Title: Osteoarthritis pathophysiology – therapeutic target discovery may require a multi-faceted approach.

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Introduction

Osteoarthritis (OA) is the single most common painful joint condition; knee OA alone currently affects >250 million people globally, and it is one of the fastest increasing health conditions worldwide[1,2]. The individual impact of OA includes pain and loss of both mobility and independence, 25% of patients being unable to carry out normal activities of daily life[3,4]. OA's societal burden is enormous, with current annual direct healthcare costs of knee OA alone estimated at up to $15 billion in the USA[5]. This figure is dwarfed by indirect costs of work absenteeism, early retirement, and loss of productivity associated with OA and associated medication use[6]. OA remains one of the major unresolved medical conditions, with no registered therapies that halt structural damage, and symptom-modifying interventions having only moderate long-term effect at best[7].

It is clear that developing new, safe, effective OA treatments is an international healthcare and socioeconomic priority. A key underpinning requirement for therapeutic advancement in OA, as it is for all diseases, is knowledge of the cellular and molecular pathophysiology[8]. There has been an extraordinary increase in understanding of human-relevant OA bio-molecular mechanisms over the last 15 years[9-11]. This has been associated with the recognition of OA as a joint-wide disease affecting and involving molecular and mechanical cross-talk between multiple tissues, and these with systemic processes and pathways. The complexity and breadth of new knowledge in OA pathophysiology, means the task of summarizing the key pathways is immense and crosses diverse mechano-biological domains. In the current review, we have therefore taken the approach to ask individuals with expertise in six different aspects of OA pathogenesis (cartilage matrix degradation, inflammation, fibrosis, failed cartilage repair, bone remodelling, and ageing), to provide a brief narrative review of what they consider the key disease mechanisms in their domain, with a lens to focus on those that may that offer the most promise for therapeutic targeting. The essays were written independently to avoid unintended collusion bias and are presented below, followed by a brief conclusion written after collation of the individual sections. We hope this approach will not only provide a different, interesting and more approachable review on a daunting topic but also allow identification of pathways and mechanisms that cross multiple aspects of OA and contribute to the changing crosstalk between joint tissues as disease progresses.

Targeting cartilage degradation to treat OA – Linda Troeberg

Degradation of the cartilage extracellular matrix (ECM) is appreciated to be an important feature of OA pathogenesis that, together with bone remodelling, leads to progressive joint damage and structural failure. Breakdown of type II collagen and aggrecan are thought to be most important, as these are the two most abundant cartilage matrix biomolecules and their loss reduces tensile strength and resistance to compression. A large body of evidence supports the conclusion that matrix metalloproteinases (MMPs) mediate type II collagen degradation, while related metalloproteinases, the adamalysins with thrombospondin motifs (ADAMTSs) are responsible for the degradation of aggrecan (Figure 1).

Type II collagen is a very stable molecule whose triple helical structure can only be cleaved by a handful of proteases, including cathepsin K and 4 collagenolytic MMPs (i.e. MMP1, 8, 13 and 14).

Collagen degradation occurs progressively in osteoarthritic cartilage[12,13], and can be blocked by metalloprotease inhibitors in vitro[14,15], suggesting that the collagenolytic MMPs play a central role in this catabolic process. Two key papers support the assertion that MMP13 is a key collagenase in OA. Firstly, transgenic mice overexpressing MMP-13 in cartilage exhibited increased collagen degradation by 5 months of age, along with increased cartilage erosion and joint pathology[16]. Secondly, *Mmp13*-null mice developed significantly less cartilage erosion 8 weeks after surgical induction of OA[17]. Expression of MMP13 is increased in human and murine OA cartilage, and is highly inducible in vitro by inflammatory cytokines. The catalytic domains of MMPs are structurally homologous, so it has historically been difficult to design inhibitors that effectively target a single MMP without undesirable side-effects. This is thought to be the reason that metalloproteinase inhibitors failed as cancer therapies[18], despite clear evidence showing the important roles of MMPs in cancer metastasis and progression. MMP13, however, is unusual among MMPs in that it has deep pockets in its active site, so attempts have been made to design MMP13 inhibitors as potential OA therapies - these fared well in pre-clinical models[19], but have not progressed further at present, most likely due to lingering concerns about lack of specificity and consequent toxicity.

The sequence of events in early OA is difficult to ascertain, but in vitro studies indicate that collagen breakdown starts relatively late in the pathogenesis of OA, while breakdown of aggrecan occurs earlier[20,21]. Importantly, aggrecan loss in these models is reversible, while collagen loss is not. For many years, MMPs were thought to be responsible for the pathological degradation of both collagen and aggrecan in OA cartilage, but this view was challenged by Sandy et al.[22], who showed that aggrecan fragments released into the synovial fluid of OA patients had been cleaved at the Glu^{373}~Ala^{374} bond, which is not targeted by MMPs. This sparked considerable interest in identifying the 'aggrecanases' or enzyme(s) responsible for pathological breakdown of aggrecan, as targets for development of OA therapies. The first 'aggrecanase' was purified from IL-1-stimulated bovine cartilage by Tortorella et al.[23], and named A Disintegrin And Metalloproteinase with Thrombospondin motifs 4 (ADAMTS4) based on its homology to ADAMTS1. Another aggrecanase, ADAMTS5, was cloned shortly afterwards[24,25], and subsequent studies indicated this is the main murine aggrecanase, since mice lacking *Adamts5* were protected against aggrecan degradation and cartilage damage in 2 pre-clinical models of OA[26,27]. ADAMTS5 may also be the primary human aggrecanase[28], although ADAMTS4 may also play a role.

Aggrecanase inhibitors have been designed by several groups, with some of these showing promising efficacy in pre-clinical models[29]. For example, Galapagos and Servier developed an ADAMTS5 catalytic domain inhibitor, GLPG1972/S201086, with good selectivity for ADAMTS5 and efficacy in preclinical rat and mouse OA models[30,31]. However, this inhibitor failed to meet its primary outcome (reduction in cartilage loss over 1 year by qMRI) or secondary outcomes, including pain and structural progression in a clinical trial (https://clinicaltrials.gov, NCT03595618). Some groups have taken the approach of designing inhibitors that target the non-catalytic domains of ADAMTSs, to reduce the potential for cross-reactivity and off-target inhibition of homeostatic MMPs and related metalloproteases such as ADAMs. For example, Santamaria et al.[32] recently generated small molecule exosite inhibitors of ADAMTS5, and Merck generated a cross-domain bi-specific nanobody with good efficacy in a murine OA model[33]. However, a word of caution was raised by GlaxoSmithKline[34], who

found that their antibody against ADAMTS5 caused cardiac abnormalities in cynomolgus monkeys, which they suggest may relate to expression of ADAMTS5 in cardiovascular tissue. ADAMTS5 also has homeostatic roles in other tissues (reviewed by Santamaria[35]), suggesting further challenges for inhibitor design.

