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Commentary

New molecular targets for the treatment of osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a chronic degenerative joint disorder characterized by destruction of the articular cartilage, subchondral bone alterations and synovitis. Current treatments are focused on symptomatic relief but they lack efficacy to control the progression of this disease which is a leading cause of disability. Therefore, the development of effective disease-modifying drugs is urgently needed. Different initiatives are in progress to define the molecular mechanisms involved in the initiation and progression of OA. These studies support the therapeutic potential of pathways relevant in joint metabolism such as Wnt/β-catenin, discoidin domain receptor 2 or proteinase-activated receptor 2. The dysregulation in cartilage catabolism and subchondral bone remodeling could be improved by selective inhibitors of matrix metalloproteinases, aggrecanases and other proteases. Another approach would favor the activity of anabolic processes by using growth factors or regulatory molecules. Recent studies have also revealed the role of oxidative stress and synovitis in the progression of this disease, supporting the development of a number of inhibitory strategies. Novel targets in OA are represented by genes involved in OA pathophysiology discovered using gene network, epigenetic and microRNA approaches. Further insights into the molecular mechanisms involved in OA initiation and progression may lead to the development of new therapies able to control joint destruction and repair.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disorder of a high prevalence that remains the leading cause of disability in aged people. This multifactorial disease is characterized by destruction of the articular cartilage, subchondral bone alterations and synovitis [1]. Current available drugs to treat OA are predominantly directed towards the symptomatic relief of pain and inflammation but they do little to reduce joint destruction. Effective prevention of the structural damage must be a key objective of new therapeutic approaches.

The mechanisms responsible for OA progression are very complex and poorly understood. A balance between anabolic and catabolic mechanisms maintains extracellular matrix homeostasis in articular cartilage, and shifts toward degradation are associated with OA. Chondrocytes constitute the unique cellular component of articular cartilage. These cells synthesize the components of extracellular matrix, including collagens, proteoglycans and non-collagen proteins. Mechanical loading and biochemical factors are believed to play important roles in disease progression, although chondrocyte responses to molecular signals vary in different regions and at different stages of disease (reviewed in [2]).

A number of polymorphisms or mutations in genes encoding extracellular matrix components and signaling pathways have been identified in relation with OA susceptibility. Genomic and proteomic analyses in chondrocytes and cartilage from OA patients have revealed some mechanisms of disease. Models using genetically modified animals have helped to understand a number of molecular pathways involved in the underlying pathobiology of OA.

Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; APC, adenomatous polyposis coli; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; CaPPS, calcium pentosan polysulfate; CK1α, casein kinase 1α; CORM-2, tricarbonyldichlororuthenium(II) dimer; DDR2, discoidin domain receptor 2; Dkk, Dikkopf related proteins; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FRZB, frizzled related protein; GSK-3β, glycogen synthase kinase 3β; HDAC, histone deacetylase; Hh, hedgehog; HIF-1α, hypoxia-inducible factor 1 α; HMGB2, high mobility group box 2; HO-1, heme oxygenase-1; IGF-1, insulin-like growth factor-1; IGFBP, IGF binding protein; IκBα, inhibitor of κB; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; JNK, c-Jun N-terminal kinase; LAP, latency-associated peptide; LRP, low-density lipoprotein receptor; LTBP, latent TGFβ binding proteins; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; OA, osteoarthritis; PAR-2, proteinase-activated receptor-2; PDGF, platelet-derived growth factor; PI3-K, phosphoinositide 3-kinase; RANKL, receptor activator for NF-κB ligand; ROS, reactive oxygen species; Runx2, runt-related transcription factor 2; SFRP, secreted frizzled related protein; SLRP, small leucine-rich repeat proteoglycan; Smurf, Smad–ubiquitin regulatory factor; SOD, superoxide dismutase; SOST, sclerostatin; TCF/LEF, resident lymphoid enhancer factor/T-cell; TIMP, tissue inhibitor of metalloproteinases; TNFα, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; WISP-1, Wnt-induced secreted protein-1; TGFβ, transforming growth factor-β.

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2. Matrix synthesis and degradation

2.1. Growth factors

Growth factors have been extensively studied for OA and cartilage repair due to their ability to enhance matrix synthesis. Efficacy of growth factors in cartilage repair is related to the recruitment of chondrogenic cells, stimulation of proliferation and enhancement of cartilage matrix synthesis (reviewed in [3]). Although growth factor therapy could be an attractive method for stimulating the repair of damaged cartilage matrix, there is evidence that with aging and/or with the development of OA, articular chondrocytes may become unresponsive to growth factor stimulation [4], and altered responses have also been observed in osteoblasts [5]. In vivo administration of these agents leads to variable results. In some cases, the new cartilage is formed mainly of fibrous tissue that does not present the biomechanical properties of hyaline cartilage. In addition, the half-life of growth factors is too short to sustain therapeutic effects, and carrier systems are needed to achieve a controlled release into the joint [3].

Insulin-like growth factor-1 (IGF-1) is the main anabolic mediator in articular cartilage (Fig. 1). It is believed that sequestration of IGF-1 by high levels of extracellular IGF-binding proteins (IGFBPs) results in a reduced response of chondrocytes. Some strategies have been proposed to restore the anabolic responses to IGF-1. Therefore, small molecules such as NBI-31772 are able to inhibit the binding of IGF-1 to IGFBPs, and restore proteoglycan synthesis by human OA chondrocytes [6]. In addition, gene therapy using viral vectors is an approach feasible in synovial tissues resulting in IGF-1 expression and up-regulation of matrix molecules for several weeks [7]. Combination of growth factors strongly induces proteoglycan synthesis with applications in cartilage repair. Osteogenic protein-1 stimulates proteoglycan production by OA chondrocytes better than IGF-1 [8], and combined therapy with this growth factor may be an effective strategy for treating OA cartilage damage [9].

