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Review

Targeting inflammasome-dependent mechanisms as an emerging pharmacological approach for osteoarthritis therapy

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SUMMARY

Arthritic diseases have attracted enormous scientific interest because of increased worldwide prevalence and represent a significant socioeconomic burden. Osteoarthritis (OA) is the most prevalent form of arthritis. It is a disorder of the diarthrodial joints, characterized by degeneration and loss of articular cartilage associated with adjacent subchondral bone changes. Chronic and unresolving inflammation has been identified as a critical factor driving joint degeneration and pain in OA. Despite numerous attempts at therapeutic intervention, no effective disease-modifying agents targeting OA inflammation are available to the patients. Inflammasomes are protein complexes known to play a critical role in the inflammatory pathology of several diseases, and their roles in OA pathogenesis have become evident over the last decade. In this sense, it is relevant to evaluate the vital role of inflammasomes as potential modulators of pathogenic features in OA. This review will provide an overview and perspectives on why understanding inflammasome activation is critical for identifying effective OA therapies. We elaborate on the contribution of extracellular mediators from the circulatory system and synovial fluid as well as intracellular activators within the synovial fibroblasts and articular chondrocytes toward invoking the inflammasome in OA. We further discuss the merits of emerging inflammasome targeting therapies and speculate on the potential strategies for inflammasome blockade for OA therapy.

INTRODUCTION

Arthritic diseases have attracted enormous scientific interest because of their increased worldwide prevalence. In particular, osteoarthritis (OA) displays the highest prevalence among arthritic diseases and represents an enormous economic cost to society, government, and health care systems.[1] Globally the prevalence of OA has rapidly increased by 113.25% in nearly 3 decades. The number of cases doubled from 247.51 million in 1990 to 527.81 million cases in 2019.[2] OA is considered a complex degenerative disorder of the diarthrodial joints, characterized by degradation and loss of articular cartilage with pathological changes in the adjacent subchondral bone.[3] Traditionally, OA is considered a non-inflammatory disease. Age-related joint degeneration, because of stress from trauma or metabolic dysregulation was attributed as the causative factor for the development of this disease.[4,5] However, increasing evidence has accumulated showing that low-grade inflammation is a critical factor driving OA pathogenesis.[6] Advanced diagnostic techniques, such as ultrasound and magnetic resonance imaging (MRI) to detect synovitis in osteoarthritic joints, set the tone for a more comprehensive understanding of inflammation in OA.[7–10] The presence of diverse inflammatory mediators in the synovial fluid of OA patients has strengthened the vital role of inflammation in OA pathogenesis.[11] Certainly, cytokines and adipokines have been proposed as biomarkers for OA. These molecules actively participate in OA by driving articular cartilage degeneration, bone marrow lesions, and increased Western Ontario and McMaster (WOMAC) scores indicative of pain.[12,13] Despite increasing evidence regarding proinflammatory mediators and their correlation with clinical and radiographical features, the exact pathogenic mechanisms leading to OA remain unclear.

The most prominent contribution of these inflammatory mediators in OA is to promote cartilage breakdown. Several studies have supported this hypothesis by demonstrating that IL-1β, IL-6, TNF-α, and

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IL-18 belong to the select group of proinflammatory cytokines that remain elevated both systemically and locally in OA.[13] This group of cytokines participates not only in chronic inflammation but also in low-grade inflammation, also known as inflammaging, a critical process underlying OA.[14] Cartilage breakdown is characterized by the release of cartilage matrix fragments, such as type II collagen and advanced glycation end products (AGEs). Once cleavage occurs, these components converge on local stimulation of chondrocytes, synovial fibroblasts, and immune cells.[15–17] Eventually, local stimulation and activation of synovial and immune cells initiate the release of matrix metalloproteinases (MMPs) and damage-associated molecular patterns (DAMPs), generating a positive activation loop that enhances and promotes OA development. The accumulation of numerous DAMPs induces the activation of diverse inflammatory processes. Over the past few years, emerging evidence has indicated that inflammasome activation is one of the main pathogenic processes activated by DAMPs in OA.[18–22]

Inflammasomes are multi-protein complexes that play a critical role in the innate immune response. Activation of these complexes is mediated by the binding of DAMPs to pattern recognition receptors (PPRs), which is followed by the activation of cytosolic caspases.[23] Together, they promote apoptosis while also driving the upregulation and activation of proinflammatory cytokines such as IL-1β and IL-18. Dysregulation of the inflammasome activity has been shown to be a major disruptor of tissue homeostasis leading to several inflammatory and metabolic diseases such as diabetes, obesity, atherosclerosis, chronic neurodegenerative diseases, and some cancers. The corresponding pathological mechanisms of these diseases have been extensively discussed in several review articles.[24–26] In OA, NLRP3 and NLRP1 are important inflammasome components, which act as the triggers of pathogenic processes in macrophages.[27,28] Because inflammation has been identified as a critical factor driving radiographic progression and pain in OA, the role of inflammasomes presents a promising strategy to target these molecules in distinct cell types, such as chondrocytes and fibroblast-like synoviocytes (FLS), which are key players in OA.[27] Evaluation of inflammasome regulation might provide relevant evidence concerning the vital role of these multi-protein complexes as potential modulators of pathogenic features in OA. This review will therefore elaborate on the emerging roles of inflammasomes in OA and describe the diverse strategies targeting inflammasome-dependent mechanisms being developed as OA disease-modifying therapies.

PATHOLOGICAL ROLES OF INFLAMMASOMES IN ARTHRITIS AND ASSOCIATED CONDITIONS

Inflammation and tissue damage are critical hallmarks of arthritic diseases (Figure 1). The role of inflammasomes in the most common arthritic diseases, for instance, rheumatoid arthritis (RA), Gout, and juvenile idiopathic arthritis (JIA) has been described in detail in several reviews pinpointing how understanding their pathogenic mechanisms can lead to the development of novel therapeutic options.[29–31]

Spondyloarthropathies are a form of arthritic diseases that affect the spine in addition to joints. They are characterized by chronic pain, joint inflammation, and bone changes. Ankylosing spondylitis (AS) and psoriatic arthritis (PsA) are examples of spondyloarthropathies in which inflammasome activation has been implicated. In AS, decreased expression of TNFAIP3 and DEPTOR in peripheral blood monocytes correlated with NLRP3 inflammasome activation and high expression of *IL1B* and *IL18*.[32] High mRNA expression of *NLRP3* and *IL1B* in PBMC from AS patients compared with healthy blood donors was also reported by an independent study, supporting the role of inflammasomes in AS pathogenesis.[33] High protein levels of NLRP3 inflammasome components and IL-1β production were observed in PBMC from AS mice.[34] In addition, Guggino et al. provided interesting evidence about the critical role of the NLRP3, NLRC4, and AIM2 inflammasomes in the gut of both AS mice and AS patients.[35] The overexpression of inflammasome components in PBMC from AS patients was additionally associated with increased serum levels of IL-1β and IL-18, which positively correlated with the AS Disease Activity Score (ASDAS).[35] Regarding PsA, several single-nucleotide polymorphisms (SNP) in inflammasome and inflammasome-related genes have been reported. These SNPs are located in *NLRP1*, *NLRP3*, and *CARD8* genes and were involved with PsA risk and the disease phenotype.[36] CLIC1 is a chloride intracellular channel protein that functions as an activator of NLRP3 in macrophages.[37] High CLIC1 expression in the skin from PsA was involved with increased angiogenesis and might suggest NLRP3 involvement in increased angiogenesis of PsA joints.[38]

The pathogenic processes involving inflammasome activation are not only restricted to arthritic diseases but also arthritic-associated conditions. One of the most common pathologies in inflammatory arthritis patients is osteoporosis, which causes bone loss and increases fracture risk (Jiang et al., 2021a). The global