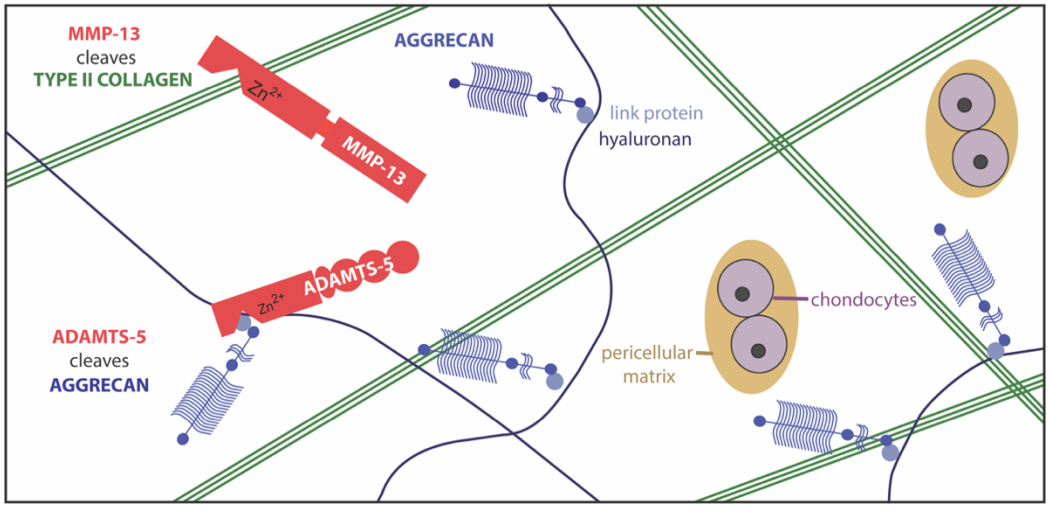

Figure 1: MMPs and ADAMTSs metalloproteases cleave type II collagen and aggrecan in the OA cartilage extracellular matrix. Chondrocytes secrete metalloproteases that degrade the cartilage extracellular matrix in OA. Studies on transgenic mice suggest that MMP13 is the key collagenase in cartilage, while ADAMTS5 is the main 'aggrecanase'.

Targeting inflammation to treat OA - Christopher B. Little

Historically osteoarthritis (OA) was considered a non-inflammatory "degenerative-joint-disease", and alternative names such as osteoarth**rosis** were proposed. However, just as the concept of passive "wear-and-tear" OA cartilage loss has been replaced with an understanding of a dynamic balance between bio-cellular repair and destruction (see sections by Troeberg and Vincent), the presence of synovial inflammation is now a well-recognized and consistent finding in OA patients and pre-clinical animal models[10,36-38]. OA synovium, even in early-stage disease, displays focal hyperplasia and hypertrophy of synovial lining cells, subintimal accumulation of inflammatory cells (macrophages, lymphocytes, plasma cells) and increased vascularity[39,40], along with progressive fibrosis of the joint capsule (see section by Kapoor). In the OA knee, the infrapatellar fat pad as part of the functional synovial unit also has increased inflammatory cells and fibrotic changes, although notably with some unique characteristics compared with other synovial tissues[41-43].

Synovial inflammation in OA is associated not only with symptoms but structural disease severity and progression in patients[44-48]. Beyond simply being a secondary response to late-stage joint tissue breakdown, synovial inflammatory mediators are more elevated acutely after OA-inducing joint injury[49] and in early compared with late OA[39,46,50,51]. Importantly, synovitis/joint-effusion is associated not only with faster progression of established disease, but also more incident OA[44] and increased risk of post-traumatic OA following joint injury[52]. In light of this, it seems clear that the **"itis"** in OA is indeed

appropriate, not only from the perspective of correctly describing the presence of synovial inflammation but also its potential pathophysiological role in initiation and progression of structural and symptomatic disease.

Activation of the innate inflammatory/immune response in OA has been well-described and may be triggered by mechanical injury directly, as has been proposed for articular cartilage[53]. It is characterized by the influx of blood-derived monocytes and macrophages, which may contribute directly to increases in cytokines, growth factors and pathologically-relevant enzymes (e.g. IL1, IL6, TNF, TGFβ, MMP1, MMP13, ADAMTS4, ADAMTS5)[54-56]. Lymphocytes, particularly CD4 and CD8 T-cells are also increased in OA synovium even in early disease stages, these cells producing cytokines, chemokines and enzymes implicated in disease progression (e.g. IL8, IL17, TNF, CCL2, MMP1, MMP3, MMP9)[39,41,43,57-59]. Stromal cells in the synovium and other joint tissues (e.g. injured cruciate ligament) also increase synthesis of cytokines and chemokines[60-62], and OA chondrocytes themselves increase expression of pro-catabolic cytokines (e.g. IL8, IL12, IL17) that may act in an autocrine or paracrine manner to promote cartilage degradation[63]. Finally, systemic inflammation, particularly circulating cytokines (e.g. IL6, TNF) and activated monocytes (associated for example, with obesity/metabolic-syndrome), further contribute to the pro-inflammatory milieux and complex cellular cross-talk that may initiate, perpetuate and exacerbate joint-wide OA structural pathology and pain (Figure 2)[54,64-67].

