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Review

A current review of molecular mechanisms regarding osteoarthritis and pain

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ABSTRACT

Osteoarthritis afflicts millions of individuals across the world resulting in impaired quality of life and increased health costs. To understand this disease, physicians have been studying risk factors, such as genetic predisposition, aging, obesity, and joint malalignment; however have been unable to conclusively determine the direct etiology. Current treatment options are short-term or ineffective and fail to address pathophysiological and biochemical mechanisms involved with cartilage degeneration and the induction of pain in arthritic joints. OA pain involves a complex integration of sensory, affective, and cognitive processes that integrate a variety of abnormal cellular mechanisms at both peripheral and central (spinal and supraspinal) levels of the nervous system Through studies examined by investigators, the role of growth factors and cytokines has increasingly become more relevant in examining their effects on articular cartilage homeostasis and the development of osteoarthritis and osteoarthritis-associated pain. Catabolic factors involved in both cartilage degradation *in vitro* and nociceptive stimulation include IL-1, IL-6, TNF-α, PGE2, FGF-2 and PKCδ, and pharmacologic inhibitors to these mediators, as well as compounds such as RSV and LfcinB, may potentially be used as biological treatments in the future. This review explores several biochemical mediators involved in OA and pain, and provides a framework for the understanding of potential biologic therapies in the treatment of degenerative joint disease in the future.

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Abbreviations: ADAMTS-4, a disintergrin and metalloproteinase with thrombospondin motifs; Anti-IL-1, anti-interleukin 1; BMP-7, bone morphogenetic protein 7; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; DRG, dorsal root ganglion; ECM, extracellular matrix; EP, E prostanoid receptor; ERK, extracellular signal-regulated kinase; FGFR1-Ras, fibroblast growth factor receptor 1-Ras; FGF-2, fibroblast growth factor 2; Fn-f, fibronectin fragment; IGF-1, insulin-like growth factor 1; IL, interleukin; IL-1β, interleukin-1 beta; IL-1ra, interleukin-1 receptor antagonist; iNOS, inducible nitric oxide synthase; IVD, intervertebral disk; JNK, c-Jun N-terminal kinase; Lf, lactoferrin; LfcinB, bovine lactoferrin; LIF, leukemia inducing factor; MAPKs, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mPGES-1, microsomal prostaglandin E synthase-1; mRNA, messenger ribonucleic acid; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NSAIDS, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; PG, proteoglycan; PGD2, prostaglandin D2; PGI2, prostaglandin I2; PGE2, prostaglandin E2; PGES, PGE synthase; PGF2Fa, prostaglandin fibroblast growth factor alpha; PKCδ, protein kinase C alpha; RA, rheumatoid arthritis; RNA, ribonucleic acid; RSV, resveratrol; ROS, reactive oxygen species; SP, substance P; TNFR, tumor necrosis factor receptor; TNF-α, tumor necrosis factor alpha.

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1. Introduction

Osteoarthritis (OA), a debilitating degenerative joint disease predominantly found in elderly individuals, is the principal source of physical disability resulting in increased health care costs and impaired quality of life in the United States (Buckwalter et al., 2004a). The disease imparts a profound economic impact on today's society, with healthcare costs exceeding $60 billion per year and OA aggregate costs increasing to $185.5 billion per year based on 2007 data (Kotlarz et al., 2009; Pereira et al., 2011). By the year 2030, an estimated 25% of the adult population in the United States will be afflicted with OA resulting in some form of disability (Buckwalter et al., 2004b; Issa and Sharma, 2006). While several risk factors have been associated with OA, including genetic predisposition (Valdes and Spector, 2010), aging (Issa and Sharma, 2006), obesity (Richette et al., 2010), and joint malalignment (Tanamas et al., 2009), the pathogenesis of OA remains largely unknown (Buckwalter et al., 2004b; Dieppe and Lohmander, 2005; Issa and Sharma, 2006; Lane et al., 2011; Sandell and Aigner, 2001; Valdes and Spector, 2010).

Current non-arthroplasty treatment options for OA are short-term or ineffective, and fail to adequately address the underlying pathophysiological and biochemical mechanisms involved with cartilage degeneration and the induction of pain in arthritic joints. Investigations have been undertaken to focus on understanding many of these processes, with the goal of developing novel biological therapies that may slow and/or reverse cartilage degradation and provide pain relief. Here, we will review several biochemical mediators involved in OA, with an emphasis on factors mediating cartilage breakdown and the induction of pain in degenerative conditions.

1.1. Pathophysiology of osteoarthritis

Under normal conditions, articular chondrocytes maintain a dynamic equilibrium between synthesis and degradation of extracellular matrix (ECM) components, including collagen type II and aggrecan, the most abundant proteoglycan (PG) in articular cartilage (Nakata et al., 1993; Sandell and Aigner, 2001). In osteoarthritic states, however, a disruption of matrix equilibrium leads to progressive loss of cartilage tissue, clonal expansion of chondrocytes in the depleted regions, induction of oxidative states in a stressful cellular environment, and eventually, apoptosis of cells (Bauer et al., 2006; Lane et al., 2011). With progression, there is usually an increase in both degradation and synthesis of ECM molecules within the joint, with an overall shift toward catabolism over anabolism. Chondrocyte metabolism is unbalanced due to excessive production of inflammatory cytokines and matrix-degrading enzymes, in conjunction with a downregulation of anabolic signaling, eventually leading to the destruction of ECM and subsequent cartilage degradation. Oxidative stress elicited by reactive oxygen species (ROS) further disturbs cartilage homeostasis and promotes catabolism *via* induction of cell death, breakdown of matrix components (Im et al., 2008), upregulation of latent matrix-degrading enzyme production (Im et al., 2007a), inhibition of matrix synthesis, and oxidation of intracellular and extracellular molecules (Goldring and Berenbaum, 2004; Sandell and Aigner, 2001). Clinically, degradation of the ECM results in the gradual impairment of articular cartilage, often accompanied by pain and physical disability. Such cartilaginous changes also elicit profound pathological remodeling in the subchondral bone, typically in the form of sclerosis and osteophyte formation (Fig. 1).

A series of catabolic and anabolic mediators have been found to play key roles in articular cartilage homeostasis and the development of OA (Fig. 2). Many of the specific signaling cascades underlying the effects induced by catabolic and anabolic growth factors and cytokines remain inadequately characterized, but recent efforts have begun to further our understanding. Upregulation of catabolic processes and/or downregulation of anabolic processes leads to disruption of matrix equilibrium and subsequent cartilage degradation (Goldring and Berenbaum, 2004; Im et al., 2008; Loeser, 2008; Sundman et al., 2011). The goal of biologic therapies is to impede joint destruction *via* inhibition of catabolic activity and/or upregulation of anabolic activity, thereby slowing or preventing the progression of OA. Previously, Ellman and colleagues presented a concise review of the literature on important factors involved in cartilage homeostasis (Ellman et al., 2008). Here, we focus on specific mediators that not only stimulate the induction of cartilage degradation, but also participate in nociceptive sensitization.

