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Review

Osteoarthritis year in review 2023: Biology

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SUMMARY

Great progress continues to be made in our understanding of the multiple facets of osteoarthritis (OA) biology. Here, we review the major advances in this field and progress towards therapy development over the past year, highlighting a selection of relevant published literature from a PubMed search covering the year from the end of April 2022 to the end of April 2023. The selected articles have been arranged in themes. These include 1) molecular regulation of articular cartilage and implications for OA, 2) mechanisms of subchondral bone remodelling, 3) role of synovium and inflammation, 4) role of age-related changes including cartilage matrix stiffening, cellular senescence, mitochondrial dysfunction, metabolic dysfunction, and impaired autophagy, and 5) peripheral mechanisms of OA pain. Progress in the understanding of the cellular and molecular mechanisms responsible for the multiple aspects of OA biology is unravelling novel therapeutic targets for disease modification.

Introduction

The electronic PubMed database was searched for papers published online between 25 April 2022 and 30 April 2023 that included the keyword 'osteoarthritis' and were written in English. Out of approximately 8700 PubMed entries, papers were pre-selected based on their relevance to OA biology with a focus on preclinical mechanistic studies. A thematic analysis was performed to define topics, and final papers for inclusion were selected based on the personal opinions of the authors with regard to relevance to topics, originality, and scientific rigour. The authors apologise to those whose work was not cited because of space constraints.

Molecular regulation of articular cartilage with implications for OA and therapeutic development

Transcription factors

The runt-related transcription factor (RUNX) family plays important roles in chondrocytes. RUNX2 contributes to post-traumatic (PT)OA development by promoting chondrocyte hypertrophy and matrix metallopeptidase (MMP)-13 expression.[1] Nagata et al.[2] further dissected the roles of RUNX2 and RUNX3 in articular cartilage during PTOA development. They show that RUNX2 not only exerted pro-catabolic effects by stimulating MMP-13 expression but also bound TG(T/C)GGT target sequences near the sex-determining-region-Y-related high-mobility-group-box (SOX)9 binding site in the intron 6 enhancer of the collagen type 2 (COL2)A1 gene, which can sustain COL2 expression when SOX9 levels are low, as demonstrated in response to interleukin (IL)-1β exposure. This may explain why partial loss, via heterozygous knockout (KO), of RUNX2 from adult cartilage resulted in decreased MMP-13 expression but sustained COL2 expression and protection from PTOA, while homozygous KO resulted in concomitant loss of COL2 expression and exacerbated PTOA. They further demonstrate a protective role for RUNX3 in articular cartilage and its decreased expression during PTOA and with age. Homozygous RUNX3 deletion from either the superficial zone or entire adult articular cartilage exacerbated PTOA but did not induce spontaneous OA with age, while adenoviral RUNX3 overexpression ameliorated PTOA. The cartilage protective effects of RUNX3 could at least in part be explained with the identification of the joint lubricant proteoglycan-4 (PRG4) and aggrecan as direct RUNX3 target genes in chondrocytes.[2] Abou-Jaoude et al.[3] show that deletion of Src homology and collagen A (SHCA), a cytosolic adaptor protein that binds to the cytoplasmic tail of activated receptor tyrosine kinases and activates RUNX2 via extracellular signal-regulated kinase (ERK) signaling, in osteochondro-lineage cells during development impaired chondrocyte hypertrophy and led to dwarfism. Further, SHCA conditional KO mice were protected from spontaneous articular cartilage damage at 2 years of age, indicating that this signalling pathway acts upstream

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of RUNX2 to promote chondrocyte hypertrophy.[3] Consistent with this, Dong et al.[4] show that rapidly accelerated fibrosarcoma family member B (B-RAF) and ERK1/2 were activated in damaged human articular cartilage and intra-articular administration of a B-RAF inhibitor attenuated anterior cruciate ligament transection (ACLT)-induced OA in mice. Finally, Zhang et al.[5] report that mice with conditional deletion of RUNX1 in cartilage during embryonic development or at 3 weeks postnatally showed spontaneous cartilage degradation by 3–4 months of age and exacerbated damage following destabilisation of the medial meniscus (DMM). Conversely, adeno-associated virus (AAV)-mediated RUNX1 overexpression partly protected the cartilage from damage following ACLT. RUNX1 conditional deletion in cartilage was associated with dysregulation of transforming growth factor (TGF)-β, wingless-related integration site (WNT), and Hippo signalling, indicative of a central role of RUNX1 in the regulation of chondrogenesis and the chondrocyte phenotype.[5]

Kawata et al.[6] identify krüppel-like factor (KLF)2 and KLF4 as important chondrogenic transcription factors with pro-anabolic, anti-inflammatory, and anti-catabolic effects that were downregulated in cartilage during ageing and in OA. Deletion of KLF2 in adult cartilage exacerbated OA following DMM, while intra-articular delivery of AAV-KLF4 after DMM protected from joint damage. Mechanistically, KLF4 overexpression in TC28a2 cells upregulated transcription of chondrogenic genes, including PRG4, directly via interaction with KLF binding motifs in gene regulatory regions, as well as indirectly via activation of protein kinase A (PKA) and downstream cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB). Overexpression of KLF2 or KLF4 had similar effects in meniscal cells and bone marrow or synovial fibroblasts, suggestive of broad joint protective effects.[6]

Forkhead-box protein (FOX)O1 and FOXO3 are downregulated in articular cartilage with age and in OA.[7] FOXO1 protects against oxidative stress in chondrocytes and promotes expression of autophagy genes and PRG4, and FOXO1 or FOXO3 deficient mice show early onset OA.[8] Using a reporter cell line with FOXO1 promoter sequences for drug screening, Ohzono et al.[9] identify the histone deacetylase (HDAC) inhibitor panobinostat as the most promising small molecule to upregulate FOXO1. It increased expression of autophagy genes and PRG4 in vitro while downregulating inflammatory and catabolic gene expression and it ameliorated OA in a mouse model.[9]