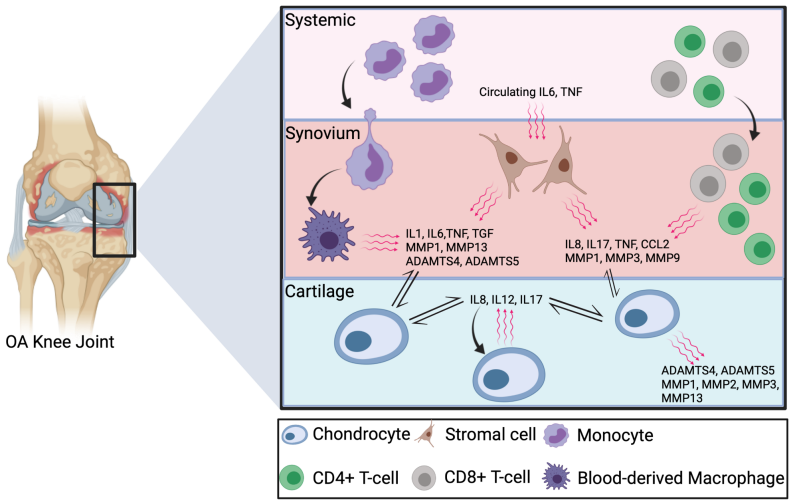

Figure 2: Schematic image depicting the key inflammatory cells and soluble mediators and pathways implicated in osteoarthritis pathogenesis.

The discussion above provides a glimpse of the burgeoning evidence for up-regulation of a multitude of inflammatory pathways locally and systemically in OA, involving innate and adaptive immune cells, and numerous cytokines, chemokines and growth factors. *Dysregulation does not equal causality however, so is there data supporting the therapeutic potential of targeting "inflammation" in OA, and which if any of the pathways may hold the most promise?* Notwithstanding that samples are predominantly from late-stage disease, unbiased genome-wide mRNA expression

and network analyses of different human joint tissues have identified highly-relevant/hub genes and/or inflammatory processes in OA[54,57,60,63,68]. While there are, not surprisingly, some differences between joint-tissue compartments and even cells with a given tissue, the commonly identified dysregulated inflammatory pathways in OA include: IL1, IL6, IL8, IL12, IL17, TNF, CCL2, M1/M2 macrophage polarization and Th1 and Th17 CD4 T-cells. There is supporting evidence from pre-clinical tissue culture and/or animal models, that inhibiting or ablating any of the above identified inflammatory pathways can modify onset, progression and/or severity of various aspects of joint-wide structural and/or symptomatic OA[10,38].

Despite the above evidence, clinical trials targeting some of these pathways in OA patients have been disappointing[69,70]. *Does this mean inflammation is not as important an OA-therapeutic target as it appeared?* In answering this, it is noteworthy that variable outcomes are reported in different OA disease models and model systems e.g. IL1 in mono-idodacetate-, meniscal destabilization-, meniscectomy- and collagenase-induced OA[71-74]; IL6 in post-traumatic and age-associated OA[75,76]. This pre-clinical data suggests that the specific inflammatory pathways involved and therefore usefully therapeutically targeted, may differ depending on the disease model i.e. it is disease-phenotype-dependent. This is consistent with human OA patient data showing differences in inflammatory dysregulation e.g. in hip versus knee OA[77], in knee OA in males versus females[78], and the cytokines that correlate with different aspects of knee OA pain[46,79]. Even within a given OA population, distinct inflammatory cell/cytokine patient clusters can be identified e.g. in those presenting for knee replacement[80].

This OA inflammatory heterogeneity may, at least in part explain the poor outcomes from clinical trials[70]. Just as in recognized inflammatory arthropathies[81,82], a more nuanced approach to anti-inflammatory therapy in OA may be needed for example selecting patients with a more inflammatory clinical phenotype, or potentially using biomarker analysis to identify particular inflammatory molecular endotypes within OA sub-populations. This is supported by serendipitous data from a large trial of IL1β inhibition for myocardial infarction in patients with elevated C-reactive protein, that demonstrated a significant reduction of incident or worsening OA symptoms and rates of total knee and hip replacement[83]. While not designed with OA-relevant structure and symptom outcomes, this study strongly suggests that targeting the right inflammatory pathways in the right patients at the right time may make significant inroads to successfully treating OA (Table 1). As with many of the potential OA therapeutic approaches based on targeting pathophysiologic pathways, developing biomarkers to identify different patient cohorts is a key research imperative.

Table 1. Evidence in favour of targeting inflammation in osteoarthritis
• Human and preclinical animal model data consistently shows upregulation and activation of inflammatory and immune pathways in the joint and systemically. • Data from selective preclinical in vitro and in vivo models confirm that genetically or pharmacologically inhibiting specific inflammatory cytokines and immune cells can reduce structural and/or symptomatic OA.

- High priority, and potentially joint-tissue and OA-phenotype specific inflammatory pathways identified from unbiased genome-wide human OA expression studies.
- Many efficacious therapeutics already developed, approved and in clinical use in other diseases, could be repurposed for specific OA phenotypes.
- Treating systemically with an inhibitor of IL1β (canakinumab) shows disease modification in patients with a systemic inflammatory phenotype.

Targeting synovial fibrosis to treat OA – Mohit Kapoor

The synovial membrane is a thin membrane that surrounds articular joints and comprises two main layers; a cellular intima and underlying collagen I-rich sub-intima[84,85]. The synovium is required to maintain joint integrity, lubrication and homeostasis. While the majority of OA research has focused on mechanisms associated with articular cartilage degeneration, it is now believed that changes in the synovium may play an active role in driving OA pathogenesis. During OA, synovium presents with different synoviopathies, including inflammatory (see preceding section), hyperplastic, fibrotic and detritus-rich forms[86].

Synovial fibrosis is characterized by excessive ECM deposition and contributes to the joint stiffness and pain associated with OA. Underlying endogenous mechanisms associated with synovial fibrosis are not well characterized and several critical questions remain to be answered: (*1) Why and how does synovial fibrosis occur?; (2) Which cell types are responsible for the initiation and progression of synovial fibrosis?; (3) How can we control fibrosis to reduce structural and symptomatic OA?*

Fibrosis is speculated to occur due to uncontrolled tissue repair responses, prolonged inflammatory insults, and cross talk between a variety of endogenous pro-fibrotic molecular and cellular mechanisms. The synovium consists of cells including, but not limited to, fibroblast-like synoviocytes (FLS) and macrophages. FLS are the key cell type of the synovium that is responsible for maintaining homeostatic functions and promoting inflammatory and fibrogenic responses (reviewed in [87]). FLS respond to a wide array of stimuli in the OA joint microenvironment resulting in increased proliferation, migratory capacity and acquiring a myofibroblast like phenotype (Figure 3), all contributing towards increased ECM deposition and fibrogenic responses in the synovium. Some of the key triggers associated with FLS activation include transforming growth factor-beta (TGFβ), cartilage wear products, Wnt/beta-catenin signaling pathway, and hypoxia inducible factor-1 alpha[88-94], among others. TGFβ is a major pro-fibrotic mediator known to activate FLS and induce their transition to highly contractile myofibroblast-like cells that are believed to be involved in excessive ECM accumulation in the synovium[95]. Targeting TGFβ and its signaling to achieve anti-fibrotic effects has proved to be complex due to its homeostatic roles in other joint tissues such as the articular cartilage[96,97].