1.2. Osteoarthritis and pain

Clinically, pain is the most prominent and disabling symptom of OA. Arthritic pain is associated with inferior functional outcomes and reduced quality of life compared with a range of other chronic conditions (Hunter et al., 2008). OA pain involves a complex integration of sensory, affective, and cognitive processes that integrate a variety of abnormal cellular mechanisms at both peripheral (joints) and central (spinal and supraspinal) levels of the nervous system (Dieppe and Lohmander, 2005; Lee et al., 2011; Li et al., 2011a) (Fig. 3). Acute, adaptive pain, such as that following injury or surgery, serves a protective function and generally disappears after the injury heals (Lee et al., 2011). In contrast, maladaptive chronic pain that persists beyond normal healing time or for more than 3–6 months may be considered pathologic as a symptom of ongoing disease. As OA-associated pain continues, severity and functional disability worsen due to a lack of effective preventative measures (Buckwalter et al., 2004b). Research efforts have recently focused on the pain pathways involved in OA, as a better understanding of these molecular mechanisms may allow for the development of new therapeutic strategies to improve function and rein in the associated increase in healthcare costs (Im et al., 2010; Imamura et al., 2008).

Nociceptors are located throughout the joint in tissues peripheral to cartilage, including the joint capsule, ligaments, periosteum and subchondral bone (Buckwalter et al., 2004a; Felson, 2005). Joint cartilage and synovial injury influences peripheral afferent and dorsal root ganglion (DRG) neurons and sensitizes symptomatic pain perception through the dynamic interactions between neuropathic pathways and OA tissues (Li et al., 2011b). Nociceptive input from the joint is processed *via* different spinal cord pathways, and inflammation may potentially reduce the threshold for nociceptive stimulus. These triggers are transmitted through the DRG, where they then travel up the spinothalamic tract to cortical centers for processing. The relative contribution of these processes into peripheral and central pathways appears to be strongly segmented, with intra-articular anesthetic studies in hip and knee OA suggestive of a peripheral

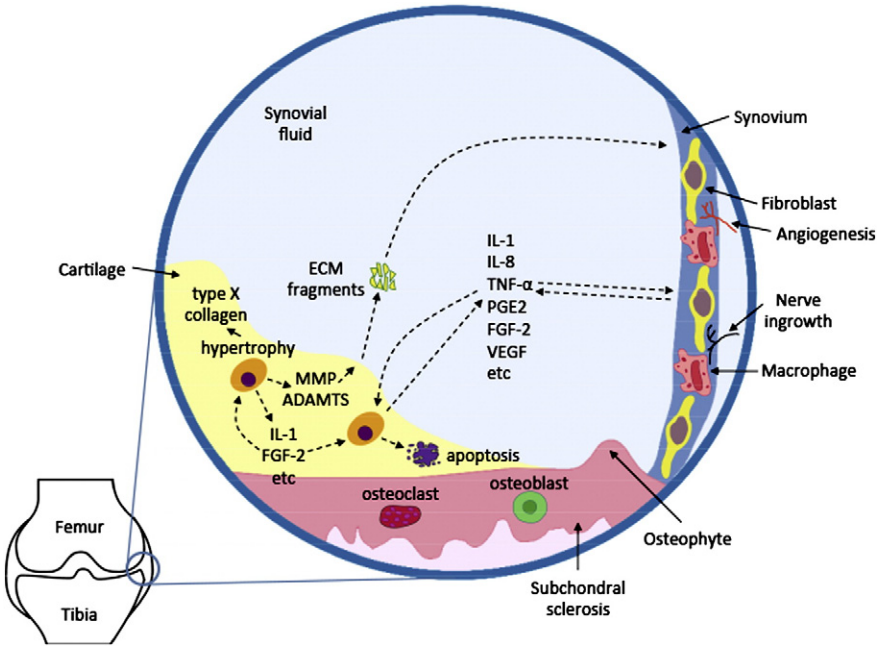

Fig. 1. Complex cellular interplay in synovial joint. In osteoarthritic state, aberrantly activated chondrocytes produce ECM-degrading proteases (MMPs, aggrecanases), pro-inflammatory cytokines (*e.g.* IL-1), and catabolic growth factors (*e.g.* FGF-2). These proteins can be secreted into synovial fluid, and subsequently act upon synoviocytes. Fragments derived from ECM degradation (*e.g.* Fn-f) are also present in the synovial fluid as catabolic inducers. In OA, a subpopulation of chondrocytes undergoes hypertrophic changes, as manifested by their expression of type X collagen. Chondrocytes may also upregulate apoptosis, resulting in diminished local cellularity. In response to cartilage loss, pathological remodeling of subchondral bone gives rise to sclerosis and osteophyte formation. Synoviocytes (fibroblasts and macrophages) also actively synthesize proteases and cytokines which can negatively effect on the articular cartilage and synovium. Pathophysiological changes in synoviocytes pave the way for angiogenesis and innervations, which may account for OA pain.
Adapted from S. B. Abramson and M. Attur, *Arthritis Res Ther* 2009;11(3):227.

drive to pain in approximately 60% to 80% of patients, depending on the affected joint (Dray and Read, 2007; Valdes and Spector, 2010). In some individuals, however, central mechanisms such as dysfunction of descending inhibitory control or altered cortical processing of noxious information, may play a greater role (Kosek and Ordeberg, 2000). Therefore, research and pharmacotherapy for OA pain need to investigate two broad classes: central sensitization and peripheral sensitization, both leading to one final outcome: pain in a patient with OA.

Current 'central' targets of pharmacotherapy for OA pain are numerous and include opioids, kinins, cannabinoids, and their respective receptors, in addition to adrenergic receptors, glutamate receptors, specific ion channels, and neurotrophins. The literature is replete with data on the alteration of pain pathways *via* inhibition of central processes (Dray and Read, 2007). By contrast, the peripheral processes linking pain with OA are lesser-known, and a better understanding of causative pro-inflammatory signaling that links OA with pain may allow clinicians to develop localized therapeutic regimens to treat OA symptoms *via* intraarticular injections (Hunter et al., 2009). Currently, there is a lack of a 'gold standard' to relieve pain caused by OA, prompting recent research to focus more on understanding the pathophysiological mechanisms leading to joint degeneration and pain symptoms, with the aim of developing more long-term solutions *via* prevention and/or reversal of OA in the future (Buckwalter et al., 2004b; Chubinskaya et al., 2005; Ellman et al., 2008, 2011; Im et al., 2007a,b, 2008; Li et al., 2008; Loeser et al., 2005). While much of this research is pre-translational, the potential for an injection of a particular mediator to induce anabolic, anti-catabolic, anti-inflammatory, and anti-pain effects in an arthritic joint *via* peripheral application is promising. Here, we will focus on select pro-inflammatory cytokines and mediators known to play a peripheral role in both cartilage degradation and pain processing, and briefly discuss two mediators with exciting therapeutic potential in the treatment of OA in the future, resveratrol (RSV) (Im et al., 2012) and bovine lactoferricin (LfcinB) (Kim et al., 2012).

Inflammatory mediators	Signaling Mediators	Proteases
• TNF	• NFκB	• MMP-1
• IL-1β	• ERK1/2	• MMP-3
• IL-6	• p38	• MMP-9
• IL-8	• JNK	• MMP-13
• IL-15	• PKCδ	• ADAMTS-4
• IL-17	• TLRs	• ADAMTS-5
• IL-21	• β-catenin	• TACE
• PGE2	• Gli1	
• Substance P	• Ptch	
• NGF	• HHIP	
• EGF	• HIF-2α	
• VEGF	• iNOS	
• FGF-2	• RUNX2	

Fig. 2. Notable mediators in OA.