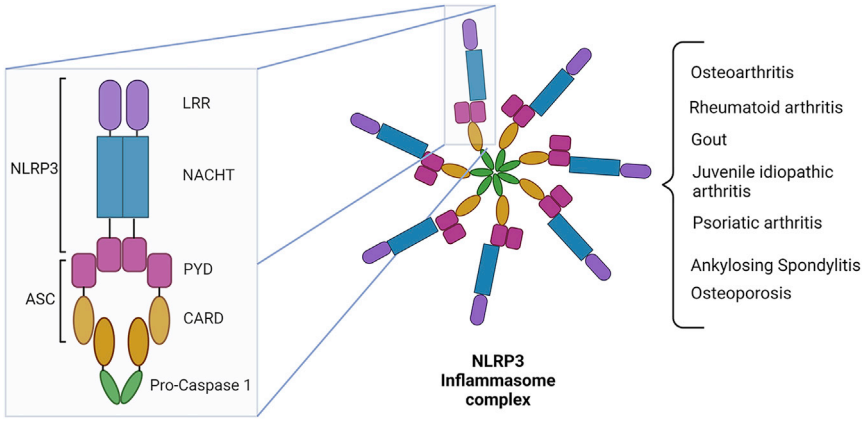

Figure 1. Schematic overview of the NLRP3 inflammasome complex

Inflammasomes are multi-protein complexes formed by the oligomerization of distinct domains. NLRP3 inflammasomes have an LRR domain at the C-terminus, a NACHT domain in the middle, and a PYD domain at the N-terminus. The PYD domain from NLRP3 interacts with the PYD domain from ASC, and CARD from ASC protein recruits pro-caspase-1 through interaction with its CARD domain. In the canonical inflammasome pathway, the NLRP3 is assembled after PAMPs or DAMPs are recognized by cytosolic PRRs or inflammasome sensors. This recognition leads to an increase in the transcriptional activity of the nuclear factor-κB (NF-κB), caspase-1-dependent cleave of GSDMD, and the release of proinflammatory cytokines, such as IL-1β and IL-18. NLRP3 inflammasome activation promotes inflammatory and pathogenic processes involved in the pathogenesis of arthritic diseases, for instance, osteoarthritis, rheumatoid arthritis, gout, and juvenile idiopathic arthritis. NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein containing CARD; LRR, leucine-rich repeat; PYD, pyrin domain; CARD, caspase recruitment domain.

strategy for osteoporosis patients has focused on improving bone mineral density (BMD); however, specialized pharmacological approaches are still required.[39] In this sense, NLRP3 inactivation has emerged as a determining process to ameliorate osteoporosis hallmarks. A study performed in the ovariectomized (OVX) mouse model, used for studying postmenopausal osteoporosis, indicated that OVX mice highly expressed NLRP3 inflammasome components in femoral bone, an effect that was successfully suppressed by melatonin treatment.[40] In addition, repression of NLRP3 resulted in the restoration of osteogenic differentiation by upregulating Runx2 and osteocalcin.[40]

OA is a disease classified as a chronic low-grade inflammatory pathology (Figure 2). Increased expression of inflammatory mediators in the OA joint supports the induction of locally active non-resolving inflammatory processes.[41,42] A role for inflammasome activation in non-resolving inflammation has started to emerge.[43] It has been proposed that NLRP1 and NLRP3 containing inflammasomes not only promote the production of IL-18 and IL-1β from various cell types within the joints but also induce a unique form of cell death known as pyroptosis in the articular chondrocytes. In the following sections of this review, we will discuss in detail the discoveries made over the past decade that demonstrate a prominent role of inflammasome activation in driving the chronic inflammatory pathology of OA.

PATHOLOGICAL MECHANISMS DRIVING INFLAMMASOME ACTIVATION IN OA

Extracellular factors drive inflammasome activation in OA

Numerous systemic and local soluble factors have been reported to be involved in driving inflammasome activation in arthritic diseases, which happens in two steps.[44,45] The first step requires a primary signal to activate intracellular cascades to transcribe and translate the inflammasome factor NLRP3 and the proinflammatory cytokines pro-IL-1β and pro-IL-18. Signal one is mediated through PAMPs (Pathogen-associated molecular patterns) or DAMPs binding to their specific PRRs. Consequently, a second step is triggered resulting in inflammasome assembly, caspase-1 activation, and finally, the release of the active form of IL-1β and IL-18 to promote inflammatory and pathogenic mechanisms.

In OA, the primary signal is predominantly constituted by TLRs agonists.[44–46] The second signal for NLRP3 inflammasome activation can be mediated via several factors. One of these factors is hydroxyapatite (HA), a

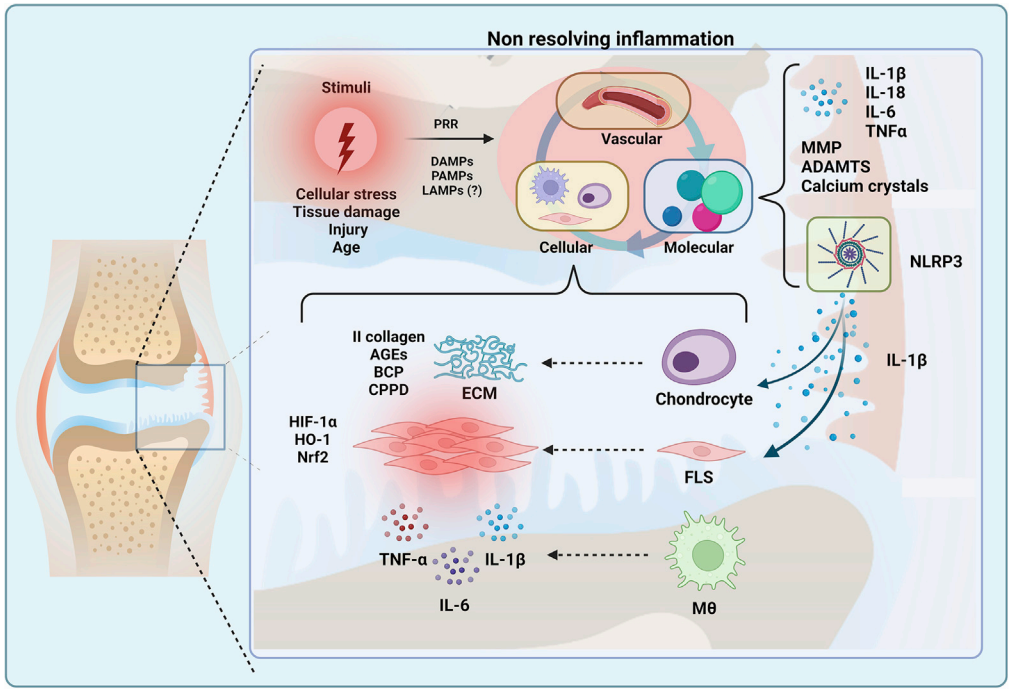

Figure 2. Overview of non-resolving inflammatory processes in osteoarthritis

Several active processes drive inflammation in OA. For instance, cellular stress, tissue damage, and trauma are critical processes commonly involved with the inflammation onset. These processes produce molecules associated with damage (DAMPs), pathogens (PAMPs), and lifestyle (LAMPs), which are recognized by the innate immune system through PRRs. This recognition synergizes the activation of the immune system in the vascular, molecular, and cellular environment to provide the appropriate defense conditions. However, in inflammatory diseases such as OA, homeostatic processes fail and the resolution model is not achieved, thus, the inflammation is perpetuated. In this scenario, the proinflammatory cytokine profile increases; MMPs, ADAMTs, and calcium crystals are produced. Moreover, the role of NLRP3 in maintaining and promoting inflammation has been described as critical in OA pathogenesis due to the regulation of IL-1β, which activates chondrocytes and FLS. Both cell subsets actively participate in mediating pathogenic processes converging in ECM degradation, cartilage breakdown, fibrosis, synovitis, and production of inflammatory mediators. In addition, macrophages display pathogenic features after TLR-4 activation, promoting NLRP3-dependent responses, and nuclear translocation of NF-κB and IL-1β/IL-18 production. Abbreviations: PRRs, Pattern Recognition Receptors; DAMPs, Damage-associated molecular patterns; PAMPs, Pathogen-associated molecular patterns; LAMPs, Lifestyle-associated molecular patterns; MMP, Matrix metalloproteinases; ADAMTs, Disintegrin and metalloproteinase with thrombospondin motifs; NLRP3, NLR family pyrin domain containing 3; ECM, Extracellular matrix; AGEs, Advanced glycation end products; BCP, Basic calcium phosphatase; CPPD, Calcium pyrophosphatase dihydrate; HIF-1α, Hypoxia-inducible factor- 1α; HO-1, heme oxygenase-1; Nrf2, nuclear factor erythroid 2 related factor 2; FLS, Fibroblast-like synoviocytes; Mθ, macrophage.