Transcription factor regulatory proteins

Genetic variants in acidic leucine-rich nuclear phosphoprotein (ANP)32A, a member of the inhibitor of histone acetyltransferase complex that limits transcription by binding to histones, are associated with OA.[10] Monteagudo et al.[11] previously showed that ANP32A protects cartilage against oxidative stress. They add to this by showing that ANP32A is a negative regulator of WNT signalling.[12] Activation of WNT signalling in chondrocytes induced release of ANP32A from the scaffold protein AXIN1 and its nuclear translocation together with β-catenin, where it repressed WNT target genes by inhibiting histone acetylation.[12] Accordingly, in addition to increased oxidative stress,[11] ANP32A KO mice displayed WNT hyperactivation in articular cartilage.[12] Interestingly, treatment of DMM-induced ANP32A KO mice with the antioxidant N-acetyl cysteine (NAC) protected against cartilage damage but not osteophyte formation, while treatment with the WNT inhibitor XAV939 had the opposite effects. The combination of XAV939 and NAC ameliorated both cartilage damage and osteophyte formation, suggesting that pathological consequences of ANP32A deficiency may be mediated via different pathways.[12] The same team identify hypoxia and WNT signalling pathways as positive and negative regulators of ANP32A expression in articular chondrocytes, respectively.[13]

Protein phosphatase magnesium-dependent (PPM)1A dephosphorylates mothers against decapentaplegic homologue (SMAD)2 and promotes its nuclear export, thereby terminating TGF-β signalling.[14] Ge et al.[15] report that PPM1A expression increased concomitant with decreased phosphorylated SMAD2 in human and mouse cartilage during OA. Mice with genetic ablation or pharmacological inhibition of PPM1A were partly protected from DMM-induced OA. The protective effects of genetic PPM1A ablation were abolished upon intra-articular treatment with SD-208, a TGF-β type I receptor inhibitor, indicating that protection was mediated by enhanced TGF-β signalling in the absence of PPM1A.[15]

Other signalling pathways

Polymorphic variants in aldehyde dehydrogenase 1 (ALDH1)A2, encoding the enzyme that synthesises all-trans retinoic acid (atRA) from retinal, are associated with severe hand OA.[16] Zhu et al.[17] confirm this association in a large UK cohort. They further show that ALDH1A2 messenger RNA was significantly lower in OA cartilage from individuals carrying the risk variants and this was associated with a more inflammatory cartilage phenotype, indicating that ALDH1A2 is cartilage protective. Cartilage injury downregulated atRA-inducible genes and upregulated inflammatory genes, and this could be prevented with talarozole, a cytochrome P450 (CYP)-26 inhibitor that blocks retinoic acid metabolism, through a mechanism involving peroxisome proliferator-activated receptor-γ and retinoid X receptor. Accordingly, subcutaneous administration of talarozole in mice with surgically induced OA reduced cartilage degradation and osteophyte formation.[17]

Jin et al.[18] report that cartilage-specific genetic ablation of the prostaglandin E2 receptor 4 (EP4) in mice promoted repair in a cartilage defect model and prevented cartilage damage in the DMM model by enhancing cartilage anabolism and reducing cartilage catabolism. Similar results were obtained by oral administration of the novel EP4 antagonist HL-43, which increased anabolism through up regulation of SOX9 expression via cAMP/PKA/CREB signalling and reduced catabolism mediated by signal transducer and activator of transcription (STAT)3 signalling. These data support the potential for the EP4 antagonist HL-43 to be used for cartilage repair and OA.[18]

The C-X-C motif chemokine receptor (CXCR)-2 is essential for cartilage homoeostasis.[19] Caxaria et al.[20] show that the CXCR2 ligand granulocyte chemotactic protein (GCP)-2 is expressed in articular and not growth plate cartilage and stimulated chondrogenesis in vitro and in an ectopic cartilage formation model in vivo. Intra-articular adenoviral overexpression of a GCP-2 mutant with retained chondrogenic but disrupted chemotactic activity reduced pain and cartilage loss in a model of instability-induced OA in mice.[20]

Gerwin et al.[21] identify angiopoietin-like 3 (ANGPTL3) as a chondrogenic molecule and generated a deletion mutant, LNA043, which was devoid of the pro-angiogenic effect of ANGPTL3 while maintaining its chondrogenic activity. The binding of LNA043 to α5β1 integrin was necessary for its anabolic effect. Intra-articular injections of LNA043 improved cartilage integrity in rodent models of OA. In a phase 1 study conducted in patients with knee OA before total knee replacement, intra-articular injection of LNA043 was safe and induced transcriptional changes in the cartilage indicative of expression of hyaline cartilage matrix components and activation of anabolic pathways while reversing the regulation of some of the genes associated with OA progression.[21]

Mechanisms of subchondral bone remodelling in OA

Subchondral bone deterioration can act as a trigger for degeneration of the overlying articular cartilage.[22] Since the discovery of a capillary subtype, termed type-H blood vessel, that supports

osteogenesis,[23] several studies have implicated increased type-H vessel formation in subchondral bone remodelling during OA and identified factors and molecular pathways involved.[24]