1.3. Catabolic & pain mediators in osteoarthritis

Numerous mediators contribute to both degradative and nociceptive pathways associated with the progression of OA. Inflammatory stimuli initiate a cascade of events, including the release of cytokines by chondrocytes, leading to complex biochemical and mechanical interplay with other biological mediators to induce OA and promote pain (Dray and Read, 2007; Schaible et al., 2011). Particular mediators then stimulate hyperalgesia by a number of direct and indirect actions, including the sensitization of primary afferent fibers for mechanical stimuli. Examples include pro-inflammatory members from the interleukin family (IL-1, IL-6, and IL-17), tumor necrosis factor-α (TNF-α), and prostaglandin E2 (PGE2) (Fig. 2). Each of these mediators not only stimulates the production of cartilage-degrading proteases to induce ECM degradation, but also contributes to OA-associated pain pathways. While many of the specific mechanisms involved in nociceptive sensitization by peripheral mediators remain largely unknown, the

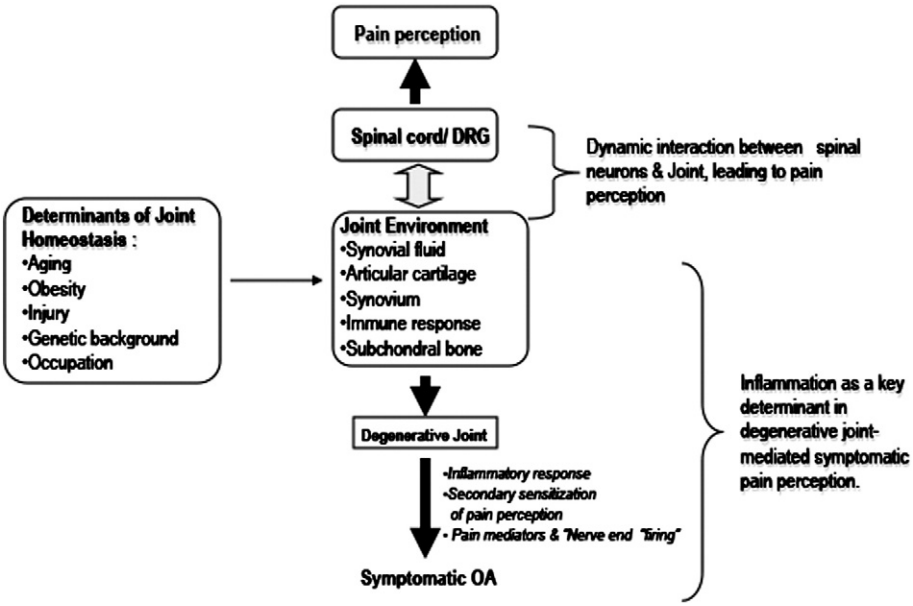

Fig. 3. Pathophysiological status of each component in synovial joint is linked to joint degeneration and related pain perception. Local homeostasis inside the joint can be perturbed by various factors, such as aging, injury, and genetic predisposition. Low grade chronic inflammation in the joint not only contributes to accelerated cartilage damage and synovitis, but also renders the joint susceptible to peripheral sensitization and, in some cases, central sensitization.

available literature demonstrates that these factors may be potential targets for novel biological therapies that may be used for prevention of OA and pain in the future (Bauer et al., 2006; Dieppe and Lohmander, 2005; Lee et al., 2011; Schaible et al., 2011).

1.3.1. Interleukin-1

One of the most well-studied cytokines involved in OA, IL-1, has been shown to play a prominent role in both cartilage degradation and stimulation of nociceptive pathways (Benito et al., 2005; Youssef et al., 1997). IL-1 demonstrates potent bioactivities in inhibiting ECM synthesis and promoting cartilage breakdown, represses the expression of essential ECM components (i.e. aggrecan and collagen type II) in chondrocytes (Goldring et al., 1988; Lefebvre et al., 1990; Richardson and Dodge, 2000), and induces a spectrum of proteolytic enzymes such as collagenases (MMP-1 and MMP-13) and ADAMTS-4, in both chondrocytes and synovial fibroblasts. Aside from these direct effects, IL-1β also induces a variety of other cytokines, including IL-6, IL-8, and leukemia inducing factor (LIF), which interact to induce additive or synergistic effects in the catabolic cascade. IL-1β has also been shown to activate nociceptors directly *via* intracellular kinase activation, and may also induce indirect nociceptive sensitization *via* the production of kinins and prostanoids (Sommer, 2004). This relationship is relevant to clinicians, as studies have demonstrated the possibility of associating patient cytokine levels with subjective outcome, pain perception, and radiographic findings of knee OA patients (Orita et al., 2011).

The significance of IL-1 in OA was further corroborated by *in vivo* studies and pharmaceutical efforts using IL-1 receptor antagonist (IL-1ra) as a potential therapeutic factor to prevent cartilage degeneration. As an inhibitory molecule of IL-1β, IL-1ra not only showed efficacy in OA animal models, but also improved clinical outcomes (Evans et al., 2006). Both gene delivery and IL-1ra intra-articular injection models have been shown to impede OA progression, indicating anti-IL-1 therapy may be a viable option in OA disease modification (Caron et al., 1996; Evans et al., 2006).

1.3.2. IL-6

IL-6, another familiar pro-inflammatory cytokine with known involvement in cartilage degradation, has also been associated with hyperalgesia and hypersensitivity in joint tissues (Brenn et al., 2007). IL-6 plays an important role in the pathogenesis of rheumatoid arthritis, and its concentration is elevated in the serum and synovial fluid of arthritic patients (Arvidson et al., 1994; Silacci et al., 1998). Interestingly, primary afferent neurons also respond to IL-6 (Obreja et al., 2005), suggesting a role of IL-6 in pain propagation in arthritic states. Indeed, more studies are warranted to elucidate the potential role of IL-6 in pain pathways associated with OA.

1.3.3. TNF-α

In addition to its potent catabolic effects in the pathophysiology of OA, TNF-α activates sensory neurons directly *via* the receptors TNFR1 and TNFR2, and initiates a cascade of inflammatory reactions *via* the production of IL-1, IL-6 and IL-8 (Aoki et al., 2004; Sommer, 2004). Direct TNF-α application in the periphery induces neuropathic pain, and this pain may be blocked by anti-inflammatory medications such as ibuprofen and celecoxib (Schäfers et al., 2004). Anti-TNF-α treatment with a TNF antibody produces a prolonged reduction of pain symptoms in OA (Grunke and Schulze-Koops, 2006), and neutralization of TNF-α in mice rescues both mechanical hyperalgesia (testing of withdrawal responses in behavioral experiments) and the inflammatory process (Inglis et al., 2007). Taken together, TNF-α induces an analgesic effect, at least in part, *via* both neuronal and inflammatory stimulation. Antagonists to TNF-α, such as etanercept or infliximab, may serve as a potential therapeutic strategy to decrease OA pain clinically (Dray and Read, 2007). Further well-designed and controlled studies will help substantiate these promising preliminary data on TNF inhibitors in OA.

1.3.4. Prostanoids and PGE2

During pro-inflammatory states in articular cartilage, numerous prostanoid cyclooxygenase (COX) enzyme products are produced and released, including PGE2, PGD2, PGF2a, thromboxane, and PGI2 (Dray and Read, 2007). These factors serve as the premise for blocking the major synthetic enzymes COX-1 and COX-2 with either selective or non-selective COX-inhibitor medications (i.e. NSAIDs, dexamethasone, or selective COX-2 inhibitors) (Yaksh et al., 2001). COX activation has been shown to enhance production of matrix metalloproteinase-3, inhibit PG and collagen synthesis, and stimulate chondrocyte apoptosis.