type of calcium phosphatase crystal found in synovial fluid samples from OA patients.[18] In addition, basic calcium phosphatase (BCP) and calcium pyrophosphatase dihydrate (CPPD) crystals, commonly found in cartilage, synovial fluid, and menisci of OA patients, activate the inflammasome.[19] The mechanism of action for these calcium crystals is NLRP3-dependent and independent; however, IL-1β production and aggravation of joint degeneration appear to take place after NLRP3-dependent calcium crystal activation (Figure 3). Purine metabolites have been proposed as an inflammasome activator in OA. It is well known that chronic local and systemic production of free fatty acids, ATP, and purine are critical factors that trigger inflammasome-dependent inflammation in OA from the stress of obesity-associated metainflammation, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS).[47]

Controversial has been the systemic role of inflammasomes in OA. Although different studies have reported increased expression of inflammasome components in the cartilage and synovium, these findings differ in blood cell studies. In this sense, NLRP3 and IL-1β were evaluated in two cohorts of erosive (EHOA) and non-erosive (NEHOA) hand OA patients. However, the study was unable to demonstrate increased

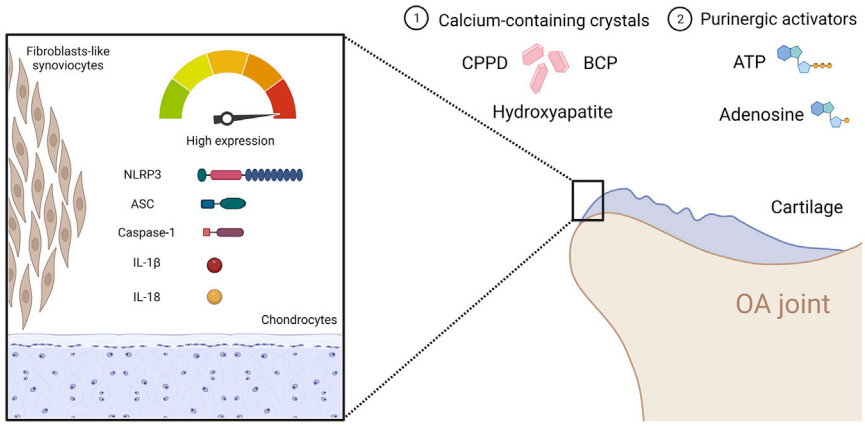

Figure 3. Multiple extracellular factors activate the NLRP3 inflammasome in OA patients

The main reported promoters of NLRP3 inflammasome activation include calcium-containing crystals, i.e., CPPD, BCP, and hydroxyapatite. Moreover, purinergic activators of the NLRP3 are ATP and adenosine. These promoters have been involved in the high expression of NLRP3 inflammasome components such as NLRP3, ASC, active caspase-1, and the production of IL-1β and IL-18 in cartilage and synovium from OA patients. Abbreviations: BCP, Basic calcium phosphatase; CPPD, Calcium pyrophosphatase dihydrate; ATP, Adenosine triphosphate; NLRP3, NLR family pyrin domain containing 3; ASC, Apoptosis-associated speck-like protein containing a CARD.

secretion of IL-1β in serum samples from hand OA (HOA) patients when compared with control subjects.[20] In addition, PBMC from HOA patients exerted a decreased gene expression of both *IL1B* and *NLRP3*. Of interest, the NLRP3/beta-actin ratio significantly increased in NEHOA compared with EHOA and the control group.[20] On the contrary, an independent study reported high soluble levels of ASC (apoptosis-associated speck-like protein containing a CARD), caspase-1, and IL-1β in OA patients compared with healthy blood donors; however, its consequence on OA phenotype was not discussed.[48] Soluble factors associated with inflammasome activation have also been observed locally. Synovial fluid from temporomandibular OA patients showed high concentrations of NLRP3, caspase-1, IL-1β, and IL-18, suggesting these molecules initiate degenerative processes *in situ*.[49] High NLRP3 expression was also reported in synovium from OA patients,[21] and LPS-primed OA FLS were able to highly express pro-IL-1β[22]; however, whether this expression is NLRP3-dependent is yet to be elucidated.

Inflammasome activation driven by chondrocytes in OA

Cartilage breakdown is the primary pathogenic process observed in OA. The breakdown is mediated by several mechanisms, for instance, overexpression of MMP and ADAMTS (A disintegrin and metalloproteinase with thrombospondin motifs) that promote sustained degradation of ECM (extracellular matrix) components, increased apoptosis of articular chondrocytes and cell senescence.

Inflammasome factors, in particular NLRP3, have been extensively investigated in the OA articular chondrocytes (Figure 4); Two independent studies have reported that deficiency of NLRP3$^{-/-}$ did not prevent cartilage damage in two different animal models for OA,[50,51] even after performing biomechanical load stimulation and inflammatory stress in chondrocytes.[50] Low-grade mechanical loading in anterior cruciate ligament transection (ACLT)-induced OA model reduced NLRP3, caspase-1, and IL-1β in chondrocytes.[52] In fact, low-grade mechanical loading had a significant effect in ameliorating disease hallmarks, for instance, cartilage degeneration, reducing OARSI (Osteoarthritis Research Society International) score and abnormal bone remodeling, and finally, decreasing the expression of joint catabolic factors (*ADAMTS5*, *MMP3*, *MMP13*).[52] In another study, increased expression of NLRP3 and caspase-1 with increased expression of IL-1β and IL-18 was detected in cartilage from the ACLT- and the monosodium iodoacetate-induced OA models, strongly supporting a role for inflammasome activation in joint degeneration.[53,54] Similarly, LPS-primed chondrocytes significantly produced and released IL-1β and IL-18 but also activated pyroptosis, an inflammatory type of lytic cell death, via the NLRP3-caspase-1-GSDMD axis.[54] Also, supporting a role for inflammasome in papain-induced experimental OA model, overexpression of the NLRP3-ASC-caspase-1-IL-1β/IL-18 axis in the chondrocytes was associated with cartilage degradation and NF-κB activation.[55] Taken together, these studies show that the downregulation of

