long-term pathology.

Our data suggests that tissue-specific responses to joint injury are critical to the long-term consequences and risk of OA. Better defining how different joint tissues respond to injury and what factors regulate this will not only provide therapeutic targets, but improved diagnostics enabling better patient stratification (phenotyping) and appropriate treatment selection following joint injury.

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