Secretion of platelet-derived growth factor (PDGF)-BB by pre-osteoclasts stimulates type-H vessel formation,[25] and elevated PDGF-BB in subchondral bone has been implicated in OA development.[26] Cui et al.[27] show that conditional KO of PDGF receptor (PDGFR)-β in endothelial cells decreased subchondral type-H vessels and ameliorated ACLT-induced and spontaneous age-related OA, confirming endothelial cells as direct targets of PDGF-BB in the pathophysiology of OA. They identify Talin-1 as intracellular PDGFR-β binding protein, and PDGF-BB/PDGFR-β engagement on endothelial cells induced Talin-1 and focal adhesion kinase (FAK) phosphorylation and activation, stimulating angiogenesis. Injecting the subchondral bone of rats with an AAV containing a PDGFR-β short-hairpin (sh)RNA sequence under the control of a tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (Tie-1) promoter to selectively knock down PDGFR-β in endothelial cells decreased ACLT-induced type-H vessel formation and OA.[27]

Prostaglandin E2 (PGE2), synthesised by cyclooxygenase-2, is highly secreted by the osteoblast lineage in subchondral bone during OA and its inhibition ameliorated OA development in STR/Ort mice.[28] Jiang et al.[29] report that PGE2 activated the EP4 receptor and downstream phosphoinositide 3-kinase (PI3K)/AKT/mitogen-activated protein kinase (MAPK) signalling in osteoclasts, and conditional KO of EP4 in the myeloid lineage (including osteoclasts) ameliorated ACLT-induced OA. EP4 conditional KO mice displayed decreased expression of PDGF-BB as well as decreased Netrin-1 secretion by osteoclasts, and reduced calcitonin gene-related peptide (CGRP)+ sensory neurons in subchondral bone marrow,[29] implicated in osteoclast-mediated OA pain.[30] They identify a novel EP4 antagonist, HL-43, able to suppress osteoclast and osteoblast numbers in subchondral bone and to improve pain and structure following ACLT, similar to celexocib.[29]

Zhang et al.[31] show that KO of lymphocyte cytosolic protein 1 (LCP1), which impairs osteoclast formation, ameliorated subchondral bone remodelling and cartilage degradation following ACLT. This was associated with partial prevention of ACLT-induced subchondral type-H blood vessel formation and the resulting increased oxygen and decreased expression of hypoxia-inducible factor (HIF)-1α in cartilage. Knockdown of HIF-1α via intra-articular administration of AAV-HIF-1α shRNA to LCP1-deficient mice at 4 and 8 weeks after ACLT abrogated the protective effects of LCP1 KO on cartilage. Conversely, stabilisation of HIF-1α by administration of dimethyloxallyl glycine (DMOG) ameliorated cartilage degradation following ACLT. Treatment with Oroxylin A to pharmacologically inhibit L-plastin, encoded by the LCP1 gene, alleviated OA progression, indicating therapeutic potential.[31]

Role of synovium and inflammation in OA

Macrophages in synovium play a role in modulating joint inflammation and OA severity. M1 macrophages secrete pro-inflammatory cytokines, while M2 macrophages secrete anti-inflammatory cytokines associated with resolution of inflammation.[32] Zhang et al.[33] developed a strategy to target mitochondrial dysfunction in synovial inflammatory M1 macrophages and induce reprogramming to an anti-inflammatory M2 phenotype. Poly(lactic-co-glycolic acid), or PLGA, nanoparticles loaded with a reactive oxygen species (ROS) scavenger, manganese dioxide, and an inducible nitric oxide synthase inhibitor, S-methylisothiourea, were encapsulated with macrophage membrane decorated with dextran sulphate, which binds to scavenger receptor A on M1 macrophages, and tri-phenyl-phosphonium for mitochondrial targeting. These nanoparticles decreased ROS and nitric oxide production and restored oxidative phosphorylation and adenosine triphosphate (ATP) production in M1 macrophages, and induced a repolarisation towards an M2 phenotype. Intravenous administration in mice with collagenase-induced arthritis resulted in targeting of the inflamed synovium and amelioration of cartilage damage.[33] Similarly, Zhou et al.[34] developed a nanotherapeutic system to induce M1-to-M2 macrophage repolarization. M2 macrophage membranes were used to coat nanoparticles, prepared by condensing KAFAK (an anti-inflammatory peptide) and shRNA against the long isoform of the leptin receptor with polyethylenimine to form a complex, modified with hyaluronic acid for a negative charge. At 3 months after surgical induction of OA in rats, nanoparticle-treated knees showed decreased cartilage damage and synovial inflammation, with increased numbers of synovial M2-like macrophages.[34]

Macrophages contribute to maintaining tissue homoeostasis and preventing inflammation through efferocytosis. Del Sordo et al.[35] report an accumulation of apoptotic cells in the synovium of late-stage OA patients, which they showed may be due to impaired clearance by macrophages. Synovium-derived, but not blood-derived, macrophages of late-stage OA patients showed impaired efferocytosis of apoptotic Jurkat T cells in vitro. Conversely, exposure of peripheral blood-derived macrophages from healthy individuals to OA synovial fluid suppressed efferocytosis, indicating the impairment is due to exposure to factors in the microenvironment.[35]

Progress has been made in understanding synovial fibroblast populations and their response to injury and role in OA using single-cell RNA-sequencing. Knights et al.[36] reveal distinct functional subsets of mouse knee synovial fibroblasts and show overactive WNT/β-catenin signalling in synovial fibroblasts in a non-invasive ACL rupture-based model of PTOA. They identify the WNT agonist R-Spondin-2 as highly secreted by PRG4-expressing lining fibroblasts during PTOA, which interacted with leucine-rich repeat-containing G-protein coupled receptor (LGR)4–6 expressed on neighbouring cells. Intra-articular injections of R-spondin-2 induced OA-like changes.[36] Using a joint surface injury surgical mouse model,[37] Collins et al.[38] identify similar synovial fibroblast populations, and unravel two fibroblast stem/progenitor populations, one located near blood vessels in sublining, and one in the PRG4-expressing lining giving rise to mature fibroblast-like synoviocytes (FLS) that play critical roles in maintaining joint lubrication and homoeostasis. Trajectory and gene regulatory network analysis identified SOX5 and FOXO1 as candidate transcription factors regulating the FLS phenotype,[36,38] indicating overlap in molecular regulation between FLS and superficial zone chondrocytes, and identifying putative targets for modulation of the synovial fibroblast phenotype. In human OA synovium, Wijesinghe et al.[39] identify fibroblast subsets in various joints, and highlight a differential effect of obesity and mechanical loading on their inflammatory phenotype.