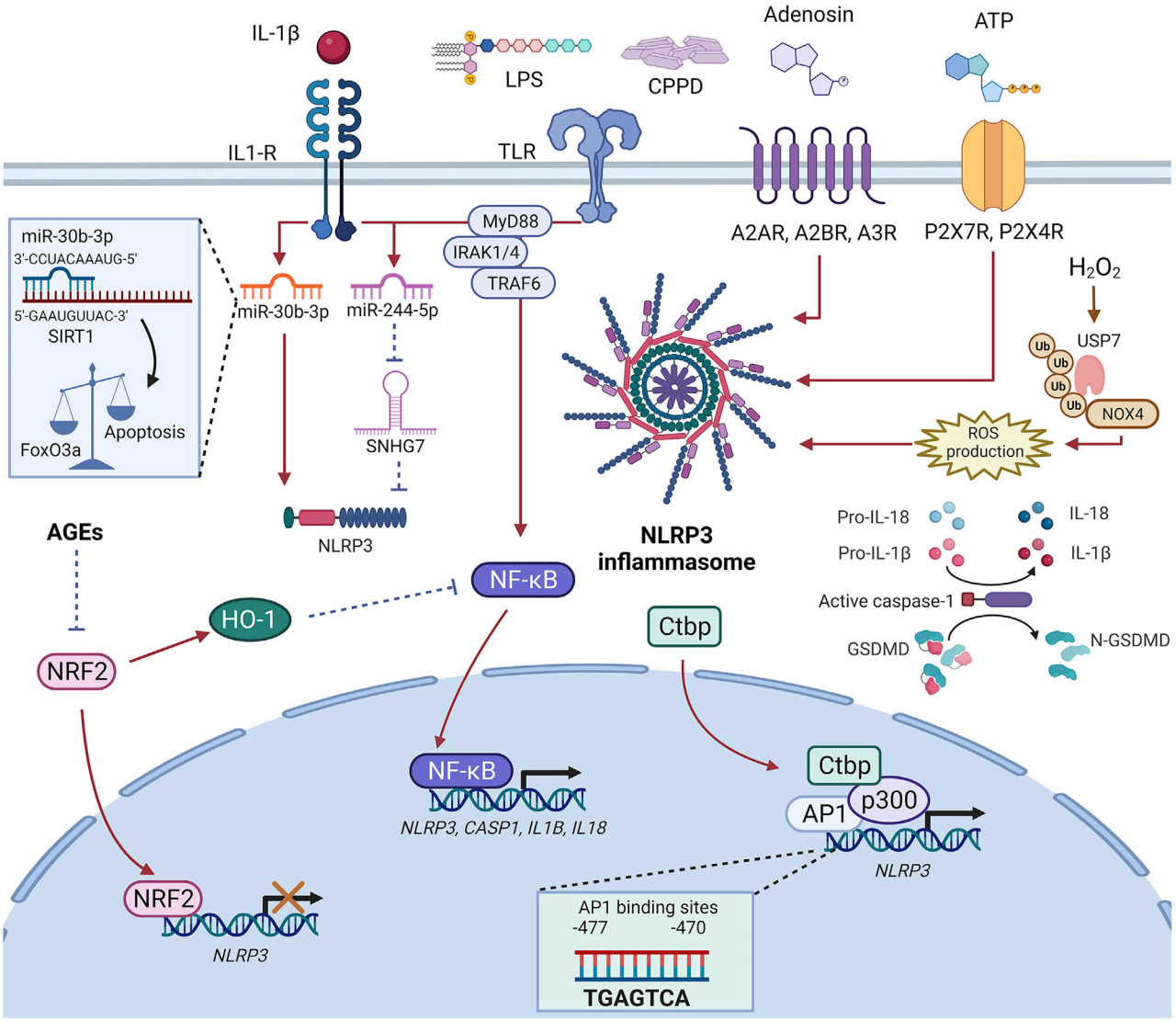

Figure 4. Mechanisms of NLRP3 activation in OA chondrocytes

Chondrocytes have become active players driving NLRP3 inflammasome-dependent mechanisms. Stimulation of these cells with LPS or calcium-containing crystals, which are critical activators of the NF-κB signaling pathway favors the transcription of *NLRP3*, *ASC*, *CASP1*, *IL1B*, and *IL18*. Moreover, IL-1β can also activate several pathogenic processes, for instance, NF-κB activation and overexpression of miRNAs that function as activators of NLRP3. The miR-30b-3p directly induces the activation of NLRP3 and binds to SIRT1 mRNA, which is involved in chondrocyte apoptosis induction. The miR-244-5p act by blocking the inhibitory effect of the long-non-coding RNA SNHG7, an inhibitor of NLRP3. Adenosine is another NLRP3 inductor after binding with A2AR, A2BR, and A3R. Similarly, ATP can activate the NLRP3 inflammasome by interacting with P2X7R and P2X4R. Complex mechanisms have also been described for NLRP3 activation, i.e., the stimulation of chondrocytes with H_2O_2 caused upregulation of USP7. USP7 is a ubiquitin protease capable of deubiquitinating NOX4, thus, preventing NOX4 proteasomal degradation. NOX4 promotes the release of ROS, a process that leads to oxidative stress and converges in NLRP3 activation. An additional mechanism for NLRP3 activation is mediated by the upregulation of Ctbp in cartilage from OA patients, where Ctbp proteins act as transcriptional co-activators of the AP1 transcription factor. The active complex Ctbp-p300-AP1 specifically binds to the promoter region of the *NLRP3* gene promoting its transcription in OA chondrocytes. On the other hand, NRF2 functions as a transcriptional repressor of *NLRP3* and NF-κB in an HO-1-dependent mechanism; however, in OA several molecules such as AGEs inhibit the effect of NRF2 and contribute to the promotion and maintenance of the inflammatory process. Abbreviations: IL-1β, Interleukin-1 beta; IL-1R, Interleukin-1 receptor; LPS, Lipopolysaccharide; TLR, Toll-like receptors; CPPD, Calcium pyrophosphatase dihydrate; ATP, Adenosine triphosphate; NLRP3, NLR family pyrin domain containing 3; A2AR, Adenosine A2A receptor; A2BR, Adenosine A2B receptor; A3R, Adenosine A3 receptor; P2X7R, P2X purinoceptor 7; P2X4R, P2X purinoceptor 4; USP7, Ubiquitin specific protease 7; NOX4, NADPH oxidase 4; MyD88, Myeloid differentiation primary response 88; IRAK1/4, Interleukin 1 receptor-associated kinase 1/4; TRAF6, TNF receptor-associated factor 6; SIRT, Sirtuin 1; FoxO3a, Forkhead box class O 3a; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; CASP1, Caspase-1; ASC, Apoptosis-associated speck-like protein containing a CARD; GSDMD, Gasdermin D; ROS, Reactive oxygen species; AGEs, Advanced glycation end products; Ctbp, C-terminal-binding proteins; p300, E1A binding protein p300; AP1, Activator protein 1 complex; NRF2, Nuclear factor erythroid 2–related factor 2; HO-1, Heme oxygenase-1.

NLRP3-inflammasome is either inconsequential or protective in articular cartilage. However, its upregulation is detrimental to joint homeostasis, making it a highly attractive therapeutic target.

The mechanisms underlying inflammasome activation in OA chondrocytes have also been reported. Increased levels of C-terminal-binding proteins (CtBPs; CtBP1 and CtBP2) in the cartilage of OA patients was put forth as one such mechanism. The CtBPs directly interact with p300 and subsequently bind with AP1 subunits.[56] The CtBP-p300-AP1 transactivation complex interacts with the *NLRP3* promoter to activate its transcription and subsequently, cleavage of caspase-1 and increased expression of IL-1β in chondrocytes occurs. This study also reported that increased expression of CtBPs in OA chondrocytes is mediated by the downregulation of DNA methyltransferases (DNMT1 and DNMT3), which causes the demethylation of *CTBP1* and *CTBP2* genes.[56]

The role of long non-coding RNAs (lncRNA) and micro-RNAs have also been implicated in the regulation of inflammasome activity in OA. In this regard, the small nuclear RNA host gene 7 (SNHG7) has been reported as a critical downregulator of NLRP3 inflammasome activation, which is downregulated in OA cartilage.[57] The mechanism mediating SNHG7 downregulation is presumably mediated by the overexpression of miR-214-5p, which to some extent can partially inhibit the cartilage-protecting effects of SNHG7 in IL-1β-stimulated chondrocytes.[57] The miR-30b-3p is another interesting microRNA proposed as a critical driver of inflammation in chondrocytes. The miR-30b-3p was found to be upregulated in joint tissues from OA patients and in chondrocytes obtained from DMM rats.[58] miR-30b-3p likely targets SIRT1 in chondrocytes and promotes apoptosis and activation of NRLP3 inflammasome components via inhibition of the chondroprotective SIRT1/FoxO3 axis.[58]

Nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor member of the leucine zipper family, is yet another regulator of NLRP3 inflammasome activation in articular chondrocytes.[59,60] A balance between NRLP3 and NRF2 activity in the chondrocytes was found to regulate OA pathogenesis; for instance, NRF2 was found to be downregulated after stimulation with AGEs in the SW1353 chondrocyte cell line, thereby resulting in the upregulation of NLRP3 and ASC, with the secretion of IL-1β and IL-18.[59] ROS production and oxidative stress downstream of NADPH oxidase 4 (NOX4) are also known to regulate NLRP3 levels in chondrocytes.[59] Oxidative stress upregulates ubiquitin-specific protease (USP7) causing the deubiquitination of NOX4. As a result, NOX4 escapes from the proteasomal degradation enhancing ROS production and NLRP3 and caspase-1 activation.[61] This model of oxidative stress-induced OA was characterized by IL-1β and IL-18 secretion, GSDMD-dependent pyroptosis of chondrocytes, and MMP production.[61] The increased expression of USP7 and NOX4 in human OA cartilage further supports that the USP7-NOX4-ROS-NLRP3 axis may be relevant to joint degeneration in OA patients.