Some growth factors such as bone morphogenetic proteins (BMPs) and transforming growth factor-β (TGFβ) promote the synthesis of proteoglycan in cartilage but they may also induce chondrocyte hypertrophy, osteophyte formation and fibrosis, which may prevent their application in cartilage repair [10]. Modulation of the BMP pathway represents an approach for the development of therapeutic agents against bone disorders. BMP-2 signaling stimulates p300-mediated runt-related transcription factor 2 (Runx2) acetylation, increasing transactivation activity and inhibiting Smad–ubiquitin regulatory factor (smurf)1-mediated degradation of Runx2. Inhibition of histone deacetylase (HDAC) increases Runx2 acetylation, BMP-2-stimulated osteoblast differentiation and bone formation [11].

Endogenous BMP antagonists, follistatin, gremlin, chordin, noggin, are expressed in OA cartilage and synovium and can be differentially regulated by cytokines and growth factors. The increased activin/BMP-binding activities of these antagonists could affect tissue remodeling. In particular, follistatin, gremlin

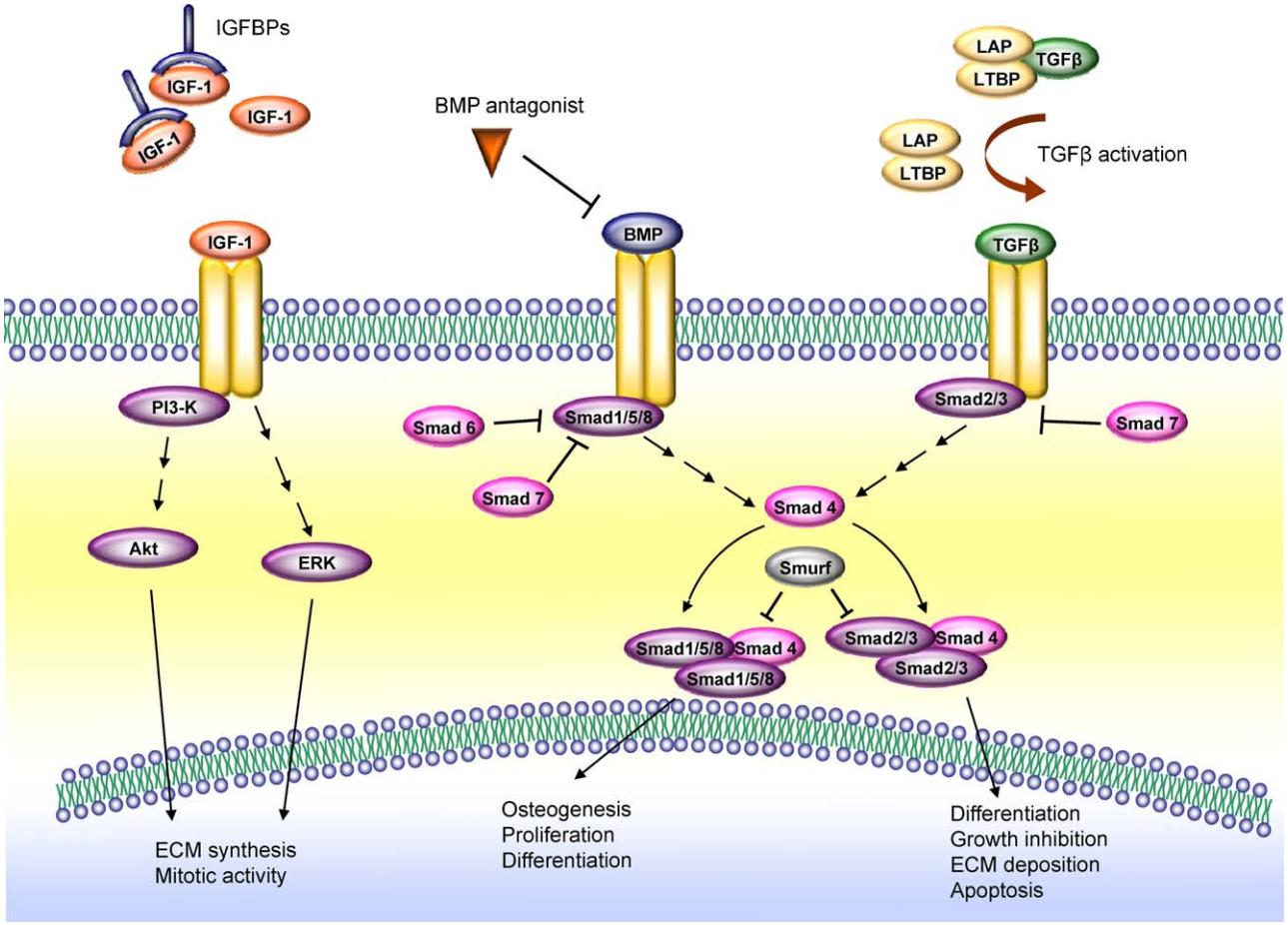

Fig. 1. Some of the best characterized growth factors in OA. IGF-1 activity is prevented by binding of IGFBPs. BMP antagonists block BMPs activity; and TGFβ is released by activation of LAP/LTBP/TGFβ complex. Both BMPs and TGFβ activate Smads; that form complexes and act as transcription factors in the nucleus. Smurfs inhibit Smads actions. BMP, bone morphogenetic protein; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; IGF, insulin-like growth factor; IGFBP, IGF binding protein; LAP, latency-associated peptide; LTBP, latent TGFβ binding proteins; PI3-K, phosphoinositide 3-kinase; Smurf, Smad–ubiquitin regulatory factor; TGFβ, transforming growth factor-β.

and chordin appear at different stages during the OA process and are attractive targets for therapeutic intervention [12].