At this point, clinical evidence to support efficacy of anti-fibrotic therapies to minimize the degree of joint destruction during OA requires further investigation. One could speculate that controlling inflammation during early stages of OA initiation and development could indirectly minimize the pro-fibrotic events and associated pathological mechanisms. Another potential therapeutic modality may

include the simultaneous targeting of inflammation and fibrosis using a combination of anti-inflammatory and anti-fibrotic agent(s). In this context, Pirfenidone, an anti-inflammatory and anti-fibrotic drug currently approved for the treatment of idiopathic pulmonary fibrosis[98], has been shown to attenuate synovial fibrosis and delay the progression of OA in a preclinical model[99]. Future clinical trials would help determine the therapeutic efficacy of such drugs and agents in reducing fibrosis and minimizing the degree of joint destruction during OA.

The Wnt family of proteins are also involved in OA pathogenesis[100-102] and have drawn significant attention in the OA field. For instance, a phase II study of Lorecivivint (SM04690) an inhibitor of intranuclear kinases CDC-like kinase 2 (CLK2) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) that modulates the Wnt pathway, shows initial efficacy in improving pain, function and joint space narrowing in patients with unilateral moderate to severe symptomatic knee OA[103], with phase III trials currently underway[104]. Preclinical studies using SM04690, shows cartilage protective effects *in vivo*[105]. It would therefore be of interest to investigate the potential of SM04690 to reduce synovial inflammation and fibrosis in preclinical animal models and in clinical trials. In this context, intra-articular injection with XAV-939, a small-molecule inhibitor of Wnt/β-catenin signaling, reduces the degree of synovitis and cartilage degeneration in a mouse model of knee OA *in vivo*, and reduces proliferation and collagen synthesis of FLS treated with XAV-939 *in vitro*[90]; however, it remains to be determined if XAV-939-induced cartilage protective effects are driven by reductions in synovitis or vice versa.

Research on synovium as a key driver of OA pathogenesis is garnering significant attention in the OA field. To better understand the contribution of synovium and to devise adequate therapeutic strategies to control processes such as synovial fibrosis and inflammation in joint destruction, it is essential to identify and understand the roles of individual synovial cell types and subpopulations that are involved in the initiation and progression of OA. The emergence of single cell sequencing, high throughput omics technologies and advanced bioinformatics provides an excellent opportunity to deep dive into the role of the synovium in OA pathogenesis. Applying these technologies to investigations using pre-clinical animal models and well characterized human OA synovial samples will allow for the identification of putative therapeutic targets that may limit pathological processes in OA, including synovial fibrosis.

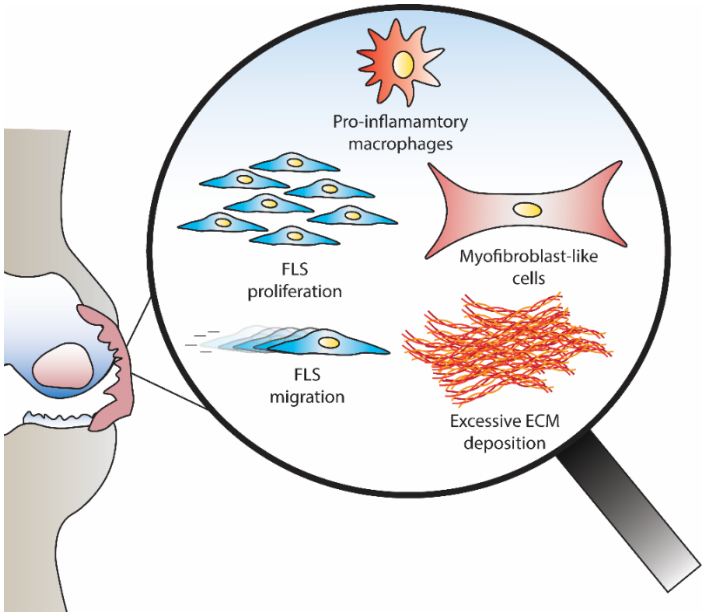

Figure 3: *Fibroblast like synoviocytes (FLS) and macrophages are key cell types present in the synovium. FLS exhibit increased proliferation and migration, and also acquire a myofibroblast like phenotype, resulting in the excessive ECM deposition in the synovium during osteoarthritis.*

Targeting regeneration of cartilage to treat osteoarthritis - Tonia L. Vincent

OA textbooks frequently describe OA as a disease determined by the balance between catabolic and anabolic pathways activated within the tissue. Historically this was based on the observation that some chondrocytes appeared to display an exuberant synthetic response in OA tissue when measuring uptake of radiolabelled sulfate (indicative of synthesis of sulfated proteoglycans), whilst other chondrocytes were in regions of the matrix completely devoid of proteoglycan[106]. Later evidence was based on transcriptomic analyses where evidence of new matrix synthesis was often upregulated alongside catabolic enzymes and other inflammatory molecules[107,108]. The textbooks shied away from describing the anabolic response as evidence for regenerative activities, as the pervasive view had been that articular cartilage was incapable of repairing itself.

Mounting evidence in the past 10 years indicates that this paradigm is incorrect. Not only is there evidence from careful prospective arthroscopy studies that many focal cartilage lesions heal spontaneously (reviewed in[109]), but also that established OA can repair if the hostile mechanical environment of the joint is corrected e.g. by joint distraction, using an external frame attached above and below the joint, or by high tibial osteotomy[110,111]. Such studies demonstrate MRI-proven regeneration of cartilage-like tissue even where the erosion was down to the underlying bone[112]. In the case of joint distraction, which is typically in situ for 6 weeks, this tissue appears to be maintained up to 2 years after removal of the frame and is associated with a sustained clinical benefit over longer periods[113]. Whether the tissue that is produced is true hyaline cartilage with newly synthesized type II collagen or fibrocartilage (type I collagen rich) is unclear. This fact may also be irrelevant so long as it shows resilience over time with associated symptom improvement. Several studies point to minimal type II collagen incorporation after skeletal maturity, which does not appear to change with OA[114,115].