Activated fibroblast-like synoviocytes mediate NLRP3 activation in OA

Tissue fibrosis that occurs in OA shows an increase in the production of ECM and angiogenesis, leading the synovial tissue to a process of chronic inflammation that has repercussions on surrounding tissues.[62,63] In addition, the synovium components allow the joints' nutrition, lubrication, and structure; therefore, the synovium becomes a relevant tissue in OA.[64] Fibroblast-like synoviocytes (FLS) are a key player in inflammation and joint destruction because of their activation and release of proinflammatory markers. Several studies have focused on centralizing the role that inflammasomes play directly in the joint and how their activation or inhibition can affect FLS homeostasis.[65]

One of the main cytokines responsible for FLS activation is IL-1β, which is regulated by NLRP3, and studies from NLRP3$^{-/-}$ FLS stimulated with LPS confirmed the involvement of NLRP3-dependent IL-1β and IL-18 production by FLS.[64] Increased hypoxia levels mediate HIF-1α activation in OA and other inflammatory pathologies[66]; this process has been directly related to the activation of NLPR3 and aggravation of synovitis and fibrosis in OA pathology.[63,67] Simultaneously, immunohistochemical analysis of synovium from OA patients and murine model showed higher expression of NLRP3, ASC, NRF2, and heme oxygenase-1 (HO-1) in the synovium. Although the relationship of these molecules in the activation of NLRP3 seems inconclusive, it was determined that ROS could induce their activation once stimulated with LPS *in vivo*. Even though induction by LPS is considered a mechanism of inflammatory OA; this study also suggests that beyond activation, NLRP3 is vital to trigger pathogenic OA hallmarks.[64] Cultures of human FLS isolated from knee OA (KOA) have shown a role for LPS and ATP as molecules capable of activating NLRP3. In addition, increased

mRNA levels of *NLRP3*, *ASC*, *CASP1*, and increased soluble levels of IL-1β and IL-18 suggest that LPS and ATP signaling converge in inflammasome-mediated activation mechanisms.[68]

Even though the expression of NLRP3 and the release of IL-1β and IL-18 in the synovium have been confirmed, the mechanisms by which they promote inflammation have not been related to cartilage degradation and MMP production in OA; therefore, the role of FLS expressing NLRP3, and cartilage degradation remain controversial.[64,69] Functional studies in FLS have supported this controversy by reporting that the inflammasome assembly was unsuccessful in active synoviocytes.[67] The synovium not only expresses NLRP3, but also NLRP1, NLRP6, NLRP10, NLRP12, and NLRP14, whereas NLRP3 and NLRP1 are the established mechanisms for the activation of pyroptosis.[43,65,67] A different NLRP complex may likely be involved in regulating the inflammasome pathways in OA FLS.

CASPASE ACTIVATION MEDIATES INFLAMMATION AND PYROPTOSIS IN OA

Pyroptosis is a type of highly inflammatory lytic programmed cell death prompted by inflammasomes. This form of cell death has characteristics such as DNA fragmentation, chromatin agglutination, caspase-1 activation, and flattening of the cytoplasm due to plasma membrane leakage.[70,71] Pyroptosis is mediated by pore formation (1–2 μm diameter) because of the polymerization of the Gasdermin family members, allowing mature IL-1β and IL-18, followed by the release of cell content and osmotic lysis and release of proinflammatory cytokines.[71] Inflammasome-mediated pyroptosis can occur via the canonical or the non-canonical mode of activation (Figure 5). The canonical inflammasome pathway primarily involves caspase-1-dependent cleavage of Gasdermin D (GSDMD) whereas the non-canonical pathway is primarily driven by the activation of caspase 4/5 (caspase 11 in mouse).[72] In OA pyroptosis was shown to occur in the perichondrium, triggered by the activation of different caspases where inflammasomes act as platforms for caspase activation.[28] Danger signals are present in joints of OA patients, such as high mobility box 1 (HMGB1), uric acid, ATP, S100 proteins, heat shock proteins (HSPs), and hyaluronan fragments, triggering the immune system, promoting pyroptosis, sustaining inflammation, and creating a chronic low-grade inflammation.[14,28] In OA pathogenesis, DAMPs accumulation is a driver of proinflammatory signals that promote pyroptosis and production of MMP-1, MMP-2, and MMP-13 and promote cartilage degeneration and damaged chondrocytes associated with OA morphological alterations consistent with pyroptosis.[28,73] Several Gasdermin family members were also implicated in OA pathology. GSDMD level is upregulated in OA synovium and its knockout provided protection from cartilage degeneration and synovitis in a surgically induced post-traumatic OA mouse model.[26] Similarly, Gasdermin E (GSDME) level is reported to be upregulated in the synovial macrophages and synovial fibroblasts of OA and RA synovium and its deficiency mitigated synovitis in mouse models of RA.[74,75] In addition, we speculated a role for Gasdermin C (GSDMC) in OA as its upstream regulator, caspase-6, [76] was shown to be upregulated in chondrocytes exposed to mechanical stress in the ACLT rat osteoarthritis model.[77]

Another class of molecules associated with OA pathogenesis are micro-RNAs, which have been associated with anti-inflammatory properties, specifically, miRNA-146a and miRNA-140-5p, via RNA silencing and transcriptional regulation of gene expression.[78] The miR-146a and miR-146b counteract with proinflammatory molecules, increase chondrocytes proliferation, and reduce cell death by targeting the NF-κB pathway. In addition, miR-140-5p was shown to alleviate OA cartilage injury by repressing chondrocyte pyroptosis via the CTSB/NLRP3 axis.[78,79]

TARGETING INFLAMMASOME-DEPENDENT MECHANISMS IN OA

The development of disease-modifying OA drugs (DMOADs) has become the most important challenge worldwide. Several non-steroidal anti-inflammatory drugs are currently used to ameliorate OA symptoms; however, these drugs only slightly relieve pain and are ineffective in preventing disease progression. In the following sections, we will discuss studies on various natural and synthetic compounds that inhibited inflammasome-dependent pathways in OA.

Emerging strategies for inhibiting inflammasome activation by extracellular factors

The role of calcium crystals in activating inflammasome-dependent mechanisms promoting OA severity and its inhibition might represent an attractive therapeutic strategy for OA patients. In this regard, Phosphocitrate (PC) has been proposed as an inhibitor of calcium-containing crystals, and several studies have supported its therapeutic activity in ameliorating OA hallmarks.[19,80,81] Another study revealed that PC

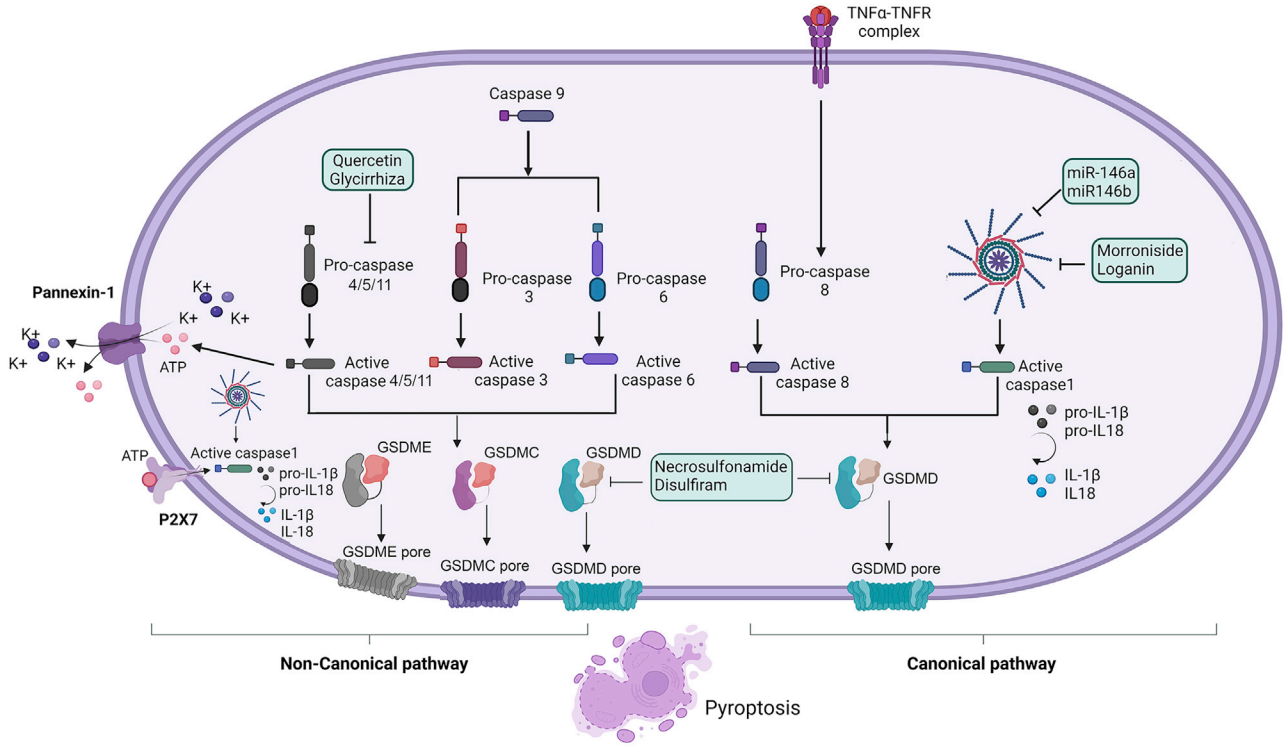

Figure 5. Pyroptotic pathways related to OA

The canonical pyroptotic pathway involves the activation of caspase-1 activity leading to the cleavage of GSDMD through the activation of nod-like receptor (NLR) proteins. GSDMD translocases to the plasma membrane and form pores that converge in cellular membrane rupturing. Caspase-8 is activated by TNF-α derived from macrophages and activates GSDMD and GSDMC, resulting in pyroptosis. In the non-canonical pyroptotic pathway, caspase-3/6 activation triggers pyroptosis by cleaving GSDMC. Moreover, p-Stat3 drives PD-L1 translocation, promoting the expression of GSDMC. Caspase-3 cleaves GSDME. Pyroptosis in the non-canonical pathway is induced by caspases-4/5/11 that activate GSDMD and is mediated by Pannexin-1 causing the release of cellular ATP and inducing pyroptotic cell death by the ion channel P2X7 receptor. Finally, the potential therapeutic agents for targeting pyroptosis in OA animal models are shown within the green boxes. Abbreviations: P2X7R, P2X purinoceptor 7; ATP, Adenosine triphosphate; GSDMD, Gasdermin D; GSDME, Gasdermin E; GSDMC, Gasdermin C.