The role of TGFβ in joint metabolism is complex. Although TGFβ3 has shown potential for the clinical enhancement of cartilage formation [13], it may lead to early osteophyte development in experimental OA [14]. A possible strategy to block TGFβ-induced fibrosis and endochondral ossification, and stimulate cartilage repair simultaneously, includes the use of antagonists. Therefore, simultaneous injections of Ad-TGFβ and Ad-Smad7 have shown beneficial effects in experimental OA [15].

Other growth factors may have potential applications to stimulate anabolic responses in cartilage. Platelet-derived growth factor (PDGF), a potent mitogenic and chemotactic factor for all cells of mesenchymal origin, stimulates meniscal cell proliferation and migration as well as cartilage synthesis by chondrocytes [3]. Expression of basic fibroblast growth factor (bFGF) in chondrocytes and release into the synovial fluid is significantly increased during OA joint disease. bFGF reduces responsiveness to BMP-7 and IGF-1 and induces matrix metalloproteinase (MMP)-13 through protein kinase Cδ-dependent activation of mitogen-activated protein kinase (MAPK), Elk-1 and nuclear factor-κB (NF-κB) signaling pathways. Inhibition of bFGF may be a strategy to control the excessive degradation of cartilage matrix in degenerative joint diseases such as OA [16].

Regenerative therapy for late OA phases would use stem cells and related cells. Chondrogenic progenitor cells exhibit stem cells with a high chondrogenic potential present in repair tissue from human articular cartilage in late OA (reviewed in [17]). Ex vivo gene transfer of IGF-1, TGFβ and BMPs has been used to facilitate the differentiation of mesenchymal stem cells into chondrocytes previously to cell implantation [18]. Recently, it has been demonstrated that antagonism of vascular endothelial growth factor (VEGF) with soluble Flt-1 gene therapy improves the BMP-4- and TGFβ3-induced chondrogenic gene expression of mouse muscle-derived stem cells in vitro, and the persistence of articular cartilage repair [19]. A similar approach may be useful in OA, as intra-articular administration of a combination of mesenchymal stem cells transduced with soluble Flt-1 and BMP-4 exerts beneficial effects on chondrogenesis, with inhibition of angiogenesis and persistent cartilage regeneration [20]. Furthermore, pluripotent human peripheral blood monocytes have multilineage potential comparable to that of mesenchymal stem cells and can differentiate into chondrocytes after stimulation with BMP-2, BMP-7, TGF-β and IGF-1, which opens the possibility for clinical application in cartilage repair after mechanical injury or in OA [21].

2.2. Matrix metalloproteinases

MMPs can degrade all components of the extracellular matrix. Although MMPs play an important role in many physiological processes, their overexpression and activation contribute to many pathologies, including joint destruction in OA. Therefore, inhibitors of MMP activity represent an attractive target in OA. Synthetic MMP inhibitors have shown beneficial effects in animal models of OA. However, results from clinical trials have been disappointing due to lack of efficacy or safety concerns. Clinical administration of broad spectrum MMP inhibitors has been implicated in severe musculoskeletal side effects. Some strategies have been discussed to optimize the development of these agents (reviewed in [22]) and small molecule MMP inhibitors with a high selectivity are under study to circumvent these problems.

The dependence of structural cartilage damage on MMP-13 (collagenase-3) activity has been demonstrated recently in a model of surgically-induced OA in knockout mice [23]. A number of selective MMP-13 inhibitors have been synthesized, such as pyrimidine dicarboxamides with a novel binding mode [24] and novel carboxylic acid derivatives that do not significantly inhibit MMP-1 (collagenase-1) or tumor necrosis factor-α (TNFα) converting enzyme and reduce proteoglycan release in a rat model of MMP-13-induced cartilage degradation [25]. In selectivity assays using catalytic domains of human MMPs, another selective MMP-13 inhibitor ALS 1-0635, potently inhibits articular cartilage degradation in vitro and also in the rat monosodium iodoacetate-induced OA. Interestingly, this agent exerts protective effects without signs of musculoskeletal toxicity in rats with surgically-induced medial meniscus tear [26]. By structure-based drug design, another orally active MMP-13 inhibitor has been generated that effectively reduces cartilage damage in vivo without induction of joint fibroplasias [27].

Tissue inhibitor of metalloproteinases (TIMP) are endogenous regulators of MMP activity and can represent another anti-catabolic strategy in OA [28]. In particular, several investigations have highlighted the therapeutic potential of TIMP-3. Inhibition kinetic studies have shown that TIMP-3 is a high affinity inhibitor of the membrane type MMP, MT3-MMP [29], while in vivo studies have demonstrated that TIMP-3 deficiency results in a process of cartilage degradation similar to OA [30].

