REVIEW

OPEN

Recent developments in emerging therapeutic targets of osteoarthritis

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Purpose of review
Despite the tremendous individual suffering and socioeconomic burden caused by osteoarthritis, there are currently no effective disease-modifying treatment options. This is in part because of our incomplete understanding of osteoarthritis disease mechanism. This review summarizes recent developments in therapeutic targets identified from surgical animal models of osteoarthritis that provide novel insight into osteoarthritis pathology and possess potential for progression into preclinical studies.

Recent findings
Several candidate pathways and processes that have been identified include chondrocyte autophagy, growth factor signaling, inflammation, and nociceptive signaling. Major strategies that possess therapeutic potential at the cellular level include inhibiting autophagy suppression and decreasing reactive oxygen species (ROS) production. Cartilage anabolism and prevention of cartilage degradation has been shown to result from growth factor signaling modulation, such as TGF-β, TGF-α, and FGF; however, the results are context-dependent and require further investigation. Pain assessment studies in rodent surgical models have demonstrated potential in employing anti-NGF strategies for minimizing osteoarthritis-associated pain.

Summary
Studies of potential therapeutic targets in osteoarthritis using animal surgical models are helping to elucidate osteoarthritis pathology and propel therapeutics development. Further studies should continue to elucidate pathological mechanisms and therapeutic targets in various joint tissues to improve overall joint health.

Keywords
animal surgical models, osteoarthritis, therapeutic targets

INTRODUCTION

Osteoarthritis is the most common type of arthritis and the primary cause of disability in elderly populations [1]. In fact, it has been estimated that 10% of men and 18% of women above 60 years of age report symptomatic osteoarthritis worldwide [2]. Although ageing is an important risk factor, osteoarthritis is multifactorial in nature and contributing sources to the pathophysiology of osteoarthritis include genetics, sex, weight, metabolism, and prior joint injury [3]. The most notable feature of osteoarthritis is articular cartilage degradation, however pathological changes can occur in all joint tissues including the underlying subchondral bone, as well as the supporting synovial membrane, ligaments, and menisci in the knee joint [3]. As articular cartilage is both aneural and avascular, changes in these tissues act as potential causes of symptomatic pain, which necessitates further research into the interplay of joint tissues as a whole in the pathology of osteoarthritis.

Despite major individual and socioeconomic burden inflicted by osteoarthritis, there are currently no effective disease-modifying therapies, and existing symptomatic treatment options are limited with unwanted side effects [4]. Our inability to diagnose osteoarthritis prior to irreversible joint

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KEY POINTS

- Lack of osteoarthritis disease-modifying treatments stem from incomplete understanding of osteoarthritis pathology. Destabilization-induced osteoarthritis models in animals are an invaluable tool for better understanding of disease onset and progression, as well as identifying novel therapeutic targets.
- Recent studies in autophagy in osteoarthritis have identified PPARγ to be an important mediator of mTOR signaling, and that suppression of mTOR signaling may have beneficial implications in metabolic osteoarthritis. However, mTOR and autophagy may also in part modulate osteoarthritis pathology independent of each other, rendering further investigation necessary.
- Recent studies examining the suppression of TGF-β and TGF-α/EGFR signaling in osteoarthritis have demonstrated beneficial roles in preventing cartilage degradation and pathological changes in subchondral bone; however, these effects seem to be context-dependent and require careful manipulation. FGFR3 signaling also exert protective effects, however due to its pleiotropic role requires further understanding to eliminate unwanted side-effects. Inhibitors of the Wnt/β-catenin signaling pathway demonstrate therapeutic potential, however their effects on the joint as a whole require further elucidation.
- Targeting inflammatory mediators, such as DAMPs, reactive oxygen species (ROS) as well as contributing sources of oxidative stress, such as dyslipidemia, possess protective effects against osteoarthritis pathology.
- Anti-NGF strategies have demonstrated promising results for minimizing osteoarthritis associated pain-like behaviors in rodent surgical models, however further research is needed to validate these effects and rule out rare adverse events as observed in human clinical trials.

damage hinders favorable management of the disease [5]. Better understanding of osteoarthritis pathogenesis is therefore crucial for identifying novel therapeutic targets. To date, the most commonly used animal models for the study of osteoarthritis pathophysiology are age-associated (spontaneous) and instability-induced through joint surgery. Among the most widely used surgical techniques include anterior cruciate ligament transection (ACLT) and destabilization of the medial meniscus (DMM) in rodents, which models human posttraumatic osteoarthritis (PTOA), and structural similarity [6]. In these modes, disease onset and progression occur with high reproducibility, which allows for the evaluation of specific genetic manipulations or pharmacological interventions on osteoarthritis progression. This review will focus on recent work on therapeutic targets identified from animal surgical models that provide novel insight into osteoarthritis pathology with potential for further progression into preclinical studies.