decreased the expression of genes involved in proliferation, angiogenesis, inflammatory response, and pain in OA FLS.[82] Considering the reported evidence about the effect of PC in OA, a new small molecule has been reported as a PC analog: Carolinas Molecule-01 (CM-01) shares structural similarities with PC and was orally administered to OA-prone Hartley guinea pigs.[83] The results suggested that CM-01 treatment protects from cartilage degeneration, osteophyte formation, and meniscal calcification; thus, preventing the development of OA, which leaves open a window for a new orally administered OA drug. The mechanism of action for PC on different tissues in aged animals needs further investigation.

Extracellular ATP levels are also known to modulate inflammasomes in OA. For instance, the purinergic receptors P2X7 and P2X4, which are primarily activated by extracellular ATP, can initiate NLRP1 and NLRP3-dependent inflammatory responses in OA.[65,84–86] AZD9056 is a potent P2X7 receptor antagonist which has shown significant efficacy by downregulating the expression of P2X7, modulating the activation of the NF-κB signaling pathway, and proinflammatory mediators in rats with monoiodoacetic acid-induced (MIA) OA.[87] Another study implemented the same MIA model to test *in vivo* the P2X7R antagonist, A740003, reporting comparable results. Moreover, A740003 was effective in downregulating the expression of NLRP3, cleaved caspase-1, IL-1β, and finally, decreasing the percentage of pyroptotic cells.[84] In the same line of evidence, 5-BDBD and A-438079, two potent antagonists of P2X4 and P2X7, respectively, were evaluated in urothelial cells, showing significant inhibition of caspase-1 activation[88]; however, the role of these modulators requires more investigation within the OA pathology.

Hyaluronic acid (HA) is another important modulator of inflammation in OA pathology; however, its role in inhibiting inflammasome components is yet to be described. In this regard, a recent study reported that

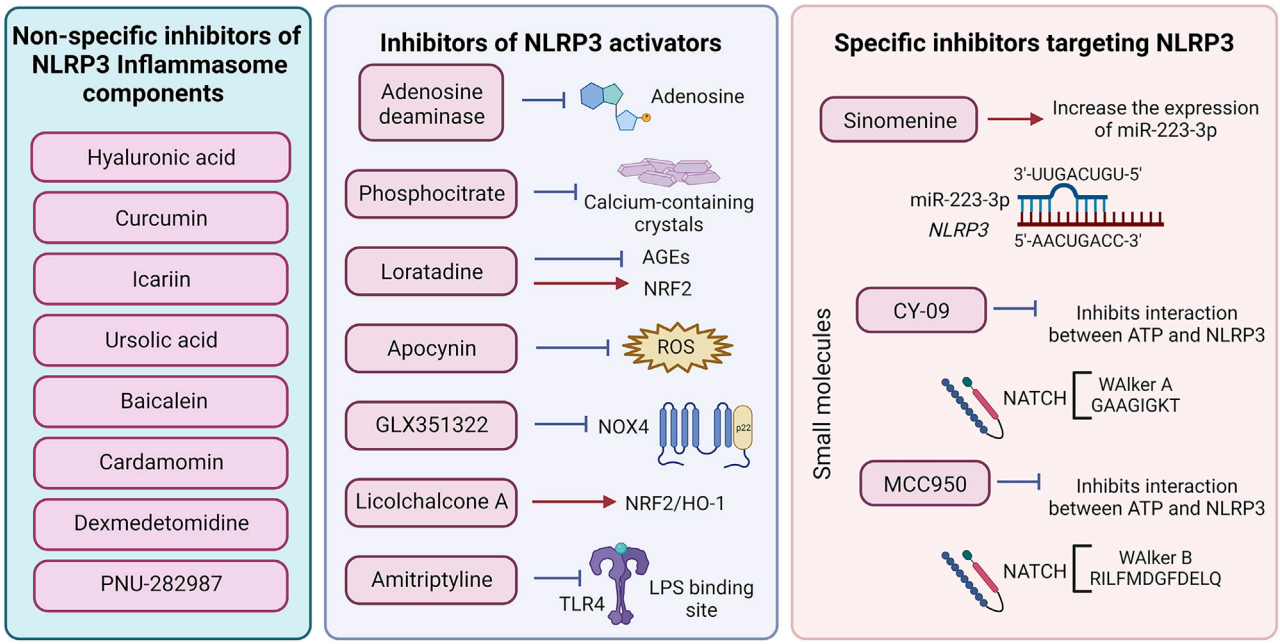

Figure 6. Targeting NLRP3 inflammasomes in OA chondrocytes

Natural and synthetic compounds have been proposed as mediators of NLRP3-dependent mechanisms in OA. Unspecific inhibitors include molecules that do not directly inhibit known inflammasome drivers or components but have a significant indirect effect downregulating the expression of NLRP3 components and critical OA hallmarks *in vivo* and *in vitro*. Inhibitors of NLRP3 activators target upstream molecules involved in NLRP3 activation, regulation, or expression. Some of these inhibitors also upregulate the expression of NRF2, an important repressor of NLRP3 expression. Specific inhibitors targeting NLRP3 include small molecules that bind to the NLRP3 NATCH motive and inhibit its structural conformation change toward an active form. Sinomenine is a natural compound that upregulates the expression of the miR-223-3p, a microRNA that directly targets the NLRP3 mRNA blocking its translation toward a functional protein.

intra-articular administration of HA in temporomandibular joint OA patients significantly inhibited the secretion of NLRP3, IL-1β, and IL-18 in FLS.[49] Curcumin has also been evaluated in the DMM OA model, demonstrating a significant reduction of OA score and inflammatory markers. Although the authors suggested that the curcumin mechanism of action targets the NLRP3-Caspase-1-IL-1β axis, those results were observed in THP-1 cells and further investigation in OA primary cells and preclinical models must be performed to corroborate these findings.[89]

Emerging treatment strategies for inflammasome inhibition in OA chondrocytes

The mechanisms mediating inflammasome activation differ between cell types within the osteoarthritic joint; for this reason, the inflammasome inhibitory mechanisms might be slightly different and dependent on the studied cell type, such as chondrocytes (Figure 6). For instance, Sinomenine, the major bioactive compound of the traditional herb *Sinomenium acutum*, has been proposed as a natural compound with anti-inflammatory effects in OA chondrocytes.[53] The suggested mechanism of action depends on the up-regulation of miR-223-3p, which has a binding site for the NLRP3 gene. miR-223-3p was found to be down-regulated in IL-1β-stimulated chondrocytes, which was reverted by Sinomenine treatment. Sinomenine also inhibited the protein levels of NLRP3, ASC, caspase-1, IL-1β, and IL-18.[53] In the same line of evidence, Icariin, an extract of the herb *Epimedium spp.*, has also shown anti-inflammatory effects in OA. The effect of Icariin was evaluated in LPS-primed chondrocytes where NLRP3 expression and caspase-1 activity were suppressed.[54] Another natural compound with anti-inflammatory properties is ursolic acid (UA). UA is a pentacyclic triterpenoid found in the peel of several fruits and medicinal plants and has shown promising results as a therapeutic agent in OA.[90] The precise mechanism of action for UA is yet to be elucidated; however, it has been proposed that UA treatment can significantly decrease the protein levels of NLRP3, ASC, and cleaved caspase-1 in rat chondrocytes stimulated with TNF-α.[90] In addition, Licochalcone A (Lico A), a flavonoid extracted from the roots of *Glycyrrhiza* spp., has demonstrated optimistic results modulating NLRP3 inflammasome-associated processes in OA. The proposed mechanism suggests an upregulation