2.3. Aggrecanases

Breakdown of aggrecan is a key early event in the development of OA. A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) are the main enzymes responsible for aggrecan degradation, and their inhibition represents an important target in OA (reviewed in [31]). Studies using the ADAMTS-5 knockout mice have demonstrated that this enzyme is the primary aggrecanase responsible for aggrecan degradation. ADAMTS-$5^{-/-}$ joints are protected from cartilage damage and show minor changes in the subchondral bone structure, suggesting links between cartilage damage and subchondral bone changes in this OA model [32].

New groups of selective ADAMTS-5 inhibitors have been synthesized in the search of new disease-modifying OA agents. Some 5-((1H-pyrazol-4-yl)methylene)-2-thioxothiazolidin-4-one derivatives can exhibit ADAMTS-5 IC_{50} of 1.1 μM and over 40-fold functional selectivity against ADAMTS-4 [33]. Similar properties have been reported for 5-benzylidene-2-thioxo-thiazolidin-4-one derivatives [34] and 5′-phenyl-3′H-spiro[indoline-3,2′-[1,3,4]thiadiazol]-2-one compounds [35], whereas *N*-((8-hydroxy-5-substituted-quinolin-7-yl)(phenyl)methyl)-2-phenyloxy/amino-acetamides show a higher potency for ADAMTS-5 inhibition and selectivity over the related metalloproteases ADAMTS-4, MMP-13, and MMP-12 [36].

Active-site inhibitors of ADAMTS-4 have been developed with activity not limited by competition with native substrate. Therefore, the hydroxamic acid SC81956 has demonstrated noncompetitive inhibition kinetics with a K_i of 23 nM [37]. In addition, *cis*-1(*S*)2(*R*)-amino-2-indanol-based compounds exhibit selectivity for ADAMTS-4 and ADAMTS-5, and crystal structures have been determined for the complex enzyme-inhibitor, leading to the establishment of structure/activity relationships [38].

TIMP-3 is unique in that it inhibits not only MMPs, but also several ADAMTS metalloproteinases. Interestingly, TIMP-3 is a potent inhibitor of both ADAMTS-4 and ADAMTS-5 [28]. Recent work indicates that calcium pentosan polysulfate (CaPPS), a chemically sulfated xylanopyranose from beechwood, may be a prototypic disease-modifying agent for OA. CaPPS interacts with the noncatalytic spacer domain of ADAMTS-4 and the cysteine-rich domain of ADAMTS-5, blocking activity against aggrecan with IC_{50} values of 10–40 nM. In addition, this agent blocks endocytosis of TIMP-3 mediated by low-density lipoprotein receptor-related protein (LRP) and increases the affinity of TIMP-3 for ADAMTS-4

and ADAMTS-5 by more than 100-fold. Studies with TIMP-3-null mouse cartilage indicate that CaPPS inhibition of aggrecan degradation is TIMP-3 dependent [39].

2.4. Small leucine-rich repeat proteoglycans

The small leucine-rich repeat proteoglycan (SLRP) family of proteins are components of the extracellular matrix that may provide a number of targets for OA treatment. The SLRP asporin, a component of cartilage and bone, inhibits the anabolic effects of TGFβ1 and is involved in the pathogenesis of OA [40]. In the subchondral bone and osteophytes of OA patients, the ratio of asporin to TGFβ1 mRNA in patients with severe cartilage damage is higher than that in patients with mild cartilage damage, suggesting that asporin may regulate TGFβ1-mediated signaling in the development of OA [41].

2.5. Syndecans

Syndecans are heparan sulfate proteoglycans expressed on the surface of adherent cells that interact with growth factors, cytokines, proteinases, adhesion receptors and extracellular matrix components, through their heparan sulfate chains. These proteoglycans modulate homeostatic processes and tissue injury [42]. Syndecan-4 is specifically induced in type X collagen-producing chondrocytes both in human OA and in murine models of disease, and controls the activation of ADAMTS-5 through direct interaction with the protease as well as by regulating mitogen-activated protein kinase (MAPK)-dependent synthesis of MMP-3 [43]. Inhibition of syndecan-4 may be a new strategy to protect cartilage destruction in OA.

2.6. Discoidin domain receptor 2

The expression of a collagen receptor, discoidin domain receptor 2 (DDR2), is increased in articular chondrocytes of mice that develop OA as a result of a heterozygous mutation in type XI collagen. Overexpression of DDR2 can be an important event in OA progression since this receptor mediates the collagen II-dependent induction of MMPs and pro-inflammatory cytokines in primary human chondrocytes. Recent studies have demonstrated the role of p38, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and the transcription factor NF-κB in intracellular collagen II signaling [44]. These findings suggest that DDR2 antagonism may lead to specific therapeutics in OA.

2.7. Proteinase-activated receptor 2

Proteinase-activated receptor 2 (PAR-2) activation participates in inflammatory reactions. Recent studies have demonstrated the potential of PAR-2 as a therapeutic target in OA. PAR-2 is significantly up-regulated in OA chondrocytes and by pro-inflammatory cytokines. Activation of PAR-2 significantly induces MMP-1, MMP-13 and cyclo-oxygenase-2 as well as the phosphorylation of ERK1/2 and p38 [45]. It has also been demonstrated that PAR-2 activation induces major bone remodeling factors and results in bone resorptive activity [46].