Das et al.[40] show that tryptase-β, a serine protease that is released by activated mast cells, cleaves PRG4, which caused loss of its lubricating properties while increasing its ability to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in target cells via binding to toll-like receptors (TLRs), adding to our understanding how inflammation contributes to loss of joint homoeostasis.

Finally, Lin et al.[41] established a method to assess synovial lymphatic function by tracing intra-articularly administered fluorescently-tagged 70-kDa dextran using live animal imaging and histology. They report reduced joint clearance, synovial influx, and lymph node draining capacity with age, associated with decreased expression of vascular endothelial growth factor (VEGF)-C and genes related to the VEGF receptor (VEGFR)-3 signalling pathway. Weekly intra-articular administration to aged mice of recombinant VEGF-C with a cysteine 156 to serine mutation,[41] which binds specifically to

VEGFR-3 and selectively promotes lymphangiogenesis,[42] improved synovial lymphatic functions and slowed the progression of spontaneous cartilage degradation.[41]

Role of age-related changes in OA development

Age is a risk factor for the development of OA. Age-related changes in the extracellular matrix (ECM), cellular senescence, mitochondrial and metabolic dysfunction, and impaired autophagy have all been implicated in the loss of cartilage homoeostasis.[43]

Cartilage matrix stiffness

Iijima et al.[44] performed a comprehensive proteomic analysis of mouse articular cartilage, which showed activation of PI3K/AKT signalling and downregulation of the longevity factor α-Klotho in male mice with age. Haploinsufficiency for α-Klotho accelerated cartilage degradation in male but not female mice, indicating downregulation of α-Klotho expression may drive age-related cartilage degradation in males. Mechanistically, ECM stiffness was found to signal via the actin cytoskeleton to induce DNA methyltransferase (DNMT)1-mediated epigenetic silencing of the α-Klotho promoter. This was associated with increased expression of lysyl oxidase (LOX) in aged cartilage,[44] an enzyme involved in collagen cross-linking previously implicated in OA pathogenesis.[45] Pharmacological inhibition of LOX in aged mice reduced cartilage stiffness, increased α-Klotho expression, and improved cartilage integrity in wild-type mice, but not in mice haploinsufficient for α-Klotho.[44]

Cellular senescence

Cellular senescence is characterised by permanent proliferative arrest, apoptotic resistance, and secretion of ECM-degrading and pro-inflammatory molecules termed the senescence-associated secretory phenotype (SASP). Selective removal of senescent cells or inhibition of SASP factors can alleviate OA in preclinical models,[46] and a more complete understanding of the mechanisms and roles of cellular senescence in OA can support therapeutic development.[47]

The SASP is sustained by the accumulation of lysosomes and mammalian target of rapamycin complex (mTORC)1 at a distinct trans-Golgi compartment, the TOR-autophagy spatial coupling compartment, where high mTORC1 activity supplies amino acids for mass synthesis of SASP proteins.[48] In an elegant study, Roh et al.[49] identify a role for increased lysosomal cholesterol in sustaining high mTORC1 activity and the SASP in senescent cells. They demonstrate that the cholesterol transporter ATP-binding cassette transporter (ABCA)1 is a transcriptional target of GATA-binding protein (GATA)4, a key regulator of the SASP.[49] ABCA1 normally functions at the plasma membrane as a cellular cholesterol exporter to high-density lipoprotein.[50] In senescent cells, ABCA1 was rerouted to the lysosome, where it functioned as a lysosomal cholesterol importer, sustaining high mTORC1 activity. Pharmacological modulation of the sub-cellular localisation or activity of ABCA1 altered OA development in male mice following DMM, indicating the potential relevance of the identified senescence molecular pathway in OA pathogenesis.[49] Increased intracellular cholesterol and enhanced cholesterol metabolism in OA chondrocytes, mediated by the retinoic acid-related orphan receptor alpha, was previously implicated in OA pathogenesis.[51] The finding that sub-cellular relocation of cholesterol into the lysosome regulates senescence-associated inflammation[49] adds further roles for intracellular cholesterol in OA pathogenesis, and highlights the lysosome as an important signalling centre in chondrocytes that determines their response to stress.