Of these mediators, PGE2 is considered to be the major contributor to inflammatory pain in arthritic conditions. PGE2 exerts its effects *via* a variety of E prostanoid (EP) receptors (EP1, EP2, EP3, EP4), which are present in both peripheral sensory neurons and the spinal cord

(Dray and Read, 2007). Activation of these receptors induces a variety of effects, ranging from calcium influx to cAMP activation or inhibition. Peripherally, sensitization of nociceptors by PGE2 is caused by the cAMP-mediated enhancement of sodium currents after ion channel phosphorylation (England et al., 1996). However, in the spinal cord, PGE2 acts *via* different receptors than peripherally, suggesting further complexity in the prostanoid regulation of pain (Bär et al., 2004). Several studies (Hardy et al., 2002; Shimpo et al., 2009) have analyzed chondrocytes derived from tissue obtained during joint replacement surgery to understand the pathway of PGE2 synthesis. IL-1β has shown to stimulate and produce high levels of PGE2 that may induce pain and the degeneration with OA. Biochemically, IL-1β is noted to enhance the expression of COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1) at the mRNA and protein levels (Shimpo et al., 2009). It has been shown that increased production of PGE2 is concurrently accompanied by increases in mPGES-1 and COX-2 derived from OA chondrocytes stimulated with as IL-1β (Shimpo et al., 2009).

Previously in our laboratory, we have studied the role of PGE2 in human adult articular cartilage homeostasis and its relation to possible pain pathways (Li et al., 2009). PGE2 utilizes the EP2 and EP4 receptors to induce its downstream catabolic effects, and PGE2 may mediate pain pathways in articular cartilage *via* its stimulatory effect on the pain-associated factors IL-6 (Brenn et al., 2007) and inducible nitric oxide synthase (iNOS) (Hardy et al., 2002). Further, when combined with the catabolic cytokine IL-1, PGE2 synergistically upregulates both IL-6 and iNOS mRNA levels *in vitro* (Li et al., 2009). Similar synergistic results were found with iNOS expression as well. Therefore, the EP2/4 receptor may be an important signaling initiator of the PGE2-signaling cascade and a potential target for therapeutic strategies aimed at preventing progression of arthritic disease and pain in the future.

As opposed to PGE2 EP receptor blockade, an alternative route of PGE2 inhibition is *via* the blockade of PGE synthase (PGES), a major route of conversion of prostaglandin H2 to PGE2 (Dray and Read, 2007). Two isoforms of the enzyme have been identified, membrane or microsomal associated (mPGES-1) and cytosolic (cPGES/p23), which are respectively linked with COX-2 and COX-1 dependent PGE2 production (Claveau et al., 2003). Both isoforms are upregulated by inflammatory mediators, and gene deletion studies in mice indicate an important role for mPGES in acute and chronic inflammation and inflammatory pain, revealing a potential target for pain treatment in OA (Dray and Read, 2007; Trebino et al., 2003).

1.3.5. FGF-2 and PKCδ

A consecutive line of evidence from our laboratory has demonstrated a potent catabolic and anti-anabolic role of FGF-2 in human cartilage homeostasis (Li et al., 2012). FGF-2 is released in supraphysiological amounts during loading and/or injury of the cartilage matrix and activates multiple transduction signal pathways (MAPKs), such as ERK, p38, and JNK (Im et al., 2007b). These kinases in turn phosphorylate a set of transcription factors to regulate gene expression and modify cellular function, resulting in a decrease in PG synthesis and antagonism against anabolic growth factors, such as insulin-like growth factor 1 (IGF-1) and bone morphogenetic protein (BMP-7) in articular cartilage (Im et al., 2007b). FGF-2 potently stimulates MMP-13 expression, which is the major type II collagen-degrading enzyme (Im et al., 2007b). The FGFR1-Ras/PKCδ–Raf–MEK1/2–ERK1/2 signaling pathway is activated after FGF-2 stimulation, which mediates upregulation of matrix-degrading enzyme expression (ADAMTS-5 and MMP-13), as well as downregulation of aggrecan expression (Fig. 4). Correspondingly, PKCδ inhibition significantly impairs these detrimental effects mediated by FGF-2 (Ellman et al., 2008, 2011; Li et al., 2012; Yan et al., 2012).

It is worth noting that controversy over the role of FGF-2 in joint homeostasis does exist. Anabolic activity of FGF-2 in articular cartilage has been reported (Ellman et al., 2008). For example, unlike in human articular cartilage, intraarticular administration of FGF-2 demonstrates protective effects on cartilage in a murine OA animal model (Li et al., 2012). Such an apparent discrepancy can be explained by our recent finding that human and murine articular cartilage bear distinct FGFR expression profiles, where FGF-2 appeared to differentially regulate the expression of FGFR subtypes between human and murine cartilage. Further, despite the successful regeneration of cartilage in the murine model, FGF-2 fails to relieve symptomatic joint pain *in vivo* as determined by behavioral tests, perhaps due to FGF-2-promoted angiogenesis and inflammation in murine synovium (Li et al., 2012). These findings suggest that there are fundamental differences in cellular responses between human and murine tissues that eventually determine biological outcomes of FGF-2, possibly due to the complex interplay of FGFRs and their downstream signaling cascades. Our data pertaining to FGF-2 in human cartilage should add caution to the use of this particular growth factor for biological therapy in the future.

Multiple studies have implicated PKCδ as the rate-limiting factor in which PKCδ is situated at the convergent point of multiple signaling inputs, including FGF-2, substance P (SP), TNF-α, IL-1, and fibronectin fragment (Fn-f). PKCδ activation can lead to NFκB activation in addition to MAPK activation, and these pathways work in concert to inhibit anabolic signaling and stimulate ECM degeneration (Fig. 5). Recently, PKCδ has also been shown to play a nociceptive role as a regulator of both peripheral knee joint tissues (cartilage, synovium, meniscus, subchondral bone) and spinal glial activity. Utilizing an established *in vivo* model for OA, research has shown that glial activity may be controlled by the PKCδ axis which integrates key nociceptive signals that promote central knee OA pain (Ellman et al., 2011). While many of these concepts are novel and pre-translational in nature, they provide deeper insights into the feasibility of utilizing downstream FGF-2 pathway-specific inhibitors in prevention and/or treatment of degenerative joint diseases and OA-associated pain. Future focuses may be toward elucidating

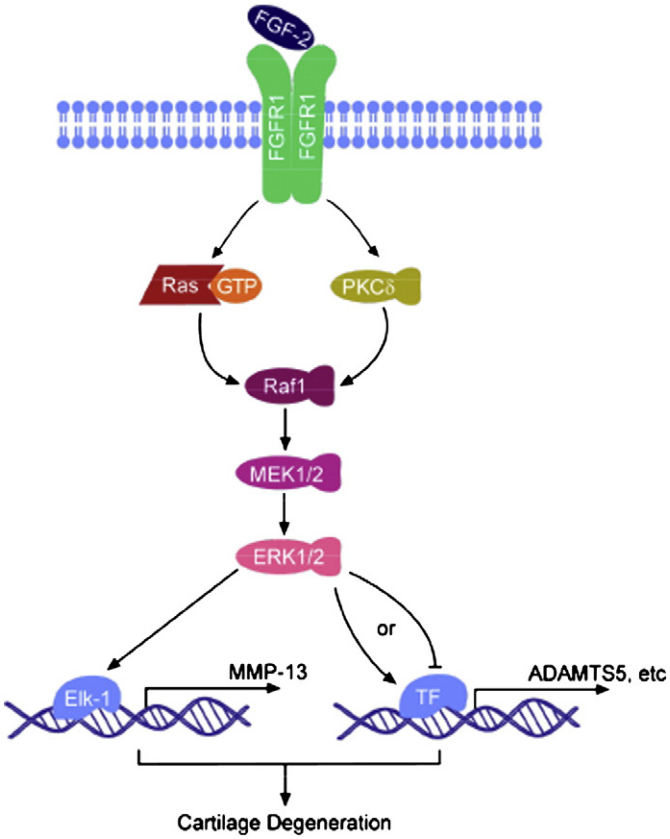

Fig. 4. Schematic model of FGF-2 signaling in articular chondrocytes. FGF-2 binds to FGFR1, which in turn activates both Ras and PKCδ. The signaling inputs then converge on the Raf1–MEK1/2–ERK1/2 axis. Activated ERK1/2 elicits transcription or repression of target genes mediated by a subset of transcription factors, including Elk-1. Adapted from D. Yan and H. J. Im et al., *J Cell Biochem* 2012.