3. Inflammatory processes

3.1. Cytokines

Pro-inflammatory cytokines such as interleukin(IL)-1β and TNFα are involved in synovial inflammation and cartilage degradation in OA. These cytokines are produced by mononuclear cells, chondrocytes or synoviocytes, leading to up-regulation of catabolic factors and down-regulation of anabolic mediators [47]. Some drugs for OA treatment may prevent the actions of pro-inflammatory cytokines. An example can be pralnacasan, the orally bioavailable pro-drug of a potent non-peptide inhibitor of IL-1β converting enzyme, RU 36384/VRT-18858. This molecule has the potential to become a disease-modifying drug for the treatment of OA, because of its ability to reduce joint damage in experimental models [48]. IL-1 receptor antagonist (IL-1Ra) has shown beneficial effects on cartilage degradation in vitro and in vivo [49]. Intra-articular injection of this protein or transfer of IL-1Ra cDNA to human joints are possible strategies [50]. An alternative therapy provides important symptomatic relief by intra-articular injection of autologous conditioned serum (Orthokine), which is generated by incubating venous blood with etched glass beads to induce the production of IL-1Ra and other anti-inflammatory mediators by peripheral blood leukocytes [51]. In addition, it has been demonstrated that human articular OA chondrocytes express a higher number of p55 TNFα receptors and produce more TNFα and its converting enzyme than normal cartilage, suggesting potential therapeutic targets related with this cytokine [47].

In OA, the subchondral bone undergoes a remodeling process involving factors synthesized by osteoblasts. Recently, the regulation of the osteoprotegerin/receptor activator for NF-κB ligand (RANKL) pathway has been suggested as a new strategy in OA treatment [52]. The protective effect of endogenous osteoprotegerin against the cartilage destruction that occurs during OA progression, can be reproduced by administration of the recombinant human protein in an experimental murine model of OA via prevention of chondrocyte apoptosis [53]. Nevertheless, the role of osteoprotegerin in cartilage has not been clearly established, as in human OA chondrocytes, exogenous osteoprotegerin can induce two catabolic factors, MMP-13 and PAR-2 [54].

Ephrin B2 and its specific receptor EphB4 participate in bone homeostasis. EphB4 activation by ephrin B2 in OA subchondral bone significantly inhibits the expression of IL-1β, IL-6, MMP-1, MMP-9, MMP-13, and RANKL. Therefore, ephrin B2 could be targeted as a specific therapeutic approach in the development of a disease-modifying OA drug [55].

Anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 can control the production of pro-inflammatory mediators in certain cells and have been proposed as potential targets in OA [47]. Interestingly, IL-4 inhibits MMPs and ADAMTS-4 expression and exerts cartilage protective effects in animal models of OA [56]. Nevertheless, IL-4 administration as a cartilage protective therapy may be limited by the suppressive effects of this cytokine on TIMP-3 [57].

3.2. Oxidative stress

In OA, continuous oxidative stress to cells and matrix is one major mechanism underlying pathogenesis. Increased oxidative stress with aging may represent an important factor to the development of OA, as chondrocytes become more susceptible to oxidant-mediated cell death through the dysregulation of antioxidant systems [58]. Nitric oxide and reactive oxygen species (ROS) are present in OA cartilage and play a role in the chondrocyte insensitivity to anabolic actions of IGF-1 [59]. Oxidative stress results in mitochondrial DNA damage, mitochondrial dysfunction, apoptosis and senescence of chondrocytes [60]. Differential gene expression studies have identified in OA cartilage important oxidative defense genes as potential targets, including genes for superoxide dismutase (SOD) 2, SOD 3, and glutathion peroxidase 3 [61]. Another endogenous antioxidant peroxiredoxin 5, is up-regulated in OA cartilage and may play a protective role against oxidative stress [62].

Free radical scavengers have been suggested as potential therapeutic agents for the protection of articular cartilage against progression of OA. An example can be the water-soluble fullerene (C60) which inhibits the catabolic stress-induced production of MMP-1, MMP-3 and MMP-13, down-regulation of matrix production, apoptosis and premature senescence in human chondrocytes, and exerts protective effects in a rabbit model of surgically-induced OA [63].

In human OA cartilage, IL-1 β, TNFα and oxidative stress induce the expression of hypoxia-inducible factor 1α (HIF-1α) in chondrocytes. This transcription factor increases the expression of genes relevant for survival in hypoxia [64] and participates in cartilage homeostasis [65]. Expression of HIF-1α in OA cartilage is associated with the progression of articular cartilage degeneration [66]. Small-molecule inhibitors of HIF prolyl hydroxylation stabilize HIF/VEGF production and increase angiogenesis, improving bone regeneration [67].

3.3. Heme oxygenase-1

Heme oxygenase-1 (HO-1) is induced as a protective response against oxidative stress in many cell types (reviewed in [68]). HO-1 expression in OA chondrocytes is down-regulated by pro-inflammatory cytokines such as IL-1β, IL-17 and TNFα, but up-regulated by the anti-inflammatory cytokine IL-10 [69]. Ex vivo studies using cartilage explants of primary chondrocytes from OA patients have demonstrated a protective role of HO-1 induction or carbon monoxide (CO) release by the CO-releasing molecule tricarbonyldichlororuthenium(II) dimer (CORM-2), against the deleterious effects of IL-1β stimulation. Both treatments result in a diminished proteoglycan release with increased synthesis and expression of aggrecan and type II collagen. The protective effects of HO-1 induction could be dependent on the down-regulation of MMP-1 and MMP-13, as well as the increased expression of the IGF-1/IGFBP3 ratio. CORM-2 treatment significantly down-regulates MMP-1, MMP-3, MMP-10, MMP-13 and ADAMTS-5 [70,71]. Our data indicate that HO-1 represents an anti-inflammatory strategy to reduce the production of oxidative stress and prostaglandin E_2, whereas CORM-2 in addition reduces TNFα and enhances IL-1Ra production. We have also shown that inhibition of NF-κB, ERK1/2 and p38 activation plays an important role in the protective effects of the HO-1 pathway in OA cartilage (Fig. 2) [72,73].