Molecular mechanisms that underly regenerative activities in articular cartilage are being revealed. These fall into two broad areas: the identity, control and activity of progenitor cells in the joint that mediate cartilage repair, and tissue factors that signal the injury and activate the tissue repair response. Meachim famously described two distinct repair responses in articular cartilage; one which was 'intrinsic' to the cartilage, mediated by cells that resided within the substance of the tissue, and a second which was mediated by cells migrating from the underlying bone marrow (especially where the osteochondral junction had been breached), which he called 'extrinsic' repair. Intrinsic repair was thought to be mechanically superior, producing excellent integrated hyaline cartilage compared with the fibrocartilaginous response elicited by extrinsic bone marrow derived cells. He concluded that, as extrinsic repair was rapid, you needed to suppress this to enable intrinsic repair to occur (reviewed in[116]). Several groups since this time have described pluripotent progenitor cells that can be expanded in vitro from cells derived from the articular cartilage[117-119]. Repair cells have also been identified in the synovium, periosteum and synovial fluid taken from human OA joints[120-122]. Such cells may be quite distinct from classical mesenchymal stem cells derived from the bone marrow. For instance, synovial derived cells are marked by being GDF5 positive, arising from those cells that originated from the joint interzone during development[123]. Collectively these results are consistent with Meachim's idea that intrinsic repair cells are distinct from those derived from the bone marrow. It also raises the possibility that orthopedic procedures such as Pridie drilling may be encouraging extrinsic repair by stimulating bone marrow derived MSCs, and this may not be in the long term interests of the tissue.

The tissue injury signals likely originate from the cartilage matrix itself. The pericellular matrix, a region immediately surrounding individual chondrocytes in the tissue is rich in the proteoglycan perlecan, upon whose heparan sulfate chains are attached a number of heparin binding growth factors [124]. Four such growth factors were identified by proteomic analysis, including FGF2, CCN2 bound to latent TGFβ, hepatoma derived growth factor and CCN1[125,126]. These are released immediately in response to mechanical injury of the tissue by a mechanism that involves a localized increase in sodium concentration as water is squeezed out of the compressed tissue[127]. This is sufficient to displace the growth factors from their pericellular matrix binding sites and allow their binding to high affinity cell surface receptors (Figure 4). In osteoarthritis, when proteolytic activity causes loss of the negatively charged aggrecan from the tissue, the sodium is no longer held in the tissue and mechanical compression is unable to generate the concentration of sodium required to release growth factors[127]. These results indicate that proteolytic loss of aggrecan in OA suppresses intrinsic repair just at the time it is most needed. FGF2 and TGFβ are the best described of these molecules and are known chondroprotective and chondrogenic molecules in preclinical and in vitro studies [128-130]. They are also implicated in repair responses in other tissues such as the skin [131].

The clinical relevance of TGFβ and FGF family members in cartilage repair is strongly supported by agnostic evidence arising from recent genome wide association studies in OA. To date, polymorphic variants associated with expression of eight members of the TGFβ family (TGFβ1, TGFβ2, LTBP1, LTBP3, GDF5, SMAD3, ACVR1, BMP5) and two members of the FGF family (FGF18, FGFR3) have been documented[132-136]. Where described, these are hypomorphic variants

associated with increased OA, thus confirming their chondroprotective role in human OA. Other growth factor families also emerge, such as the Wnts (DOT1L; WNT9a, WNT1, WNT10a) and TGFα, a ligand for the epidermal growth factor receptor (EGFR). Very few recognizable 'inflammatory' genes are identified in these analyses raising the possibility that OA could be viewed primarily as a disease of failed repair (Table 2).

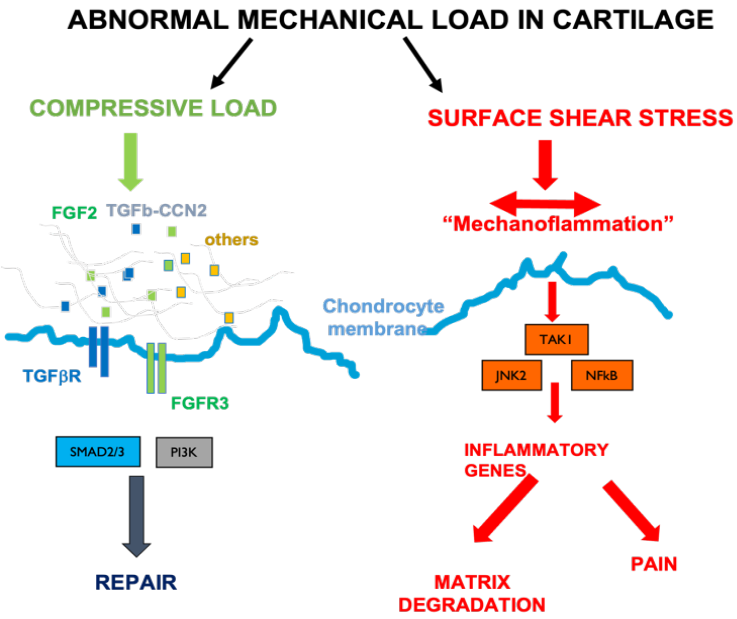

Figure 4. Balance of pro-regenerative and mechano-inflammatory responses in articular cartilage with abnormal mechanical load. *Compressive load leads to sodium dependent release of pericellular matrix growth factors, which drive repair and chondroprotection through a variety of intracellular signalling pathways. Surface shear stress (perpendicular to compressive load) leads to activation of TGFβ-activated kinase 1 (TAK1) dependent inflammatory signalling and results in nerve growth factor regulation (driving pain) and matrix degradation.*

So will this change our approach to disease modification in OA? Evidence to support this concept is already emerging. To date, the only successful structure modifying pharmacological trial is that using intraarticular injections of sprifermin, a truncated form of FGF18. In this extended 3 year trial, (the study was originally 2 years[137]), there was evidence of a delay in cartilage loss in the sprifermin group and increased cartilage thickness measured in the affected and unaffected regions of the joint[138]. Although not reaching its primary endpoint for symptoms, a recent post hoc analysis, considering a 'subgroup at risk' of progression (defined by lower joint space width and higher pain at baseline), was able to demonstrate both structural and symptomatic improvement over the study

period[139]. Collectively these data appear to represent a striking U-turn for molecular pathogenesis and target discovery in OA.