DNA damage is an important contributor to cellular senescence and dysfunction, and is increased in OA chondrocytes.[52] Copp et al.[53] reveal that chondrocytes from healthy donors accumulate DNA damage with age. This could at least in part be explained by an age-related decline in DNA repair efficiency.[54] It was previously shown that depletion of Sirtuin (SIRT)6 in human chondrocytes causes increased DNA damage and telomere dysfunction, and premature senescence.[55] Copp et al.[54] show that treatment with MDL-800, an allosteric activator of SIRT6, stimulated DNA repair in response to irradiation-induced DNA damage, and rapidly decreased the amount of DNA damage that had naturally accumulated in mouse or human chondrocytes over their lifespan.[54] Accordingly, Ji et al.[56] report that intra-articular adenoviral delivery of SIRT6, or the SIRT6 activator MDL-800 encapsulated in chondrocyte-specific functionalised nanoparticles,[57] decreased chondrocyte senescence and ameliorated OA development in DMM-operated mice.[56] The proposed mechanism involved inhibition of STAT5 phosphorylation and transcriptional activation of senescence-associated, pro-inflammatory and catabolic genes in response to IL-15 signalling, via SIRT6-mediated deacetylation of lysine 163 on STAT5.[56]

Sarkar et al.[58] established an epigenetic clock predictive of the biological age of human adult chondrocytes by performing global DNA methylation analysis of human foetal and adult chondrocytes. They demonstrate a protective role for STAT3 activation in OA chondrocytes via transcriptional repression of DNMT3B and decreased DNA methylation, which they postulate may represent an attempt to revert to a progenitor-like phenotype,[58] and contrasts with the detrimental effects of cytokine-induced activation of STAT3 and downstream NF-κB signalling.

Using single-cell transcriptomic analysis of freshly isolated and donor-matched articular and meniscal chondrocytes obtained from OA or normal donors, Swahn et al.[59] show an increased abundance of a putative pathogenic cluster of chondrocytes in OA and identify CREB3 family members and zinc finger E-box-binding homeobox (ZEB)1 as candidate transcription factors regulating this cluster. Overexpression of ZEB1 in TC28a2 cells upregulated oxidative phosphorylation and ROS production and increased cellular senescence. Of note, upregulation of senescence-associated genes and the serine protease fibroblast-activating protein (FAP) were detected more widely across clusters in chondrocytes from OA compared to normal donors.[59] Fan et al.[60] show that the FAP inhibitor osteolectin was expressed in the superficial layer of normal articular cartilage but was undetectable in human OA and downregulated in mouse DMM cartilage. Genetic deletion of FAP, or intra-articular administration of a FAP inhibitor, or of recombinant osteolectin, all ameliorated DMM-induced OA. Conversely, intra-articular administration of recombinant FAP, or genetic deletion of osteolectin, accelerated OA progression following DMM. MMP-13-degraded COL2 fragments were identified as FAP substrates, indicating that FAP works in concert with MMP-13 to degrade COL2.[60]

Mitochondrial and metabolic dysfunction

Several studies support an important role for mitochondrial and metabolic dysfunction in OA and provide novel insights into the mechanisms linking mitochondrial and metabolic dysfunction to OA development. Through a series of elegant experiments, Kim et al.[61] show that mitochondrial stress in chondrocytes led to efflux of double-stranded (ds)RNA into the cytosol, where it activated protein kinase RNA-activated (PKR) and other dsRNA sensors of the innate immune system, while the release of mitochondrial dsRNA into the extracellular environment activated TLR3 in neighbouring cells. This triggered interferon-β signalling and SASP factor expression. In addition, they implicate PKR activation downstream of mitochondrial dsRNA cytosolic efflux in the induction of chondrocyte senescence in response to H_2O_2, doxorubicin, or acute ionising radiation. The effects of mitochondrial stress could be partly rescued by activation of autophagy with torin-1 or metformin, and this rescue effect was

reduced when mitochondrial dsRNAs were depleted, indicating that autophagy could alleviate the cellular response to stressors through prevention of cytosolic dsRNA efflux from dysfunctional mitochondria or clearance of mitochondrial dsRNA from the cytosol.[61] Release of mitochondrial (mt)DNA may also act as damage-associated molecular pattern in OA, and its release after joint injury in horses could be prevented by treatment with the mitoprotective peptide SS-31.[62]

An emerging putative mitoprotective mechanism involves the transfer of mitochondria between neighbouring cells. Fahey et al.[63] show that mitochondrial transfer between mesenchymal stem cells (MSCs) and chondrocytes in vitro was increased under conditions that induce mitochondrial dysfunction, and was dependent on connexin-43. Strikingly, they show that within mechanically injured bovine cartilage explants, MSCs were capable of transferring mitochondria to chondrocytes deep within cartilage via cellular processes extending into microcracks. This raises the question whether mitochondrial transfer occurs between neighbouring cells in vivo and what roles this may play during OA.[63]

Changes in intracellular metabolism and mitochondrial dysfunction can cause increased ROS production and oxidative stress and lead to cellular senescence.[64] Three studies further clarify the importance of different sources of ROS in OA development. Inducible overexpression of peroxiredoxin-3 in chondrocytes, which functions as an antioxidant in mitochondria by scavenging H_2O_2, decreased cartilage damage at 18 months of age, supporting a role for mitochondrial ROS in age-related OA, while peroxiredoxin-3 overexpression had no effect on DMM-induced OA.[65] In contrast, ROS produced by membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) appeared more important in PTOA, with KO of NOX4[66] or treatment with the NOX inhibitor APX-115[67] conferring protection against DMM-induced OA.[66,67]

The mitochondrial deacetylase SIRT3 protects against OA by limiting oxidative stress and improving mitochondrial DNA integrity and function, at least in part by reducing acetylation of the antioxidant enzyme superoxide dismutase 2.[68,69] Unexpectedly, Zhu et al.[70] show that chondrocyte-specific deletion of SIRT3 in male mice at 5 weeks of age conferred protection from high-fat diet (HFD)-induced cartilage degradation, but not osteophyte formation. This was associated with a shift towards a glycolytic metabolic phenotype and resistance to the HFD-induced upregulation of mitochondrial fatty acid metabolism in SIRT3 conditional KO chondrocytes, possibly driven by increased HIF-1α signalling in the absence of SIRT3.[70] This highlights the complexity and context dependence of the roles of chondrocyte metabolic and mitochondrial processes in OA development in response to different stressors, and suggests that suppression of the shift towards intracellular lipid transport and fatty acid oxidation in cartilage under HFD conditions may be protective, although the loss of SIRT3 may have had pleiotropic protective effects in this study.