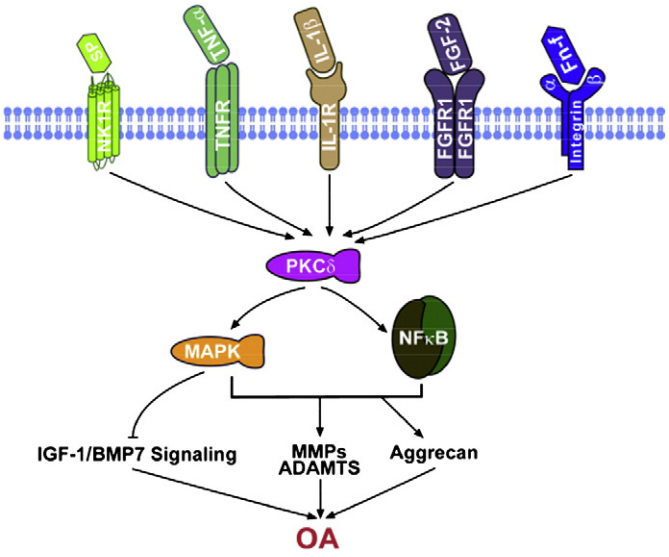

Fig. 5. PKCδ functions as the signaling node of multiple pathways. Input from each illustrated ligand–receptor complex triggers PKCδ activation. PKCδ in turn activates MAP kinases (ERK1/2, p38, and JNK) and NFκB, leading to inhibition of anabolic signaling (*e.g.* IGF-1 and BMP-7), suppression of PG production, and upregulation of catabolic proteases.

pharmacological interventions that have a high translational potential and may establish the potential efficacy of a PKCδ peptide inhibitor in the treatment of symptomatic OA.

1.4. Potential biological treatments: the future

In addition to the development of pharmacologic inhibitors of key catabolic mediators discussed above, recent research has elucidated several compounds that may also play a key role in the future treatment of symptomatic OA. Two of these compounds, resveratrol (RSV) and bovine lactoferricin (LfcinB), have been extensively studied in our laboratory and show considerable promise as an additional potential biological treatment strategy for OA in the coming years.

1.4.1. Resveratrol

The phytoestrogen resveratrol (*trans*-3,4′,5-trihydroxystilbene; RSV) is a natural polyphenol compound found in peanuts, cranberries, and the skin of red grapes, and is thought to be one of the compounds responsible for the health benefits of moderate red wine consumption (Leiro et al., 2004; Wang et al., 2002). The anti-inflammatory, anti-oxidant, cardioprotective, and anti-tumor properties of RSV have been well-documented in a variety of tissues (Bertelli et al., 1999; Bhat et al., 2001; Frémont, 2000; Haider et al., 2003; Huang et al., 2001; Ignatowicz and Baer-Dubowska, 2001; Jang et al., 1997; Leiro et al., 2004; Martinez and Moreno, 2000), with recent studies beginning to analyze the effects of RSV on cartilage homeostasis. Elmali *et al.* first reported a significant protective effect of RSV injections on articular cartilage degradation in rabbit models for OA and RA *via* histological analysis *in vivo* (Elmali et al., 2005, 2007). In human articular chondrocytes, Shakibaei et al. (2008) and Csaki et al. (2008) elucidated both anti-apoptotic and anti-inflammatory regulatory mechanisms mediated by RSV.

In our laboratory, we have demonstrated potent anabolic and anti-catabolic potential of RSV in bovine spine nucleus pulposus IVD tissue (Li et al., 2008) and human adult articular chondrocytes (Im et al., 2012) *via* inhibition of matrix-degrading enzyme expression at the transcriptional and translational level. Further, combination therapy of RSV with BMP-7 induces synergistic effects on PG accumulation, and RSV reverses the catabolic effects of FGF-2 and IL-1 on matrix-degrading enzyme expression, PG accumulation, and the expression of factors (iNOS, IL-1, IL-6) associated with oxidative stress and inflammatory states (Li et al., 2008). Future studies are needed to assess the role of RSV in nociceptive stimulation with OA, as well as its role *in-vivo* before its use in a clinical setting, but these findings reveal considerable promise for use of RSV as a unique biological therapy for treatment of cartilage degenerative diseases.

1.4.2. Lactoferricin

Bovine lactoferricin (LfcinB) is a 25-amino acid cationic peptide with an amphipathic, anti-parallel β-sheet structure that is obtained by acid-pepsin hydrolysis of the N-terminal region of lactoferrin (Lf) found in cow's milk (Baker and Baker, 2009; Gitay-Goren et al., 1992). Similar to RSV, the anti-inflammatory, anti-viral, anti-bacterial, anti-oxidant, anti-pain, and anti-cancer properties of LfcinB have been reported in a variety of tissues (Gifford et al., 2005; Mader et al., 2007). The natural anti-oxidative effect of LfcinB has also been reported, suggesting a possible chondroprotective biological role in articular cartilage (Henrotin et al., 2003), and recent studies have attempted to unravel the role of LfcinB in musculoskeletal disease. In a mouse collagen-induced and septic arthritis model, periarticular injection of human Lf substantially suppresses local inflammation (Guillen et al., 2000). Further, in a rat adjuvant arthritis model, oral administration of bovine Lf suppresses the development of arthritis and hyperalgesia in the adjuvant-injected paw, suggesting Lf has preventative and therapeutic effects on the adjuvant-induced inflammation and pain (Hayashida et al., 2004).

In our laboratory, LfcinB was found to exert potent anabolic and anti-catabolic effects in bovine nucleus pulposus matrix homeostasis in the IVD (Kim et al., 2012). Further, we found similar anabolic and anti-catabolic effects of LfcinB in human articular cartilage (Im et al., unpublished data). LfcinB reverses the catabolic effects of FGF-2 and IL-1 on matrix-degrading enzyme production, PG accumulation, and expression of factors associated with oxidative stress and inflammation, suggesting the promise of LfcinB as an anti-catabolic and anti-inflammatory molecule in human articular cartilage. To date, the anti-pain potential of LfcinB has yet to be studied, and caution must be advised as further studies are warranted to determine, among other things, possible detrimental effects of the use of LfcinB (and RSV) *in vivo*.

2. Conclusion

In summary, the literature reveals important roles of growth factors and cytokines in articular cartilage homeostasis and the development of OA and OA-associated pain. Upregulation of catabolic processes and/or downregulation of anabolic processes leads to disruption of equilibrium with subsequent cartilage degradation and OA, and several of these pathways are known to induce pain in OA as well. Currently, many of the underlying pathways remain unknown, but recent efforts have begun to increase our understanding. Catabolic factors involved in both cartilage degradation *in vitro* and nociceptive stimulation include IL-1, IL-6, TNF-α, PGE2, FGF-2 and PKCδ, and pharmacologic inhibitors to these mediators, as well as compounds such as RSV and LfcinB, may potentially be used as biological treatments in the future.