Table 2. Evidence in favour of targeting cartilage regeneration in osteoarthritis
• Experimental data in preclinical models show that articular cartilage makes a strong synthetic response after injury and in some instances can repair. • Human data show spontaneous cartilage repair, especially when the hostile mechanical environment in OA is corrected. • Articular cartilage matrix is full of chondroprotective growth factors that are released upon tissue injury. This response is lost in OA when the tissue loses aggrecan. • Growth factor families arise from large scale agnostic genome wide association studies in OA • Delivery of a growth factor (sprifermin, modified FGF18) intra-articularly shows disease modification in Phase II clinical trials.

Targeting bone remodeling to treat OA – Tamara Alliston

In the healthy joint, subchondral bone provides mechanical and vascular support to overlying avascular cartilage[140]. Given this vital role in joint structure, function, and shape, it is not surprising that subchondral bone is thought to be both a target and a driver of osteoarthritis progression.

Human imaging studies demonstrate that changes in the subchondral bone compartment both precede and predict degradative changes in overlaying cartilage; with the effects of OA apparent on the thin subchondral bone plate, subchondral trabecular bone, and the surrounding bone marrow. First, subchondral bone loss early in OA, due to increased bone remodeling by osteoclasts and osteoblasts, is followed by radiographic detection of sclerosis, or thickening, of the subchondral bone plate and trabecular bone[141 142]. Second, machine learning analysis of magnetic resonance imaging (MRI) in the Osteoarthritis Initiative identify changes in subchondral bone shape as one of the earliest known predictors of OA, as well as joint pain[143] . Third, the appearance of bone marrow lesions (BML) in clinical MRI is associated with joint pain and increased cartilage loss[144]. Histologically, BMLs are associated with greater cartilage degeneration, increased marrow vasculature, fibrosis, and edema, and increased osteoid deposition and osteocyte density[145,146]. BMLs appear to be a response to subchondral bone microdamage, resulting from traumatic injury or mechanical insufficiency of

subchondral bone. Therefore, the bony sclerosis, changes in joint shape, and bone marrow lesions in OA subchondral bone are diagnostically and clinically significant because they can be detected early in OA and can predict OA progression and joint pain.

These changes in subchondral bone motivate bone-targeting therapies to prevent or treat OA, some of which have been tested clinically, but still with limited success. In an effort to abrogate the hyperactive subchondral bone remodeling that occurs early in OA, osteoclast-inhibitory bisphosphonates have been evaluated in clinical trials for OA. Bisphosphonates may indeed be therapeutically beneficial in a subset of non-overweight individuals with early stage OA[147], even though this clinical benefit was not observed in a meta-analysis of randomized control trials[148]. In clinical trials, cathepsin K inhibitors, which suppress bone remodeling, prevent changes in subchondral bone and cartilage, but were ineffective for treating OA pain[149]. Other bone-targeting agents with potential to impact OA progression, including estrogen, PTH, TGFβ antagonists, and calcitonin, show benefits in pre-clinical studies, but have yet to be tested in randomized clinical trials, or to show reproducible clinical benefits in diverse human cohorts[140].

Discrepancies between the success of pre-clinical and clinical studies still limit the clinical application of bone-targeting agents to treat OA. A more precise stratification of OA subtypes, perhaps with the help of new genetic, serum, and imaging biomarkers, could improve the identification of patients who would benefit from bone-targeting therapies. Another possibility is that bone-targeting therapies for OA are still missing a critical cellular target – osteocytes.

The cellular mechanisms by which changes in subchondral bone propel cartilage degeneration have largely been attributed to osteoblasts and osteoclasts. However, the contribution of osteocytes, the most abundant bone cell type, in OA has been overlooked until recently[140]. Over the past ten years, the dynamic role of bone-embedded osteocytes in bone homeostasis has become more clear. Osteocytes couple mechanical demands to bone resorption and deposition by osteoclasts and osteoblasts through mechanosensitive secretion of Rank Lignad (RANKL;TNFSf11) and Sclerostin, respectively[150-152]. Furthermore, through the process of perilacunar/canalicular remodeling (PLR), osteocytes directly resorb their local ECM) by secreting acid and proteases such as MMP13 and cathepsin K, and then later deposit new ECM. PLR maintains systemic mineral homeostasis, bone quality, and the intricate lacunocanalicular network (LCN), which enables osteocytes to communicate with one another and the vascular supply[153-155] . Since cartilage relies on

subchondral bone for mechanical and vascular support, understanding the impact of OA on osteocytes, and vice versa, became a critical question.

Several lines of evidence support a causal role for osteocytes in the progression of OA. Relative to non-OA cadaveric controls, subchondral bone from human OA surgical retrieval specimens shows several hallmarks of deregulated osteocyte function, including LCN degeneration, collagen disorganization, and heterogeneous mineralization[156]. Furthermore, osteocyte-intrinsic defects in genetically modified mice were sufficient to exacerbate cartilage degeneration and mimic several features of human OA subchondral bone. Specifically, mice with an osteocyte-targeted ablation of the PLR enzyme MMP13 exhibit cartilage degeneration, accompanied by sclerotic subchondral bone with degenerated LCN, disorganized collagen, and heterogeneous mineralization[156]. Similar results are observed upon osteocyte-intrinsic inhibition of TGFβ signaling through targeted ablation of the TGFβ type II receptor ($T\beta RII^{ocy-/-}$)[157]. Recently, several genes in the osteocyte transcriptome were shown to have significant associations with OA in a human GWAS study, including MEPE, TSKU, SEMA3F, SEMA3G and SEMA7A, which are expressed in osteocytes but not in chondrocytes[158]. Thus data from human clinical and genetic studies, as well as from mouse models with osteocyte-intrinsic mutations, support a causal role of osteocyte dysfunction in OA.