Arra et al.[71] show that glutamine was utilised by chondrocytes as an energy source to fuel the tricarboxylic acid cycle even when glucose was abundant. Unexpectedly, glutamine deprivation inhibited glycolytic activity and suppressed the enhanced glycolysis induced by IL-1β stimulation. This may represent a compensatory mechanism that forces chondrocytes to rely upon fatty acid oxidation and other energy sources to fuel the tricarboxylic acid cycle and maintain anabolic processes in the absence of glutamine. Glutamine deprivation, or blocking the conversion of glutamine to glutamate using a glutaminase inhibitor, reduced ROS production and decreased the inflammatory and catabolic response to IL-1β stimulation by suppressing NF-κB activation. These effects were associated with increased autophagy, possibly mediated by activation of activating transcription factor 4 in response to amino acid deprivation or decreased levels of ammonia, a metabolite of glutamine and autophagy inhibitor.[71] In contrast, Ma et al.[72] report that glutamine supplementation suppressed NF-κB activation and IL-1β-induced inflammatory and catabolic gene expression in rat OA chondrocytes and decreased cartilage degradation in a rat OA model.[72] Cytosolic and mitochondrial glutamine metabolism has many important functions in cells.[73] Deciphering its precise and varied roles in chondrocytes, its interactions with other metabolic pathways, and its significance in OA, remains a challenge.

Alabarse et al.[74] show a role for cyclic adenine dinucleotide phosphate (ADP) ribose hydrolase, also known as CD38, in OA development. CD38 degrades nicotinamide adenine dinucleotide (NAD)+ for the synthesis of the intracellular calcium regulators ADP-ribose, cyclic ADP-ribose and nicotinamide. NAD+ is an essential cofactor in energy metabolism and for other NAD+-dependent enzymes, including SIRTs, and declining NAD+ with age has been implicated in metabolic dysfunction and loss of tissue homoeostasis.[75] Analysis of CD38 expression in cartilage showed upregulation with age and in damaged cartilage of knee OA patients. Chondrocytes stimulated with IL-1β increased CD38 expression,[74] likely due to activation of NF-κB and STAT, which can bind to and activate the CD38 promoter,[76] and this resulted in decreased intracellular NAD + levels and increased catabolic activity. Genetic KO or pharmacological inhibition of CD38 ameliorated OA development following DMM.[74]

Metabolic syndrome, associated with hypertension, insulin resistance, and dyslipidemia, is implicated in OA pathogenesis.[77] Li et al.[78] show the importance of glucose metabolism in the protection of superficial zone chondrocytes from damage. Conditional KO of glucose transporter (GLUT)1 in PRG4-expressing superficial zone cells in 3-week-old mice exacerbated cartilage damage while transgenic expression of GLUT1 in chondrocytes protected the cartilage from DMM-induced damage. The cartilage of diabetic mice showed decreased glucose consumption and lactate production, indicative of impaired glycolysis, and this was associated with decreased anabolic gene expression and proteoglycan content and increased chondrocyte apoptosis and cartilage damage following DMM.[78] Enhancing glucose metabolism in articular chondrocytes may thus protect from cartilage degeneration in OA. Cao et al.[79] report that downregulation of low-density lipoprotein (LDL) receptor-related protein-3 in chondrocytes in response to extracellular cholesterol contributed to PT and age-related OA development by upregulating syndecan-4,[79] required for cartilage degradation mediated by a disintegrin and metalloproteinase with thrombospondin motifs-5.[80] Van Gemert et al.[81] show that cholesterol-lowering treatment combined with anti-IL-1β in a mouse model of hyperlipidemia significantly decreased spontaneous cartilage pathology, highlighting the combined role of high cholesterol and inflammation in metabolic syndrome-associated OA pathology.[81]

To restore energy deficits and enhance anabolic processes, Chen et al.[82] introduced photosynthetic machinery derived from plants, in the form of nanothylakoid units (NTUs), into mouse and human chondrocytes to provide a light-inducible source of ATP and NADPH. They used an elegant delivery system by encapsulating the NTUs in chondrocyte-derived membrane (CM-NTUs) to avoid immune elimination and enhance chondrocyte targeting. They demonstrated uptake of CM-NTUs by chondrocytes via fusion with the chondrocyte membrane, with penetration into cartilage possibly involving extracellular vesicle (EV)-dependent transcytosis. Functionally, the CM-NTUs were able to restore energy state, with improved ATP/ADP ratio and a shift from glycolysis towards oxidative phosphorylation, as well as enhanced ECM protein anabolism, in IL-1β-treated chondrocytes. Furthermore, regular intra-articular delivery of CM-NTUs and light irradiation following ACLT restored ATP and NADPH levels in cartilage and ameliorated OA development in both young and old mice.[82]