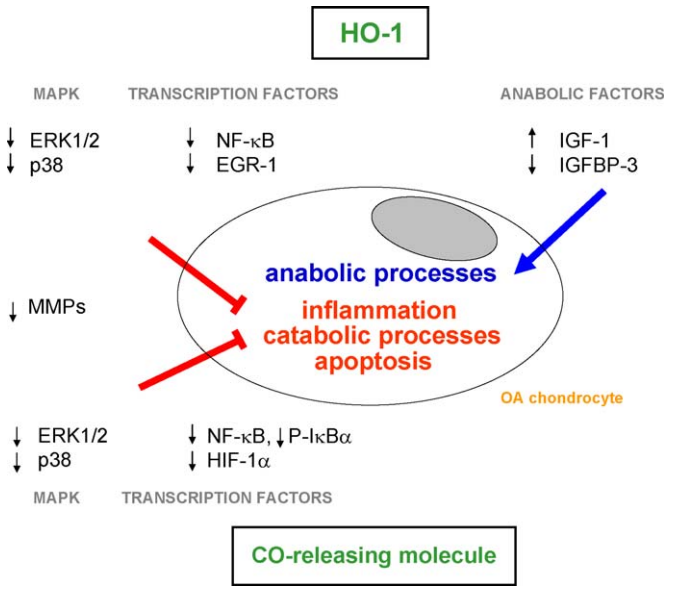

Fig. 2. Protective effects of HO-1 induction or CO release in OA chondrocytes, with inhibition of catabolic processes, inflammation and apoptosis but up-regulation of anabolic processes. EGR, early growth response transcription factor; ERK, extracellular signal-regulated kinase; HIF-1α, hypoxia-inducible factor 1 α; IGF, insulin-like growth factor; IGFBP, IGF binding protein; P-IκBα, inhibitor of κB; MAPK, mitogen-activated protein kinase; MMP, metalloproteinase; NF-κB, nuclear factor κB.

4. Signaling pathways

4.1. Mitogen-activated protein kinases

Targeting of specific signaling pathways can inhibit cartilage degradation. It has been demonstrated that in vitro inhibition of p38, ERK1/2 and Src abrogates cartilage degradation by blocking MMP synthesis and activity, although only MAPK ERK1/2 seems to be essential for aggrecanase-mediated aggrecan degradation [74]. SB-203580 and VX-745 are potent inhibitors of p38 with IC_{50}s of 136 nM and 35 nM, respectively. Both compounds administered orally at a dose of 50 mg/kg result in the significant inhibition of joint degeneration in the rat monosodium iodoacetate model and attenuate the pain response in the Hargraeves hyperalgesia assay, suggesting that inhibition of p38 may be useful for the treatment of OA [75].

4.2. Nuclear factor-κB

NF-κB activation determines the expression of a wide range of inflammatory and catabolic mediators in joint tissues. In particular, the OA synovium is a pro-inflammatory environment, with production of many cytokines and MMPs. NF-κB-targeted therapeutics may regulate disease activity and improve clinical outcome in OA, as suggested by preclinical studies [76]. Gene therapy with Ad-siRNA(NF-κBp65) suppresses the progression of early experimental OA surgically-induced in rats [77]. The interest of pharmacological inhibition of NF-κB has been demonstrated in experiments showing the effects of a novel inhibitor, RO100 at a concentration of 0.1 μM, on IL-6, MMP-1 and MMP-3 production in OA synovial fibroblasts, with equivalent efficacy as IL-1β and TNFα neutralizing antibodies [78].

Nevertheless, strategies involving inhibition of NF-κB or MAPK may present a number of complications as these pathways are key components of physiological systems.

4.3. Wnt/β-catenin

The Wnt/β-catenin pathway involves the interactions of Wnt ligands with frizzled receptors and LRP-5 or -6 co-receptors (Fig. 3) [79]. Wnt signaling is involved in embryonic development of cartilage and bone and is considered a key regulator of joint remodeling. Nevertheless, the response of chondrocytes to a canonical Wnt stimulus is affected by alterations in extracellular matrix components, as in OA cartilage. There is evidence that activation of Wnt/β-catenin is part of mechanisms leading to excessive remodeling and degradation of cartilage matrix in joint pathologies and may be related with the progression of OA [80].