While the mechanisms by which osteocytes affect cartilage remain to be determined, the importance of their participation in subchondral bone and cartilage homeostasis, and joint disease, is clear. Computational modeling predicts that degeneration of the osteocyte LCN in aged or $T\beta RII^{ocy-/-}$ mouse bone, relative to young or control bone, is sufficient to compromise bone mechanosensitivity and solute transport[159]. Either of these mechanisms could compromise cartilage integrity. Uncoupling bone remodeling from mechanical stimuli could contribute to subchondral bone sclerosis. LCN degeneration could interfere with the ability of bone vasculature to support cartilage. Interestingly, the downregulation of osteocytic TGFβ signaling and MMP13 is a common feature in human OA subchondral bone, the $T\beta RII^{ocy-/-}$ mouse model, aging mouse bone, and wild type mouse bone following meniscal ligamentous injury[156,157,159] (Table 3). Determining whether the relationship between OA and osteocytic TGFβ signaling and MMP13 are correlative or causal in aging will require further investigation. Either way, these observations highlight the need to consider the joint-compartment-specific effects of each factor. For example, agents that suppress MMP13 may protect cartilage from proteolytic degradation, while simultaneously interfering with osteocyte functions

required for cartilage homeostasis. Unfortunately, diagnostic markers of osteocyte function, or osteocyte-specific therapies currently do not exist. Although the "osteocyte transcriptome“ identifies genes that are specific to osteocytes, relative to other skeletal cell types, more work is needed[158]. Continued efforts to understand osteocyte function and regulation, in the healthy skeleton and in aging and disease, are needed to develop new strategies to monitor and target subchondral bone to prevent or treat joint disease.

Table 3. Osteocyte-intrinsic inhibition of MMP13 or TGFβ signaling is sufficient to mimic several hallmarks of human osteoarthritis

	Human OA	MMP13$^{ocy-/-}$	TβRII$^{ocy-/-}$
Cartilage Degeneration	✓	✓	✓
Subchondral Sclerosis	✓	✓	✓
Thickened Subchondral Plate	✓	✓	✓
Collagen Disorganization	✓	✓	
Mineral Heterogeneity	✓	✓	
Degenerated Osteocyte LCN	✓	✓	✓
Impaired Mechanosensitivity			✓
Altered TGFβ signaling	✓		✓
Altered MMP13 activity	✓	✓	✓

Targeting aging and cell senescence to treat OA - Richard F. Loeser

There is no doubt that aging processes, both systemic and within joint tissues, contribute to the pathophysiology of OA. The prevalence of radiographic and symptomatic OA in all the commonly affected joints, including hands, hips, knees, and spine, increases with increasing age[160]. The prevalence of OA and the pain and loss of function associated with it make OA one of the leading causes of disability in older adults worldwide[161]. What is not clear is precisely how aging promotes the development of OA or if targeting aging processes would slow or halt OA progression. This essay will

focus on cell senescence in OA and address the question of whether targeting senescent cells would be of therapeutic benefit.

Nine hallmarks of aging have been proposed that include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, stem cell exhaustion, altered intercellular communication, and perhaps most importantly, cellular senescence[162]. Many, if not all these aging hallmarks have been investigated in the context of joint tissue aging, with the majority of the published work focused on articular cartilage and its resident cell, the chondrocyte[163]. A common denominator to the hallmarks of aging is cell senescence, as the other hallmarks can either lead to senescence or result from the senescent state.

The literature to date strongly supports cell senescence as a major factor contributing to age-related diseases including OA[164,165]. Cell senescence can be defined as a state of growth arrest that prevents further cell division and results in typical phenotypic changes[162,164]. Importantly, cell senescence is not just a phenomenon seen after replicating cells have stopped dividing due to telomere shortening. Senescent cells contribute to tissue development during embryogenesis, tissue repair during wound healing, and suppress tumor formation by preventing the propagation of damaged cells[164,166]. Cell senescence can result from multiple chronic stresses that result in an accumulation of cellular damage, many of which are relevant to factors thought to contribute to OA (Figure 5). DNA damage is a central mediator of cell senescence and has been shown to induce senescence in chondrocytes[167]. The OA joint has often been referred to as a “chronic wound” with irreparable damage, the type of environment that can promote cell senescence. Chronic signaling from inflammatory factors such as cytokines has been proposed to result in “stress-induced” senescence resulting from a feed forward loop[168]. This could be a very relevant mechanism for senescence in the joint.

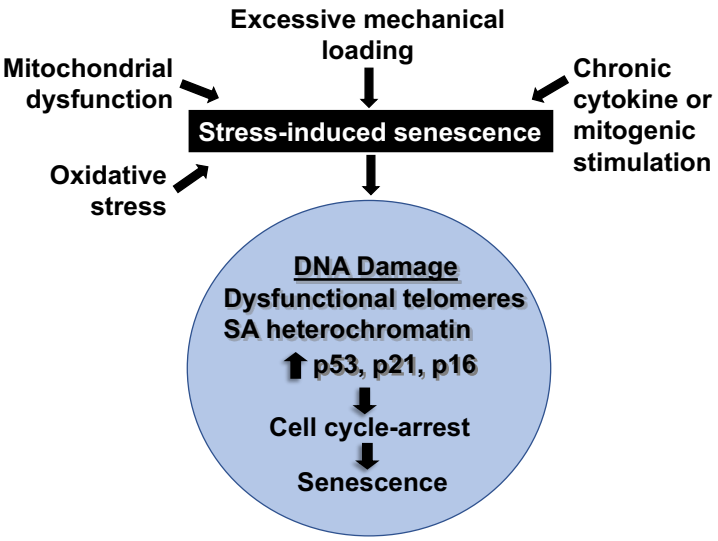

Figure 5: *Factors that promote stress-induced senescence*

A central mechanism by which senescence contributes to disease is through the production of inflammatory cytokines and matrix degrading enzymes, referred to as the senescence-associated secretory phenotype or SASP[164]. Many of the proinflammatory mediators and matrix degrading enzymes considered to be SASP factors (Table 4) are found in the OA joint[54,169,170] and may directly contribute to the tissue changes seen in OA. Increased expression of $p16^{INK4a}$, a cell cycle inhibitor, is considered one of the most reliable markers of cell senescence[164]. $p16^{INK4a}$ mRNA expression was found to be significantly increased with age in murine cartilage and in primary human chondrocytes from cadaveric tissue donors and this correlated with expression of the SASP transcripts IGFBP3, MMP1 and MMP13[171]. However, deletion of $p16^{INK4a}$ in chondrocytes of adult mice did not mitigate SASP expression and did not alter the severity of age-related OA, suggesting the effects of chondrocyte senescence on OA are most likely driven by the production of SASP factors and not by the loss of chondrocyte replicative function that occurs with increased $p16^{INK4a}$.