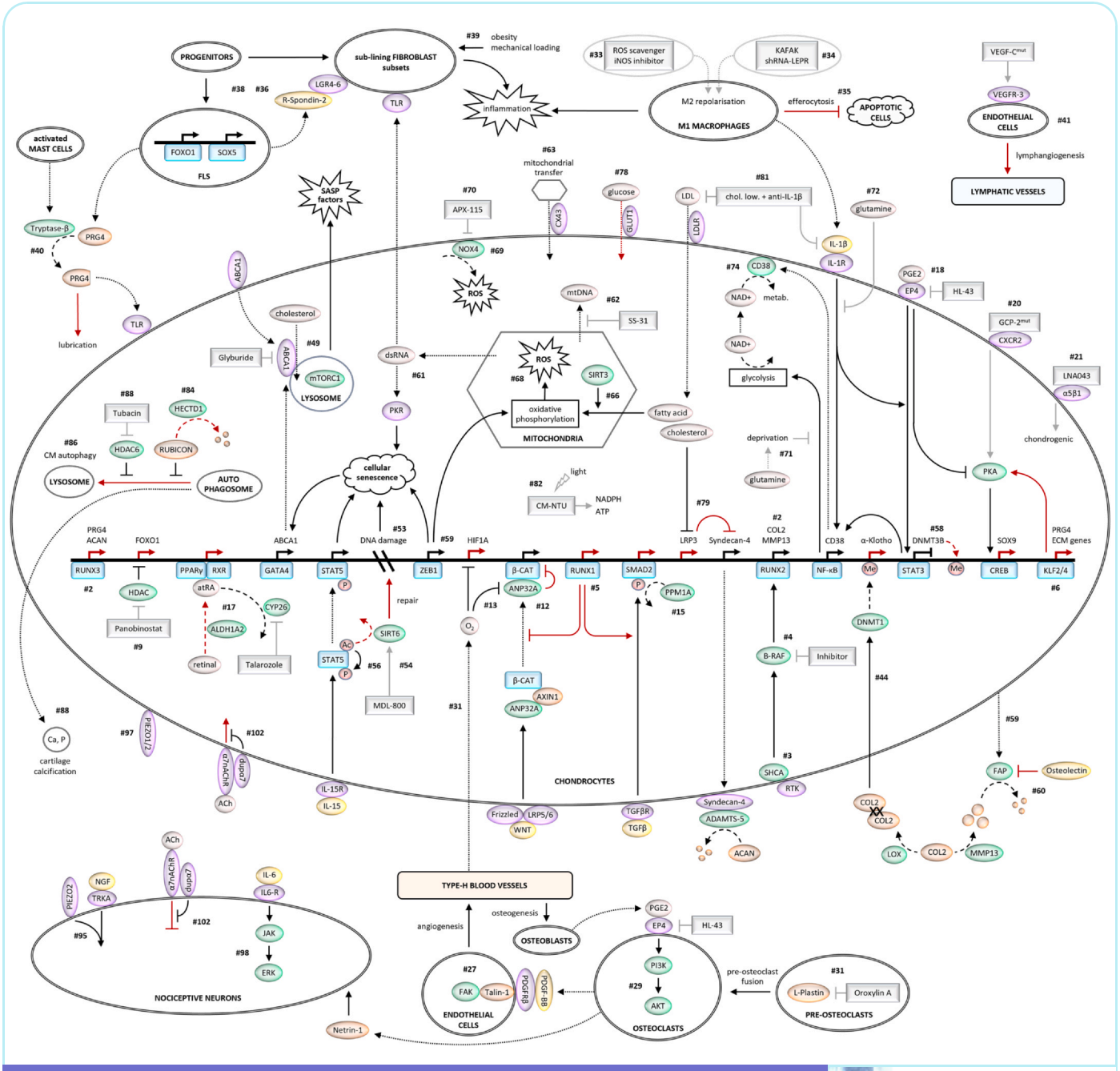

Fig. 1

Osteoarthritis and Cartilage

Overview of the molecular pathways implicated in OA. This schematic attempts to provide a cohesive illustration of the main findings of the research papers published in the last year included in this review. The reader is referred to the text and original articles for details. Citations are indicated with hashtags in bold. Red indicates downregulated pathways. Grey indicates pharmacological modulation. Ac, acetylated amino acid residue; Ca, calcium, Me, methylated DNA; P, phosphorylated amino acid residue/phosphate.

Impaired autophagy

Articular cartilage, as a post-mitotic tissue, depends on autophagy for the maintenance of homoeostasis. Autophagy declines with age and defective macroautophagy plays an important role in OA pathogenesis.[83]

Liao et al.[84] provide further insights into the molecular mechanisms underpinning defective macroautophagy in OA by identifying HECT-domain E3 ubiquitin protein ligase 1 (HECTD1) as a chondroprotective regulator of autophagy that was downregulated in OA. They show that HECTD1 functioned by inducing proteasomal degradation of run-domain beclin-1-interacting and cysteine-rich domain-containing protein (RUBICON),[84] an inhibitor of autophagy that promotes ageing and restricts lifespan.[85]

Lorenzo-Gomez et al.[86] investigated a role in OA for impaired chaperone-mediated (CM) autophagy, which mediates selective

degradation of KFERQ-like motif-containing cytosolic proteins via lysosomal translocation by a complex involving lysosome-associated membrane protein (LAMP)2A, heat shock protein (HSP)70 and HSP90A.[87] They show that HSP90AA1 and LAMP2A were down-regulated in OA and aged cartilage. Knockdown of HSP90AA1 in chondrocytes in vitro promoted senescence, inflammatory and catabolic gene expression, oxidative stress, and apoptosis, while overexpression attenuated senescence. HSP90AA1 modulation altered macroautophagy in parallel with CM autophagy, indicating autophagic cross-talk, and this may underpin some of the observed effects.[86]

Yan et al.[88] propose a link between impaired chondrocyte autophagy and cartilage calcification in temporomandibular joint (TMJ) OA by showing that calcium and phosphate-containing EVs released from OA chondrocytes could initiate calcification of the cartilage ECM. These EVs were positive for microtubule-associated proteins 1A/1B light chain 3 (LC3) and likely derived from accumulated autophagosomes resulting from decreased autophagosome-lysosome fusion and impaired autophagic flux within the chondrocytes, due to increased HDAC6-mediated α-tubulin deacetylation and microtubule destabilisation. Intra-articular administration of the HDAC6 inhibitor tubacin,[88] which restores autophagosome-lysosome fusion and autophagic flux,[89] decreased calcium-containing LC3+ EVs in cartilage and partially prevented cartilage calcification and degradation in a rat TMJ OA model.[88]