of NRF2 and HO-1, both considered regulators of inflammatory mechanisms.[91] Lico A can additionally suppress the inflammatory response in LPS-primed chondrocytes and protect mice from cartilage degeneration in the ACLT-induced OA model.[91] Similarly, Cardamonin, a biological compound extracted from *Alpinia katsumadai*, has been evaluated in IL-1β-stimulated chondrocytes exerting anti-inflammatory properties. Cardamonin is capable of suppressing NLRP3 inflammasome signaling and upregulating the expression of NRF2.[60] These findings provide reasonable evidence for a regulatory mechanism between NRF2 and NLRP3 in chondrocytes *in vitro*; nevertheless, *in vivo* models are required to corroborate those results. Baicalein, a flavonoid found in *Scutellaria spp.*, also showed promising results *in vivo* OA models by downregulating NLRP3 and caspase-1 and reducing chondrocyte damage.[92] Although the suggested mechanism of action for Baicalein indicated an association with the regulation of oxidative stress markers, the current evidence was unable to demonstrate the specific mechanism. Further research must be performed to clarify this concern. Similarly, Quercetin has exerted important anti-inflammatory and antioxidant properties. Decreased OARSI score, soluble IL-1β, and IL-18, with decreased cartilage expression of NLRP3 and caspase-3, have been reported after Quercetin administration in the ACLT-induced OA model.[93] These findings were further corroborated *in vitro* by stimulating chondrocytes with IL-1β. The mechanism of action for Quercetin was suggested to inhibit IRAK1-dependent IL-1β stimulation; thus, causing inhibition of NLRP3 activation, suppression of cell apoptosis, ROS production, and IL-1β and IL-18 secretion.[93]

Synthetic compounds have equally exerted modulatory properties in OA. Dexmedetomidine, which acts as a highly selective α2-adrenergic agonist with sedative and analgesic properties, has also been associated with pain relief and cartilage protection in OA. The reported mechanism of action suggests that dexmedetomidine might target inflammasome-dependent mechanisms in the cartilage of OA rats by significantly decreasing the expression of NLRP3, ASC, and cleaved caspase-1.[55]Of interest, histamine receptor 1 (H1R) expression and activation have also been detected in chondrocytes after stimulation with AGEs, and loratadine, a synthetic blocker for H1R, has emerged as a modulator of inflammasomes in OA.[59] AGEs-H1R interaction induced the activation of intracellular signaling cascades that converge in NLRP3 activation. Loratadine treatment seems to participate in modulating inflammasome activation in two simultaneous processes: (1) Interfering with AGEs signaling and (2) facilitating nuclear translocation of NRF2, which, as previously mentioned, can act as a repressor of NLRP3 transcription.[59] In previous sections of this review, we discussed the two-step mechanism for inflammasome activation, which requires one signal mediated by PRRs. In this sense, TLR4 belongs to those critical PRRs that trigger NLRP3 inflammasome signaling. Amitriptyline is employed as an antidepressant drug that has been repurposed as a pain modulator and has also exerted exciting results by blocking TLR4-LPS interactions in OA chondrocytes.[92] Amitriptyline could downregulate NLRP3 expression and IL-1β secretion in OA chondrocytes in an inhibitory mechanism that seems to be TLR4-dependent.[92] Repurposing available drugs represent a promising strategy to encourage researchers to follow new lines of evidence focused on ameliorating signs and symptoms in OA patients.

Earlier in this review, we explored the activation of the USP7-NOX4-ROS-NLRP3 axis as a critical driver of inflammation in OA. In this regard, an interesting study reported that inhibition of different components of this axis had a potential therapeutic effect in OA chondrocytes. Apocynin, a ROS inhibitor, along with the molecules P22077 and GLX351322, which are USP7 and NOX4 inhibitors, respectively, were able to suppress the NLRP3 inflammasome-dependent mechanisms.[61] Chondrocyte pyroptosis and expression of MMP-1 and MMP-3 were successfully targeted after inhibition of those three axis components,[61]which supports that targeting oxidative stress can indirectly suppress the inflammasome-dependent mechanism in OA.

Small synthetic molecules have emerged as potential DMOADs. CY-09 is a small molecule that interferes with the ATP-binding motif of the NLRP3 NACHT domain; thus, representing a specific inhibitor for this inflammasome component. CY-09 was evaluated in TNF-α-stimulated chondrocytes and in the DMM OA model showing that the NLRP3 inflammasome and GSDMD-mediated pyroptosis were effectively targeted both *in vivo* and *in vitro*.[94] MCC950 is another small molecule described as a specific inhibitor of NLRP3 because of its binding to the Walter B motif at the NLRP3 NACHT domain. MCC950 has shown meaningful results in IL-1β-treated chondrocytes by suppressing NLRP3 and cleaved caspase-1, a mechanism that is dependent on the activation of the Nrf2/HO-1/NQO2 axis.[95] Those findings were further corroborated by using the DMM model. MCC950 has also exerted its therapeutic effect by decreasing the expression

of several catabolic factors and suppressing ROS production in chondrocytes.[95] Some other small molecules have demonstrated an effective indirect inhibition of NLRP3, for instance, the alpha 7 nicotinic acetylcholine receptor (α7nAChR) agonist, PNU-282987. This α7nAChR agonist has been tested in the MIA-induced arthritis model, causing decreased expression of MMP-1 and MMP-13. Of interest, several NLRP3 inflammasome components were also inhibited in the cartilage of MIA rats, findings that were additionally corroborated in IL-1β-treated chondrocytes.[96] The indirect mechanism by which α7nAChR protects from OA progression is presumably through inhibition of IκBα degradation, which prevents NF-κB phosphorylation, thus indirectly targeting NLRP3 inflammasome activation and, finally, conferring chondroprotection.[96]

Emerging treatment strategies for inflammasome inhibition in OA FLS

An emerging area of interest relates to distinct natural molecules that can block the activation of NLRP3 in OA FLS (Table 1). For instance, casticin was a molecule capable of reducing the effect of LPS in FLS by decreasing the expression of caspase-1.[62] Vanillic acid was also able to decrease IL-18 and IL-1β expression,[97] a result further confirmed by studying the effect of this molecule in combination with LPS in an experimental model of OA.[98] Furthermore, Chrysin, a flavonoid found in several plants, could inhibit the NLRP3-dependent production of IL-18 and IL-1β, and suppressed the expression of NLRP3 and caspase-1.[99] In the same line of evidence, agnuside, a chemical compound isolated from *Vitex negundo L.*, has exerted inhibitory properties by decreasing the expression of NLRP3.[63]

Synthetic molecules have also been studied as important inflammasome inhibitors. KMU-1170, a novel multi-protein kinase inhibitor, has shown the ability to suppress the activation of the NLRP3 inflammasome signaling pathway in human osteoarthritic FLS.[22] Another study reported that estradiol significantly suppresses IL-18, IL-1β, and caspase-1 production and downregulated the expression of NLRP3 at the mRNA and protein levels, whereas pro-caspase-1 production was not significantly changed.[68] Moreover, the exogenous stromal cell-derived factor-1 (SDF-1) downregulated the gene expression and protein levels of NLRP3 inflammasome.[100] Limitations in knowledge regarding the role of inflammasome-dependent mechanisms in OA FLS are a critical issue that must be taken into consideration in future studies.