Table 4. *Senescence-Associated Secretory Phenotype (SASP) Factors Most Relevant to OA*

Class	Component
Cytokines	IL1, IL6, IL7, IL13, IL15, IL17, OSM
Chemokines	IL8 (CXCL15), GRO (CXCL1), MCP1 (CCL2), MIP1α (CCL3), ENA78 (CCXL5)

Other inflammatory molecules	TGFβ, MIF
Growth factors, regulators	EGF, FGF2, HGF, VEGF, SDF1 (CXCL12), NGF, IGFBP2, IGFBP3, IGFBP4, IGFBP6, IGFBP7
Proteases and regulators	MMP1, MMP3, MMP10, MMP12, MMP13, MMP14, TIMP1, TIMP2, PAI1 (SERPINE1), PAI2 (SERPINEB2), CTSB
Receptors and ligands	OPG (TNFRSF11B), sTNFRI (TNFRSF1B), sTNFRII (TNFRSF1A), FAS, uPAR (PLAUR), EGFR
Non-protein molecules	PGE2, nitric oxide, reactive oxygen species
Insoluble factors	fibronectins, collagens

Adapted from Gorgoulis et al, Cell 2019; 179:813-827. Abbreviations: bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; ENA, epithelial neutrophil-activating peptide; GRO, growth-related oncogene; HGF, hepatocyte growth factor; IGFBP, insulin-like growth factor binding protein; IL, interleukin; MCP, monocyte chemotactic protein; MIF, macrophage inhibitory factor; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; NGF, nerve growth factor; OPG, osteoprotegerin; OSM, oncostatin M; PAI, plasminogen activator inhibitor; PGE2, prostaglandin E2; SDF, stromal cell-derived factor; TGF, transforming growth factor; sTNFR, soluble tumor necrosis factor receptor; TIMP, tissue inhibitor of metalloproteinases; uPAR, urokinase-type plasminogen activator receptor; VEGF, vascular endothelial growth factor.

It has been suggested that senescent progenitor cells may be present in aged cartilage and release inflammatory mediators, including IL8, to promote the SASP[172]. Transplantation of senescent cells into mouse knee joints was shown to promote OA-like changes[173]. NFκB is considered a key regulator of the SASP[164] and a recent study found activation of NFκB signaling in mice promoted age-related OA and production of SASP factors[174]. Other important regulators of the SASP include C/EBPβ, STAT3, and GATA4, while the SASP may be inhibited by activity of FOXOs[164,166]. Importantly, all these mediators have also been implicated in OA pathogenesis[175-179], providing further support for a strong connection between SASP regulation and the development of OA.

Perhaps the strongest evidence for a causal role of senescent joint tissue cells in OA comes from studies that have demonstrated reduced OA severity in the anterior cruciate ligament transection

model of post-traumatic OA and in age-related OA in mice treated with small molecules called "senolytics" to selectively kill senescent cells or using a molecular approach to kill senescent cells expressing p16[180,181]. However, translation of this pre-clinical work to the treatment of human OA has not yet been realized. The senolytic compound UBX0101 that reduced OA severity in mice, did not achieve a significant reduction in WOMAC knee pain compared to a placebo when tested as an intra-articular therapy in a 12-week Phase 2 clinical study in humans (UNITY Biotechnology Announces 12-week data from UBX0101 Phase 2 Clinical Study in Patients with Painful Osteoarthritis of the Knee | Unity Biotechnology).

There are many possible reasons why a single injection of a senolytic drug would fail in a short-term trial with pain as the outcome. Clearly, further work is needed to: a) define an OA phenotype that may be more responsive to an intervention targeting senescent cells by discovering one or more biomarkers of joint tissue senescence; b) decide on the timing in the disease course of when such an intervention would be most useful; c) establish how many doses of the senolytic would be needed, and d) determine what outcome measures in early phase studies would best predict efficacy. Alternatives to killing senescent joint tissue cells with a senolytic also need to be developed such as "senomorphics" that target the production of SASP factors[182]. Although the link between aging and the development of OA is well established, and the underlying mechanisms are becoming clearer, the field is still not at the point where targeting a specific aging process to slow OA progression and improve symptoms is possible.

Conclusions

Molecular pathogenesis is a relatively new scientific discipline in OA. The scientific community has needed to overcome significant hurdles associated with working with matrix-rich pauci-cellular tissues, and to develop pre-clinical models of disease that are accepted as being clinically informative. In recent years, additional molecular insights have emerged from agnostic ~omic studies such as genome wide association studies. Being a highly prevalent condition, such studies can be performed in very large numbers to elucidate common pathways associated with OA risk[135]. As demonstrated above, there has been a rapid expansion of cellular and molecular pathogenic understanding across multiple tissues of the OA joint. *But how likely is it that this knowledge will deliver translational success?*

Epidemiology, perhaps the oldest discipline in OA research, has much to teach us. It reminds us that mechanical strain remains a principal driver of OA development and progression[183]. It also teaches us that the disease is heterogeneous; having a variable course and symptoms[184]. Using all sources of data available, we should be able to improve our chances of success but as independently highlighted by the authors of the individual sections, there are key questions that need constant re-inforcement if we are to translate our ever more detailed understanding of OA pathophysiology to treatment and patient care.

- *Which of the pathways are targetable?*
- *If targetable, do they deliver a clinically meaningful effect?*
- *Does the target have benefits across all tissues of the joint or is it tissue-specific (see conflicting roles of MMP13 in bone and cartilage above)?*
- *Do several targets need to be delivered in combination?*
- *Will treatments work when the adverse mechanical environment of the joint is uncorrected?*
- *Are the described processes active in all patients at all stages of disease, or will patient stratification be necessary?*

We don't have all the answers yet, but progress has been rapid, there is a recognized urgency across funders and patient groups, and as this review demonstrates, the scientific community is working collaboratively and imaginatively to combat this challenging disease.

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