Peripheral mechanisms of pain in OA

Progress has been made in recent years in understanding the molecular pathogenesis of peripheral joint pain in OA.[90] Piezo-type mechanosensitive ion channel component (PIEZO)2 is a key mechanotransducer mediating proprioception[91,92] and inflammation- and injury-induced allodynia.[93,94] Obeidat et al.[95] show that conditional deletion of PIEZO2 from nociceptors in male mice resulted in decreased joint pain during DMM-induced or spontaneous age-related OA, while cartilage damage and osteophyte formation were similar to control mice. PIEZO2 conditional KO mice were also protected from joint swelling and pain induced by intra-articular administration of nerve growth factor (NGF) that interacts with tropomyosin receptor kinase A (TRKA) expressed by a subset of PIEZO2+ nociceptive neurons, suggesting that PIEZO2 is required for NGF-mediated nociceptor sensitisation in OA. Acute silencing of PIEZO2-expressing neurons, using PIEZO2-Cre-controlled expression of the inhibitory designer receptors exclusively activated by designer drugs (DREADD) receptor and intra-articular administration of its synthetic ligand clozapine N-oxide,[96] transiently alleviated knee hyperalgesia at 9 weeks post-DMM, indicating therapeutic potential for local inhibition of PIEZO2-expressing neurons.[95] Conditional deletion of PIEZO1 and PIEZO2 in the embryonic joint interzones in mice did not affect joint development and had minimal effect on OA following DMM,[97] suggesting that PIEZO2 inhibition for pain modulation may be safe.

Liao et al.[98] report dual and sex-specific roles for IL-6 in cartilage damage and pain during PTOA. Genetic ablation of IL-6 in males protected from severe cartilage degeneration and pain following DMM, with prevention of increased CGRP+ innervation in the subchondral space in the absence of any effect on subchondral bone remodelling. In vitro experiments using pharmacological inhibitors indicated that the effects of IL-6 on cartilage degeneration involved multiple signalling pathways downstream of janus kinase (JAK) activation, while the stimulatory effects of IL-6 on nociceptive neurons were mediated via ERK signalling within dorsal root ganglion (DRG) neurons. Since female wild-type mice exhibited only mild cartilage damage after DMM, the effect of loss of IL-6 on structural OA progression in females could not be clearly ascertained. However, pain and CGRP+ nociceptive subchondral innervation, as well as activation of STAT and ERK signalling in DRG neurons, in response to DMM was not prevented by the loss of IL-6 in female mice. This indicates that nociceptive signalling in females involves other activating molecules.[98]

The cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) has anti-inflammatory and anti-nociceptive functions. It mostly assembles as an α7 nicotinic acetylcholine receptor (α7nAChR) homopentamer. However, during human evolution, a hybrid gene (CHRFAM7A) arose via partial duplication of exons 5–10 of the CHRNA7 gene fused with a copy of the family with sequence similarity (FAM)7A gene.[99] The CHRFAM7A gene product, dupα7, assembles with CHRNA7-encoded α7 subunits but lacks the agonist binding site and acts as a dominant negative inhibitor of α7nAChR receptor function.[100,101] Courties et al.[102] show that mice engineered to express the human CHRFAM7A gene (TgCHRFAM7A) developed worse cartilage damage following DMM surgery or monoiodoacetate (MIA) injection, and showed a blunted protective effect of nicotine, an α7nAChR agonist, against IL-1β-induced upregulation of IL-6 and MMP-3, showing that the presence of CHRFAM7A decreased the chondroprotective effects of endogenous acetylcholine (ACh)/α7nAChR signalling in cartilage. Further, TgCHRFAM7A mice showed increased baseline mechanical allodynia, which persisted in response to sham surgery, DMM surgery and MIA injection, and failed to show a transient anti-nociceptive effect of nicotine administration, indicating loss of α7nAChR anti-nociceptive response in the presence of CHRFAM7A. Interestingly, while the CHRFAM7A hybrid gene was expressed at similar levels across human tissues, CHRNA7 expression was higher in DRGs and lower in cartilage, resulting in a comparatively high CHRFAM7A/CHRNA7 ratio in cartilage, and this was increased in OA. In chondrocytes from OA patients carrying two copies of the CHRFAM7A gene, the protective effect of nicotine against IL-1β-induced IL6 upregulation was blunted compared to chondrocytes from patients with only one copy of the CHRFAM7A gene.[102] Thus, although the CHRFAM7A gene product, dupα7, could modulate α7nAChR-mediated anti-nociceptive responses, it may predominantly interfere with anti-inflammatory functions of α7nAChR in the joint tissues and represent a putative disease-modifying OA drug target. Tissue-specific expression of CHRFAM7A in mice could help to clarify its varying roles in OA pathophysiology.

Conclusion

This review summarised important contributions to the field of OA biology in the last year, with further exploration of the roles of many genes, pathways, and molecules in various aspects of OA pathogenesis (Fig. 1). While cartilage has remained the central focus, more holistic approaches aimed at investigating the synovial joint as an organ continue to emerge. Cutting-edge single-cell investigative tools will increasingly provide unprecedented resolution in the years ahead and are likely to identify further cellular and molecular targets for mechanistic investigations, extending the already long list of drug candidates that have proved effective in preclinical models. While technological advances are facilitating human studies, the challenge in OA research remains the translation of preclinical findings into clinical applications.

Contributors

Both authors contributed to drafting, editing, and approving the manuscript.

Conflict of interest statement

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