Despite a tremendous research effort in recent years to elucidate these processes, however, biologic therapy for OA remains experimental in nature, and several unknowns exist. Given the wide array of interactions of growth factors that are necessary for maintenance of cartilage homeostasis *in vivo*, it is unlikely that any single growth factor will lead to complete cartilage repair or affect the arthritic joint clinically, and rather a combination approach will be required (Fortier et al., 2011). Further, appropriate dosing, scaffolds, and routes of administration must be determined before biological factors play a beneficial role clinically. Nevertheless, this paper reviews several of the most well-studied biochemical mediators involved in OA and pain, and provides a framework for the understanding of potential biologic therapies in the treatment of degenerative joint disease in the future.

Conflict of interest

No conflict of interest to note.

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References

Aoki, Y., et al., Dec. 2004. Disc inflammation potentially promotes axonal regeneration of dorsal root ganglion neurons innervating lumbar intervertebral disc in rats. Spine 29 (23), 2621–2626. http://dx.doi.org/10.1097/01.brs.0000146051.11574.b4.

Arvidson, N., Gudbjörnsson, B., Elfman, L., 1994. Circadian rhythm of serum interleukin-6 in rheumatoid arthritis. Ann. Rheum. Dis. http://dx.doi.org/10.1136/ard.53.8.521.

Baker, E.N., Baker, H.M., 2009. A structural framework for understanding the multifunctional character of lactoferrin. Biochimie 91 (1), 3–10. http://dx.doi.org/10.1016/j.biochi.2008.05.006.

Bär, K.J., et al., 2004. Changes in the effect of spinal prostaglandin E2 during inflammation: prostaglandin E (EP1–EP4) receptors in spinal nociceptive processing of input from the normal or inflamed knee joint. J. Neurosci. 24 (3), 642–651. http://dx.doi.org/10.1523/JNEUROSCI.0882-03.2004.

Bauer, D.C., et al., Aug. 2006. Classification of osteoarthritis biomarkers: a proposed approach. Osteoarthr. Cartil. 14 (8), 723–727. http://dx.doi.org/10.1016/j.joca.2006.04.001.

Benito, M.J., Veale, D.J., FitzGerald, O., van den Berg, W.B., Bresnihan, B., Sep. 2005. Synovial tissue inflammation in early and late osteoarthritis. Ann. Rheum. Dis. 64 (9), 1263–1267. http://dx.doi.org/10.1136/ard.2004.025270.

Bertelli, A.A., et al., 1999. Resveratrol, a natural stilbene in grapes and wine, enhances intraphagocytosis in human promonocytes: a co-factor in antiinflammatory and anticancer chemopreventive activity. Int. J. Tissue React. 21 (4), 93–104.

Bhat, K.P., Kosmeder, J.W., Pezzuto, J.M., Dec. 2001. Biological effects of resveratrol. Antioxid. Redox Signal. 3 (6), 1041–1064. http://dx.doi.org/10.1089/152308601317203567.

Brenn, D., Richter, F., Schaible, H., 2007. Sensitization of unmyelinated sensory fibers of the joint nerve to mechanical stimuli by interleukin-6 in the rat: an inflammatory mechanism of joint pain. Arthritis Rheum. http://dx.doi.org/10.1002/art.22282.

Buckwalter, J.A., Saltzman, C., Brown, T., 2004a. The impact of osteoarthritis: implications for research. Clin. Orthop. Relat. Res. 427, S6–S15 (Oct.).

Buckwalter, J.A., Saltzman, C., Brown, T., Oct. 2004b. The impact of osteoarthritis. Clin. Orthop. Relat. Res. 427, S6–S15. http://dx.doi.org/10.1097/01.blo.0000143938.30681.9d.

Caron, J.P., et al., 1996. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis. Suppression of collagenase-1 expression. Arthritis Rheum. http://dx.doi.org/10.1002/art.1780390914.

Chubinskaya, S., Pacione, C., Im, H., 2005. Basic fibroblast growth factor inhibits the anabolic activity of insulin-like growth factor 1 and osteogenic protein 1 in adult human articular chondrocytes. Arthritis Rheum. 52 (12), 3910–3917 (Dec).

Claveau, D., et al., 2003. Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E2 production in the rat adjuvant-induced arthritis model. J. Immunol. 170 (9), 4738–4744.

Csaki, C., Keshishzadeh, N., Fischer, K., Shakibaei, M., 2008. Regulation of inflammation signalling by resveratrol in human chondrocytes *in vitro*. Biochem. Pharmacol. 75 (3), 677–687. http://dx.doi.org/10.1016/j.bcp.2007.09.014.

Dieppe, P.A., Lohmander, L.S., 2005. Pathogenesis and management of pain in osteoarthritis. Lancet 365 (9463), 965–973. http://dx.doi.org/10.1016/S0140-6736(05)71086-2 (Feb.).

Dray, A., Read, S.J., 2007. Arthritis and pain. Future targets to control osteoarthritis pain. Arthritis Res. Ther. 9 (3), 212.

Ellman, M.B., An, H.S., Muddasani, P., Im, H.-J., Aug. 2008. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene 420 (1), 82–89. http://dx.doi.org/10.1016/j.gene.2008.04.019.

Ellman, M., Kim, J., An, H., Kroin, J., 2011. The pathophysiological role of the PKCδ pathway in the intervertebral disc: *In vitro*, *ex vivo* and *in vivo* studies. Arthritis Rheum. http://dx.doi.org/10.1002/art.34337.

Elmali, N., Esenkaya, I., Harma, A., Ertem, K., Turkoz, Y., Mizrak, B., Apr. 2005. Effect of resveratrol in experimental osteoarthritis in rabbits. Inflamm. Res. 54 (4), 158–162. http://dx.doi.org/10.1007/s00011-004-1341-6.

Elmali, N., Baysal, O., Harma, A., Esenkaya, I., Mizrak, B., Apr. 2007. Effects of resveratrol in inflammatory arthritis. Inflammation 30 (1), 1–6. http://dx.doi.org/10.1007/s10753-006-9012-0.

England, S., Bevan, S., Docherty, R.J., 1996. PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones *via* the cyclic AMP-protein kinase A cascade. J. Physiol. 495 (Pt 2), 429–440.

Evans, C., Gouze, E., Gouze, J., Robbins, P., 2006. Gene therapeutic approaches—transfer *in vivo*. Adv. Drug Deliv. Rev. http://dx.doi.org/10.1016/j.addr.2006.01.009.

Felson, D.T., Sep. 2005. The sources of pain in knee osteoarthritis. Curr. Opin. Rheumatol. 17 (5), 624–628. http://dx.doi.org/10.1097/01.bor.0000172800.49120.97.

Fortier, L.A., Barker, J.U., Strauss, E.J., McCarrel, T.M., Cole, B.J., 2011. The role of growth factors in cartilage repair. Clin. Orthop. Relat. Res. http://dx.doi.org/10.1007/s11999-011-1857-3.

Frémont, L., 2000. Biological effects of resveratrol. Life Sci. 66 (8), 663–673. http://dx.doi.org/10.1016/S0024-3205(99)00410-5.

Gifford, J.L., Hunter, H.N., Vogel, H.J., Nov. 2005. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell. Mol. Life Sci. 62 (22), 2588–2598. http://dx.doi.org/10.1007/s00018-005-5373-z.