Proteins of the Wnt/β-catenin pathway are overexpressed in joint tissues from OA patients and in animal models of disease. Gene expression analyses have revealed up-regulation of Wnt-16 and β-catenin in OA cartilage with moderate to severe damage. In addition, mechanical injury to human articular cartilage results in the activation of morphogenetic pathways, with overexpression of Wnt-16, up-regulation of Wnt target genes, and nuclear localization of β-catenin. These molecules may be targets for the treatment of joint surface defect repair and the prevention of posttraumatic OA [81]. Increased Wnt-I-induced secreted protein-1 (WISP-1) expression has been demonstrated in human OA cartilage and synovium, whereas in experimental OA, WISP-1, Wnt-16 and Wnt-2B

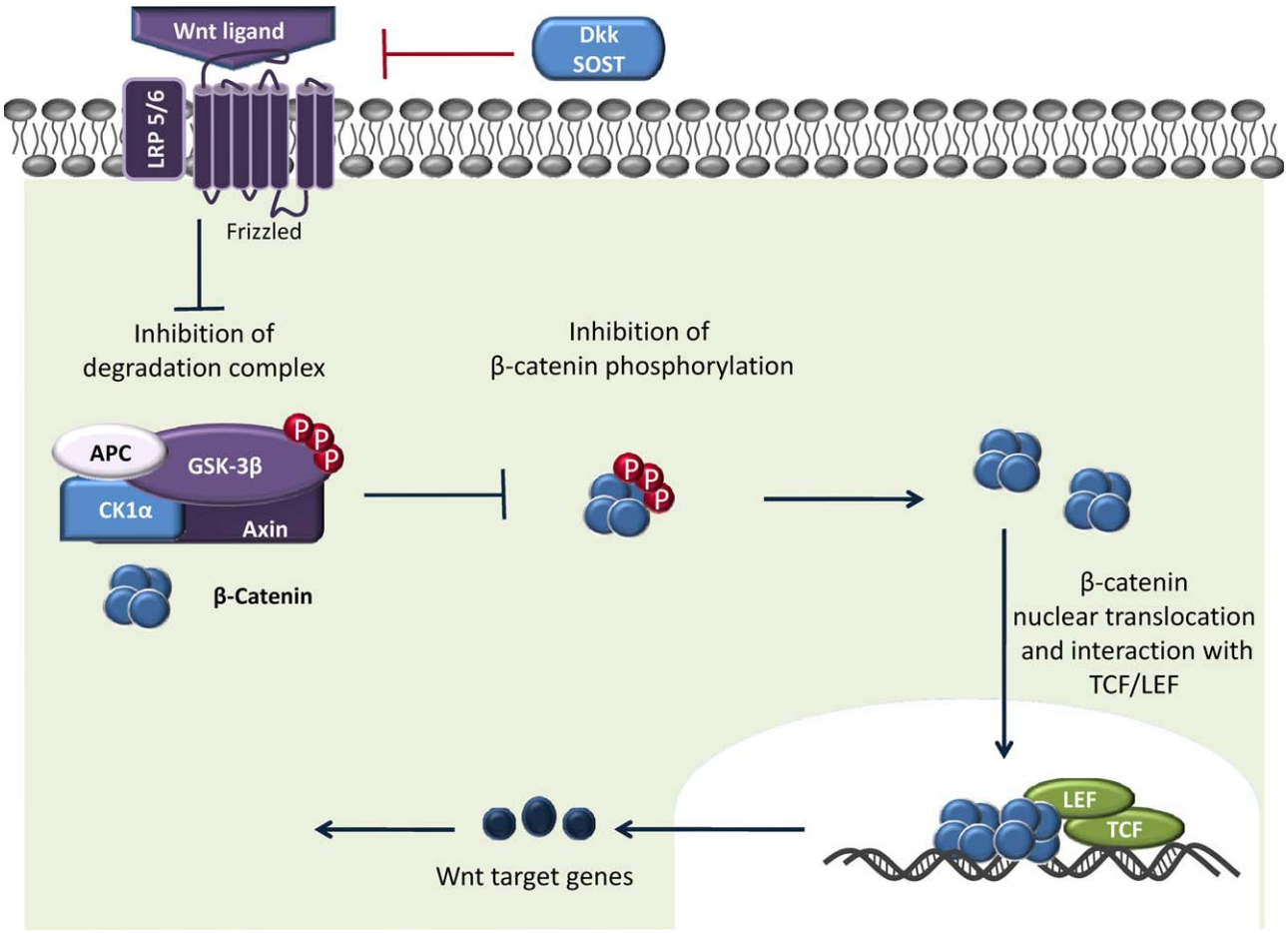

Fig. 3. The canonical Wnt signaling pathway in OA. Binding of Wnt-ligands to receptors LRP 5/6 or Frizzled promotes the inhibition of the degradation complex formed by APC, GSK-3β, CK1α and axin, thus inhibiting β-catenin phosphorylation and latter destruction. β-catenin molecules accumulate in the cytoplasm and then translocate into the nucleus, where they interact with the transcription factor TCF/LEF, that activates the transcription of Wnt target genes, causing an increase in catabolic processes during OA development. These effects of Wnt-canonical pathway can be inhibited by endogenous antagonists such as Dkk and SOST. APC, adenomatous polyposis coli; CK1α, casein kinase 1α; Dkk, Dikkopf related proteins; GSK-3β, glycogen synthase kinase 3β; LRP, low-density lipoprotein receptor; SOST, sclerostatin; TCF/LEF, resident lymphoid enhancer factor/T-cell.

expression is also strongly up-regulated in the synovium and cartilage [82]. Evidence that activation of this pathway results in the production of catabolic factors has come from in vitro and in vivo studies. In macrophages and chondrocytes cultures, WISP-1 induces MMPs and aggrecase activity dependent on IL-1β [82].