OTHER POTENTIAL FUTURE DIRECTIONS FOR INFLAMMASOME TARGETING IN OA

In addition to the research focusing on non-specific inhibitors of inflammasomes, inhibition of NLPR3 activators, and specific molecules targeting NLRP3 inflammasomes, other research directions related to chondrocytes and FLS inflammasome-dependent activation in OA should be considered. In this regard, adenosine deaminase (ADA) could represent a promising therapeutic modulator in OA because of its effect promoting the degradation of adenosine into inosine. Of interest, adenosine degradation promoted decreased NLRP3 inflammasome activation and reduction of IL-1β and ATP levels.[101]

Targeting pyroptosis in OA has been proposed in *in vitro* experimental studies, where chondrocytes were activated by LPS and ATP, and treatment with disulfiram and glycyrrhizin acid resulted in suppression of the inflammatory response and reduction of pyroptosis.[102] Another interesting finding was that treatment with Icariin also suppressed GSDMD expression, and chondrocyte pyroptosis, and protected against cartilage degeneration in the MIA rat OA model.[54] Other molecules; for instance, morroniside and loganin were assessed in a mouse model of OA, and the authors found that those two iridoid glycosides extracted from *Cornus officinalis* were protective against cartilage degradation by reducing chondrocyte pyroptosis via inhibition of NF-κB signaling.[103,104] Furthermore, the effect of the combined use of the Hedgehog signaling inhibitor, GANT-61, and indomethacin was reported in experimental OA mice. GANT-61 and indomethacin had a synergistic effect on OA via mitigation of chondrocytes pyroptosis and reduction of IL-1β, IL-18, TNF-α, IL-2, and IL-6 expression.[105] In the same line of evidence, Lico A, mitigated the progression of OA in a mouse model, attenuated LPS-induced chondrocytes pyroptosis, and reduced the expression of NLRP3, GSDMD, caspase-1, IL-1β, and IL-18. The authors concluded that Lico A may have a therapeutic potential in OA.[91] Another potential agent for the treatment of OA that has been recently described is Quercetin. This molecule was tested in different models of OA Wistar rats, showing that quercetin can reduce LPS-induced apoptosis and ECM degradation by inhibiting NLRP3-mediated pyroptosis.[106]

The role of GSDMD in mediating IL-1β secretion is well known. Several reports have pointed out that loss or inhibition of GSDMD results in attenuated IL-1β and IL-18 release. For instance, in autoimmune diseases,

Table 1. Potential therapeutic agents targeting NLRP3-dependent mechanisms in OA FLS

Therapeutic agent	Experimental model	Relevant data	Reference
Estromal cell-derived factor-1 (SDF-1)	Human FLS	SDF-1 was found to increase the proliferation of OA FLS. SDF-1 downregulated the gene and protein expression levels of NLRP3 inflammasome, GSDMD, and IL-1β	Wang et al.[100]
	Murine model of collagenase-induced OA	SDF-1 treatment delays pathological phenotypes. Intra-articular injection of 120 ng/kg SDF-1 twice a week reverses high expression of NLRP3, ASC, caspase-1, and protein markers of pyroptosis	
Agnuside (extract of *Vitex negundo L.* (Verbenaceae))	Rat FLS	Agnuside reverses NLRP3 expression	Zhang et al.[63]
	Monoiodoacetic acid (MIA)-induced KOA in rats	Agnuside relieves the state of hypoxia in KOA rats and reduces mRNA and protein levels of NLRP3 in KOA rats	
Casticin	MIA-induced KOA in rats	Upregulated NLRP3 inflammasome complex in MIA group were reduced by use of casticin significantly	Li et al.[62]
	Rat FLS	The increase of NLRP3, ASC, pro-caspase-1 expression was reduced by casticin	
Vanillic Acid	MIA-induced KOA in rats	The mRNA and protein expressions of caspase-1, NLRP3, and ASC showed a reduction after vanillic acid treatment. The use of vanillic acid promotes less inflammation.	Ma et al.[98]
	Rat FLS	The mRNA and protein expressions of IL-18 and IL-1β were decreased in the vanillic acid treatment group	
Chrysin (*Scutellariae Radix*)	MIA-induced KOA in rats	MIA modeling resulted in increased NLRP3, caspase-1 and ASC mRNA expression in synovial tissue compared with the normal group, but chrysin reverses this effect. The high IL-1β and IL-18 expression levels in serum from KOA rats were significantly suppressed by chrysin.	Liao et al.[99]
	Rat FLS	High NLRP3, caspase-1 and ASC mRNA expression is suppressed by chrysin. The expression of the proinflammatory factors IL-1β and IL-18 was reduced in the cell supernatant upon treatment with Chrysin	
Estradiol (E2)	Human FLS	NLRP3 mRNA and protein expression were significantly suppressed by E2. The cleaved caspase-1, the production of IL-1b and IL-18 were also significantly decreased by E2 treatment, whereas ASC and pro-caspase-1 was not significantly changed	Shi et al.[68]
KMU-1170 (a derivative of indolin-2-one)	Human FLS	KMU-1170 pretreatment inhibited LPS-induced phosphorylation of IKKα/β and NF-κB p65 associated with proinflammatory cytokines (IL-1β, TNF-α, and IL-6) production	Baek et al.[22]

the GSDMD inhibition attenuated synovitis and cartilage degeneration.[107] In addition, necrosulfonamide, an inhibitor of pyroptotic cell death, was investigated and proposed as a therapy in animal models of inflammatory diseases and resulted in the molecular regulation of inflammatory response by promoting GSDMD inhibition.[108] Other studies have reported inhibition of pyroptosis by blocking GSDMD using

FDA-approved drugs such as disulfiram (used to treat alcohol addiction)[109] and dimethyl fumarate.[110] Overall, these findings provide relevant evidence that points out the inhibition of GSDMD as a potential anti-inflammatory strategy in pyroptosis-driven diseases such as OA.

Metainflammation is the loss of metabolic homeostasis that involves a dysregulation of the immune system, and this process is associated with low-level inflammation.[111] Metainflammation is characterized by increased levels of catabolic mediators and proinflammatory cytokines, for instance, TNF-α, IL-6, and IL-1β.[112] Those factors contribute to chondrocyte dysfunction and aggravate OA progression.[47] Thus, evaluating the effect of distinct metainflammation inhibitors might represent a promising strategy in OA. From this perspective, the evaluation of Levornidazole, a nitroimidazole derivative, has been demonstrated to block NLRP3 activation through the reduction of ROS generation.[113] Moreover, studies evaluating the effect of resveratrol, a natural polyphenol, reported that this molecule can cause the inadequate assembly of ASC on the mitochondria[114] and inhibit NF-κB-dependent responses.[115] Therefore, resveratrol might function as a potential anti-inflammatory and antioxidant molecule, which may be used to attenuate the metainflammation observed in OA. In addition, melatonin, an endogenous indoleamine, has exerted immunomodulatory properties, and apparently, this molecule can influence energy metabolism. Melatonin has shown inhibitory properties on the expression of NLRP3 inflammasome-associated genes in adipose tissue and inhibited NF-κB phosphorylation, a key factor in metainflammation.[116] Remarkably, another study showed that certain components, such as parthenolide and vinylsulfones, could inhibit the activation of caspase-1 and NF-κB. Regarding synthetic molecules targeting inflammasome components, Bay 11–7082, displayed interesting results and might be considered a potential inhibitor of NLRP3.[117] Lastly, γ-tocotrienol, an analog of vitamin E, could also be considered a metainflammation inhibitor because this molecule can attenuate adipose tissue inflammation and insulin resistance in diet-induced obesity. The γ-tocotrienol molecule was able to repress inflammasome activation, caspase-1 cleavage, and IL-1β secretion, exerting anti-inflammatory and anti-pyroptotic properties.[118] Taken together, these findings from non-joint tissues have improved our understanding of the potential role of inflammasome in OA.

CONCLUSION

Over the last decade, the role of inflammasomes driving chronic inflammation and pathogenic hallmarks in OA has become increasingly evident. Several *in vitro* and preclinical studies have suggested that the activation of NLRP3 inflammasomes in tissue-resident cells, such as chondrocytes and FLS, plays a crucial role in OA. An abundance of the research discussed in this review, suggests that targeting the inflammasome-dependent mechanisms could slow down OA progression, but whether these approaches could reverse the pathology of fully established disease in humans remains to be established. Several studies were also limited by an independent focus on either chondrocytes or FLS rather than considering the joint as a functional unit with different cell/tissue types. Moreover, primary FLS and chondrocytes in culture are known to lack subtype diversity that is observed under *in vivo* conditions.[119–122] Therefore, profiling the gene expression of various inflammasome components from published single-cell RNA-seq datasets from human OA patient synovium and cartilage will be very informative. Finally, OA is a complex disease that can be categorized into multiple disease subtypes depending on the age, and mechanical, metabolic, inflammatory, and genetic characteristics of the patient. Hence, conducting translational research to identify potential responders and non-responders of the inflammasome regulators coupled with the development of effective delivery strategies for the identified bioactive compounds will be needed to effectively exploit the inflammasome pathway for OA therapy.

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AUTHOR CONTRIBUTIONS

S.R-P. and P.B. developed the idea. S.R-P and I.V.R-P. accomplished the review framework. S.R-P., I.V.R-P., D.E.M-F., and L.A.H-P. wrote the first draft of the manuscript. S.R-P. and P.B. supervised manuscript writing

and performed the final editing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

DECLARATION OF INTERESTS

All authors declare no conflict of interest.

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