Gitay-Goren, H., Soker, S., Vlodavsky, I., Neufeld, G., Mar. 1992. The binding of vascular endothelial growth factor to its receptors is dependent on cell surface-associated heparin-like molecules. J. Biol. Chem. 267 (9), 6093–6098.

Goldring, M.B., Berenbaum, F., 2004. The regulation of chondrocyte function by proinflammatory mediators: prostaglandins and nitric oxide. Clin. Orthop. Relat. Res. (427 Suppl.), S37–S46. http://dx.doi.org/10.1097/01.blo.0000144484.69656.e4.

Goldring, M.B., Birkhead, J., Sandell, L.J., Kimura, T., Krane, S.M., Dec. 1988. Interleukin 1 suppresses expression of cartilage-specific types II and IX collagens and increases types I and III collagens in human chondrocytes. J. Clin. Invest. 82 (6), 2026–2037. http://dx.doi.org/10.1172/JCI113823.

Grunke, M., Schulze-Koops, H., 2006. Successful treatment of inflammatory knee osteoarthritis with tumour necrosis factor blockade. Ann. Rheum. Dis. http://dx.doi.org/10.1136/ard.2006.053272.

Guillen, C., McInnes, I.B., Vaughan, D., Speekenbrink, A.B., Brock, J.H., Sep. 2000. The effects of local administration of lactoferrin on inflammation in murine autoimmune and infectious arthritis. Arthritis Rheum. 43 (9), 2073–2080. http://dx.doi.org/10.1002/1529-0131(200009)43:9<2073::AID-ANR19>3.0.CO;2-U.

Haider, U.G.B., Sorescu, D., Griendling, K.K., Vollmar, A.M., Dirsch, V.M., 2003. Resveratrol increases serine15-phosphorylated but transcriptionally impaired p53 and induces a reversible DNA replication block in serum-activated vascular smooth muscle cells. Mol. Pharmacol. 63 (4), 925–932. http://dx.doi.org/10.1124/mol.63.4.925.

Hardy, M.M., et al., Jul. 2002. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum. 46 (7), 1789–1803. http://dx.doi.org/10.1002/art.10356.

Hayashida, K.-I., Kaneko, T., Takeuchi, T., Shimizu, H., Ando, K., Harada, E., Feb. 2004. Oral administration of lactoferrin inhibits inflammation and nociception in rat adjuvant-induced arthritis. J. Vet. Med. Sci. 66 (2), 149–154. http://dx.doi.org/10.1292/jvms.66.149.

Henrotin, Y.E., Bruckner, P., Pujol, J.-P.L., Oct. 2003. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthr. Cartil. 11 (10), 747–755. http://dx.doi.org/10.1016/S1063-4584(03)00150-X.

Huang, K.S., Lin, M., Cheng, G.F., Sep. 2001. Anti-inflammatory tetramers of resveratrol from the roots of *Vitis amurensis* and the conformations of the seven-membered ring in some oligostilbenes. Phytochemistry 58 (2), 357–362. http://dx.doi.org/10.1016/S0031-9422(01)00224-2.

Hunter, D.J., McDougall, J.J., Keefe, F.J., Aug. 2008. The symptoms of osteoarthritis and the genesis of pain. Rheum. Dis. Clin. North Am. 34 (3), 623–643. http://dx.doi.org/10.1016/j.rdc.2008.05.004.

Hunter, D.J., McDougall, J.J., Keefe, F.J., 2009. The symptoms of osteoarthritis and the genesis of pain. Med. Clin. North Am. 93 (1), 83–100. http://dx.doi.org/10.1016/j.mcna.2008.08.008 (xi, Jan.).

Ignatowicz, E., Baer-Dubowska, W., 2001. Resveratrol, a natural chemopreventive agent against degenerative diseases. Pol. J. Pharmacol. 53 (6), 557–569.

Im, H.J., et al., 2007a. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 *via* the molecular cross-talk between the mitogen-activated protein kinases and protein kinase C pathways in human adult articular chondrocytes. J. Biol. Chem. 282 (15), 11110–11121. http://dx.doi.org/10.1074/jbc.M609040200.

Im, H.J., et al., Feb. 2007b. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 *via* the molecular cross-talk between the mitogen-activated protein kinases and protein kinase c pathways in human adult articular chondrocytes. J. Biol. Chem. 282 (15), 11110–11121. http://dx.doi.org/10.1074/jbc.M609040200.

Im, H.-J., et al., 2008. Basic fibroblast growth factor accelerates matrix degradation *via* a neuro-endocrine pathway in human adult articular chondrocytes. J. Cell. Physiol. 215 (2), 452–463. http://dx.doi.org/10.1002/jcp.21317.

Im, H.-J., et al., Jun. 2010. Alteration of sensory neurons and spinal response to an experimental osteoarthritis pain model. Arthritis Rheum. 62 (10), 2995–3005. http://dx.doi.org/10.1002/art.27608.

Im, H.-J., et al., 2012. Biological effects of the plant-derived polyphenol resveratrol in human articular cartilage and chondrosarcoma cells. J. Cell. Physiol. http://dx.doi.org/10.1002/jcp.24049.

Imamura, M., et al., Oct. 2008. Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: a controlled analysis. Arthritis Rheum. 59 (10), 1424–1431. http://dx.doi.org/10.1002/art.24120.

Inglis, J.J., et al., 2007. Collagen-induced arthritis as a model of hyperalgesia: functional and cellular analysis of the analgesic actions of tumor necrosis factor blockade. Arthritis Rheum. 56 (12), 4015–4023. http://dx.doi.org/10.1002/art.23063.

Issa, S.N., Sharma, L., Feb. 2006. Epidemiology of osteoarthritis: an update. Curr. Opin. Rheumatol. 8 (1), 7–15. http://dx.doi.org/10.1007/s11926-006-0019-1.

Jang, M., et al., Jan. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275 (5297), 218–220. http://dx.doi.org/10.1126/science.275.5297.218.

Kim, J., Ellman, M., An, H., Yan, D., 2012. Lactoferricin mediates anabolic and anti-catabolic effects in the intervertebral disc. J. Cell. Physiol. http://dx.doi.org/10.1002/jcp.22867.

Kosek, E., Ordeberg, G., Oct. 2000. Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. Pain 88 (1), 69–78. http://dx.doi.org/10.1016/S0304-3959(00)00310-9.

Kotlarz, H., Gunnarsson, C.L., Fang, H., Rizzo, J.A., Dec. 2009. Insurer and out-of-pocket costs of osteoarthritis in the US: evidence from national survey data. Arthritis Rheum. 60 (12), 3546–3553. http://dx.doi.org/10.1002/art.24984.