Recent data have demonstrated the interaction between the Wnt/β-catenin pathway and high mobility group box 2 (HMGB2), which regulates the maintenance of the superficial zone of cartilage. HMGB2 potentiates the transcriptional activation of the Lef-1-β-catenin complex, promotes chondrocyte survival and may be a target in aging-related cartilage pathology [83].

Secreted frizzled related proteins (SFRP)1-5 are Wnt antagonists. Mice deficient in frizzled related protein (FRZB or SFRP3) show MMPs activation and loss of proteoglycans from the knee cartilage in models of OA, as well as thicker cortical bone, which may contribute to the development of OA by producing increased strain on the articular cartilage [84]. Elevated circulating levels of Wnt inhibitor Dickkopf-1 (Dkk-1) are associated with reduced progression of radiologic hip OA in elderly women [85], whereas inhibition of this agent results in the bone-forming pattern of OA in animals [86].

There is also evidence that in Col2a1-inhibitor of β-catenin and T cell factor-transgenic mice, β-catenin signaling inhibition in chondrocytes leads to cell apoptosis and increased articular destruction [87]. Further studies are needed to establish the role of the different components of the Wnt pathway and their interaction networks. The discovery of drugs exerting selective effecs on the Wnt-β-catenin cellular system, may help to delimitate the specific roles of this pathway in cartilage and bone. A number of antagonists have been identified recently, as the small molecule XAV939, which selectively inhibits β-catenin-mediated transcription by stabilizing axin [88]. On the other hand, Wnt agonists exert anabolic effects on bone. Therefore, (hetero)arylpyrimidines show in vivo osteogenic activity in a mouse calvaria model [89] and (1-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperidin-4-yl)methanamine (WAY-262611) has excellent pharmacokinetic properties and shows a dose-dependent increase in the trabecular bone formation rate in ovariectomized rats following oral administration [90].

4.4. Other signaling pathways

Activation of the transcription factor Runx2 contributes to chondrocyte hypertrophy and matrix breakdown. In an experimental model of OA induced by instability of knee joints, Runx2 is induced in the articular cartilage of wild-type mice at the early stage of OA, and causes pathologic hypertrophic differentiation of articular chondrocytes. By contrast, Runx2$^{+/-}$ mice show decreased cartilage destruction and osteophyte formation, along with reduced type X collagen and MMP-13 expression [91]. Runx2 can also regulate ADAMTS-5 expression and mediates the effects of hedgehog (Hh) activation. In human cartilage explants and in mice

with surgically-induced OA, inhibition of Hh signaling reduces the severity of OA [92].

5. Novel approaches to control gene and protein expression

Molecular mechanisms regulating the expression of proteins relevant in OA are potential targets for rational therapeutic intervention. Recent studies have focused on the role of epigenetics in the pathogenesis of OA. Changes in DNA methylation are likely to be important in determining the complex gene expression patterns of OA chondrocytes [93]. Both primary and secondary OA are characterized by the abnormal expression of cartilage-degrading proteases that correlate with epigenetic DNA demethylation of CpG sites in the promoter regions of these enzymes [94]. Therefore, abnormal expression of MMPs 3, 9, and 13 and ADAMTS-4 by human OA chondrocytes is associated with epigenetic unsilencing. These changes may contribute to the development of OA [95]. MMP-13 activity is controled by leptin in OA chondrocytes. Recent studies indicate that epigenetic mechanisms regulate leptin expression and its downstream target MMP-13, suggesting a therapeutic potential in OA [96]. Histone deacetylase (HDAC) has also been implicated in the regulation of MMP gene expression. In human articular chondrocytes, inhibition of HDAC by trichostatin A antagonizes FGF-2 and IL-1β-induced MMP expression. Combination of FGF-2 and the HDAC inhibitor decreases both anabolic and catabolic genes, which may slow cartilage turnover and help to maintain cartilage integrity [97].

Novel strategies in OA are represented by microRNA that control genes involved in OA pathophysiology. In OA chondrocytes, miR-27a down-regulates MMP-13 and IGFBP-5, whereas the last protein is a direct target of miR-140 [98]. Gene network approaches can help to improve the understanding of OA pathogenesis and provide novel therapeutic targets. Integration of genetic, bioinformatic and proteomic approaches has led to the identification of new genes and their collaborative networks in disease. MicroRNA profiling and protein arrays of OA cartilage in comparison to normal cartilage, have revealed microRNA OA gene signature and proteins such as Sox11, FGF-23, Krueppel-like factor 6, WW domain-containing oxidoreductase and growth differentiation factor-15. Interestingly, inhibition of miR-22 blocks inflammatory and catabolic changes in OA chondrocytes. This approach is of great interest to understand the correlation of this network state with disease to develop new treatments for OA [99].

6. Conclusion

Studies of pathogenetic changes are starting to provide insights into the molecular mechanisms involved in the initiation and progression of OA. This is a very complex process, with possible different targets according to a variety of causes, disease stage and patient characteristics that need to be identified. Some pathways involved in joint metabolism such as Wnt/β-catenin, DDR2 or PAR-2 have recently attracted attention and their relevance in OA is being tested. In addition, selective inhibition of proteases offers new opportunities to therapeutic intervention. Evidence has been presented that inflammatory processes impact on OA development suggesting that anti-inflammatory strategies may find utility to complement other treatments. Finally, multidisciplinary approaches and large-scale molecular studies can play a critical role in understanding the pathobiology of this multifactorial disease and improving current therapeutic strategies.

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