Lane, N.E., Brandt, K., Hawker, G., Peeva, E., Schreyer, Tsuji, W., Hochberg, M.C., 2011. OARSI-FDA initiative: defining the disease state of osteoarthritis. Presented at

the Osteoarthritis and cartilage/OARS. , Vol. 19. Osteoarthritis Research Society, pp. 478–482. http://dx.doi.org/10.1016/j.joca.2010.09.013.
Lee, Y.C., Nassikas, N.J., Clauw, D.J., 2011. The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. Arthritis Res. Ther. 13 (2), 211. http://dx.doi.org/10.1186/ar3306.
Lefebvre, V., Peeters-Joris, C., Vaes, G., May 1990. Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochim. Biophys. Acta 1052 (3), 366–378. http://dx.doi.org/10.1172/JCI113823.
Leiro, J., Alvarez, E., Arranz, J.A., Laguna, R., Uriarte, E., Orallo, F., Jun. 2004. Effects of *cis*-resveratrol on inflammatory murine macrophages: antioxidant activity and down-regulation of inflammatory genes. J. Leukoc. Biol. 75 (6), 1156–1165. http://dx.doi.org/10.1189/jlb.1103561.
Li, X., et al., 2008. The action of resveratrol, a phytoestrogen found in grapes, on the intervertebral disc. Spine 33 (24), 2586–2595. http://dx.doi.org/10.1097/BRS.0b013e3181883883.
Li, X., et al., 2009. Prostaglandin E2 and its cognate EP receptors control human adult articular cartilage homeostasis and are linked to the pathophysiology of osteoarthritis. Arthritis Rheum. 60 (2), 513–523. http://dx.doi.org/10.1002/art.24258.
Li, X., Kim, J.S., Wijnen, A.J., Im, H.J., 2011a. Osteoarthritic tissues modulate functional properties of sensory neurons associated with symptomatic OA pain. Mol. Biol. Rep. 38 (8), 5335–5339. http://dx.doi.org/10.1007/s11033-011-0684-7.
Li, X., Kim, J.-S., Wijnen, A.J., Im, H.-J., 2011b. Osteoarthritic tissues modulate functional properties of sensory neurons associated with symptomatic OA pain. Mol. Biol. Rep. 38 (8), 5335–5339. http://dx.doi.org/10.1007/s11033-011-0684-7 (Feb.).
Li, X., et al., 2012. Species-specific biological effects of FGF-2 in articular cartilage: implication for distinct roles within the FGF receptor family. J. Cell. Biochem. 113 (7), 2532–2542. http://dx.doi.org/10.1002/jcb.24129.
Loeser, R.F., Sep. 2008. Molecular mechanisms of cartilage destruction in osteoarthritis. J. Musculoskelet. Neuronal. Interact. 8 (4), 303–306.
Loeser, R., Yammani, R., Carlson, C., 2005. Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. Arthritis Rheum. http://dx.doi.org/10.1002/art.21199.
Mader, J.S., et al., Jul. 2007. Bovine lactoferricin causes apoptosis in Jurkat T-leukemia cells by sequential permeabilization of the cell membrane and targeting of mitochondria. Exp. Cell Res. 313 (12), 2634–2650. http://dx.doi.org/10.1016/j.yexcr.2007.05.015.
Martinez, J., Moreno, J.J., 2000. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem. Pharmacol. 59 (7), 865–870. http://dx.doi.org/10.1016/S0006-2952(99)00380-9.
Nakata, K., et al., 1993. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing alpha 1(IX) collagen chains with a central deletion. Proc. Natl. Acad. Sci. U. S. A. 90 (7), 2870–2874. http://dx.doi.org/10.1073/pnas.90.7.2870.
Obreja, O., et al., Jul. 2005. Fast modulation of heat-activated ionic current by proinflammatory interleukin 6 in rat sensory neurons. Brain 128 (7), 1634–1641. http://dx.doi.org/10.1093/brain/awh490.
Orita, S., et al., 2011. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskelet. Disord. 12 (1), 144. http://dx.doi.org/10.1186/1471-2474-12-144 (Jun.).
Pereira, D., Peleteiro, B., Araújo, J., Branco, J., Santos, R.A., Ramos, E., Nov. 2011. The effect of osteoarthritis definition on prevalence and incidence estimates: a systematic review. Osteoarthr. Cartil. 19 (11), 1270–1285. http://dx.doi.org/10.1016/j.joca.2011.08.009.
Richardson, D.W., Dodge, G.R., 2000. Effects of interleukin-1β and tumor necrosis factor-α on expression of matrix-related genes by cultured equine articular chondrocytes. Am. J. Vet. Res. 61 (6), 624–630. http://dx.doi.org/10.2460/ajvr.2000.61.624 (Jun.).
Richette, P., et al., 2010. Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. Ann. Rheum. Dis. 70 (1), 139–144. http://dx.doi.org/10.1136/ard.2010.134015 (Dec.).
Sandell, L., Aigner, T., 2001. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res. http://dx.doi.org/10.1186/ar148.
Schäfers, M., Marziniak, M., Sorkin, L., Yaksh, T., 2004. Cyclooxygenase inhibition in nerve-injury- and TNF-induced hyperalgesia in the rat. Exp. Neurol. http://dx.doi.org/10.1016/j.expneurol.2003.09.015 (Jan.).
Schaible, H.G., Ebersberger, A., Natura, G., 2011. Update on peripheral mechanisms of pain: beyond prostaglandins and cytokines. Arthritis Res. Ther. 13 (2), 210. http://dx.doi.org/10.1186/ar3305.
Shakibaei, M., Csaki, C., Nebrich, S., Mobasheri, A., 2008. Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochem. Pharmacol. 76 (11), 1426–1439. http://dx.doi.org/10.1016/j.bcp.2008.05.029.
Shimpo, H., et al., 2009. Regulation of prostaglandin E2 synthesis in cells derived from chondrocytes of patients with osteoarthritis. J. Orthop. Sci. 14 (5), 611–617. http://dx.doi.org/10.1007/s00776-009-1370-7 (Oct.).
Silacci, P., Dayer, J.M., Desgeorges, A., Peter, R., Manueddu, C., Guerne, P.A., 1998. Interleukin (IL)-6 and its soluble receptor induce TIMP-1 expression in synoviocytes and chondrocytes, and block IL-1-induced collagenolytic activity. J. Biol. Chem. 273 (22), 13625–13629.
Sommer, C., 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci. Lett. http://dx.doi.org/10.1016/j.neulet.2003.12.007.
Sundman, E.A., Cole, B.J., Fortier, L.A., 2011. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am. J. Sports Med. http://dx.doi.org/10.1177/0363546511417792 (Aug.).
Tanamas, S., Hanna, F.S., Cicuttini, F.M., Wluka, A.E., Berry, P., Urquhart, D.M., 2009. Does knee malalignment increase the risk of development and progression of knee osteoarthritis? A systematic review. Arthritis Rheum. 61 (4), 459–467. http://dx.doi.org/10.1002/art.24336.
Trebino, C.E., et al., 2003. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc. Natl. Acad. Sci. U. S. A. 100 (15), 9044–9049. http://dx.doi.org/10.1073/pnas.1332766100.
Valdes, A.M., Spector, T.D., Nov. 2010. Genetic epidemiology of hip and knee osteoarthritis. Nat. Publ. Group 7 (1), 23–32. http://dx.doi.org/10.1038/nrrheum.2010.191.
Wang, Z., Huang, Y., Zou, J., Cao, K., Xu, Y., Wu, J.M., 2002. Effects of red wine and wine polyphenol resveratrol on platelet aggregation *in vivo* and *in vitro*. Int. J. Mol. Med. 9 (1), 77–79.
Yaksh, T.L., Dirig, D.M., Conway, C.M., Svensson, C., Luo, Z.D., Isakson, P.C., 2001. The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. J. Neurosci. 21 (16), 5847–5853.
Yan, D., Chen, D., Im, H.J., 2012. Fibroblast growth factor-2 promotes catabolism *via* FGFR1–Ras–Raf–MEK1/2–ERK1/2 axis that coordinates with the PKCδ pathway in human articular chondrocytes. J. Cell. Biochem. http://dx.doi.org/10.1002/jcb.24160.
Youssef, P.P., et al., 1997. Variability in cytokine and cell adhesion molecule staining in arthroscopic synovial biopsies: quantification using color video image analysis. J. Rheumatol. 24 (12), 2291–2298 (Dec.).