CHONDROCYTE SURVIVAL AND AUTOPHAGY

Macroautophagy (referred to simply as 'autophagy') is a highly conserved eukaryotic process whereby damaged or harmful cytoplasmic materials are translocated to the lysosome for degradation [7]. It is most notably known for its roles in protecting cells from conditions of cellular stress, such as oxidative stress, endoplasmic reticulum stress, hypoxia, and nutrient and growth factor withdrawal, through maintenance of nutrient and energy homeostasis [8]. Alterations in autophagy occur in a variety of disease states, thus understanding these dysregulations in the disease context may bear therapeutic implications.

Much research has been done demonstrating an extensive link between autophagy and osteoarthritis, in which a compensatory increase in key autophagy markers appears in early osteoarthritis pathogenesis but is reduced in late osteoarthritis in parallel with increased chondrocyte apoptosis [9,10]. This link was further supported when mice with cartilage-specific deletion of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that functions as a key suppressor of autophagy, exhibited protection from DMM-induced osteoarthritis [11▪▪]. Vasheghani *et al.* [12▪▪] went onto identify peroxisome proliferator-activated receptor gamma (PPARγ) to be an important player in maintaining mTOR signaling in cartilage homeostasis, where they showed cartilage-specific PPARγ deficiency to cause an accelerated osteoarthritis phenotype that was subsequently rescued in PPARγ/mTOR double knock-out mice. On the basis of PPARγs known roles in regulating metabolic homeostasis, inflammation and adipogenesis, its involvement in mTOR signaling reinforces the crosstalk between cellular metabolism, autophagy, and cell survival [13]. Indeed, intraperitoneal administration of rapamycin, an mTOR inhibitor, markedly reduced surgically induced osteoarthritis severity in wild-type as well as db/db (leptin receptor mutation) mice, suggesting therapies that suppress mTOR and upregulate autophagy to also be beneficial in metabolic osteoarthritis [14,15▪▪].

Interestingly, Alvarez-Garcia *et al.* [16▪] recently demonstrated REDD1, an endogenous inhibitor of mTOR whose expression is markedly reduced in aged and surgically induced osteoarthritis cartilage, to modulate autophagy in complex with

thioredoxin-interacting protein TXNIP in an mTOR-independent manner. Likewise, cartilage-specific deletion of Atg5, a protein essential for autophagosome formation, led to the development of ageing-associated osteoarthritis but exerted no effect on surgically induced osteoarthritis [17▪]. These studies suggest autophagy and mTOR signaling to potentially modulate osteoarthritis pathology independent of each other (at least in part), rendering further elucidation of the regulatory mechanisms of autophagy necessary for developing therapeutic strategies targeting this process.

GROWTH FACTORS, CARTILAGE ANABOLISM AND SUBCHONDRAL BONE CHANGES

The transforming growth factor-beta (TGF-β) signaling pathway has been identified to play an important role in osteoarthritis development. Despite its anabolic functions in articular cartilage homeostasis through prevention of terminal chondrocyte maturation, TGF-β is also involved in pathological changes in the subchondral bone leading to osteoarthritic cartilage degeneration [18,19]. Modulation of TGF-β signaling as a therapeutic strategy therefore needs to be conducted in an optimized manner to balance the risks and benefits of TGF-β signaling modulation in different joint tissues.

Recent research advances are allowing further clarification into this predicament whereby Xie *et al.* [20▪▪] showed systemic administration of a TGF-β neutralizing antibody to ACLT-treated mice prevented articular cartilage degeneration, normalized subchondral bone structure, and prevented uncoupled subchondral bone remodeling and angiogenesis. Additionally, articular cartilage-specific deletion of *Tgfbr-2* in adult mice subjected to DMM surgery protected from cartilage degeneration [21▪▪], suggesting inhibition of TGF-β signaling to be optimal in mature cartilage in order to avoid the harmful effects of TGF-β signaling ablation during cartilage development and homeostasis.

TGF-α is a member of the epidermal growth factor (EGF) family which binds to epidermal growth factor receptor and has been recently identified to be involved in osteoarthritis pathology, where its expression was found to be upregulated in osteoarthritic chondrocytes in a rat model of ACLT-induced osteoarthritis [22]. In line with previous in-vitro data showing that TGF-α induces catabolic activity in articular chondrocytes, TGF-α null mice experienced protection from DMM-induced osteoarthritis with significantly reduced cartilage damage, MMP13 expression, and type II collagen fragmentation [23▪▪]. However, this protective effect was not mirrored during ageing-associated osteoarthritis or DMM-induced osteoarthritis in older mice, suggesting therapeutic effects of TGF-α signaling to be context-dependent, such as in posttraumatic osteoarthritis of young individuals [23▪▪].

Additionally, another EGFR ligand-heparin-binding EGF (HB-EGF) – has also recently been shown to be increased in the knee joints of DMM operated mice, and elicits similar catabolic activities in cartilage whereas suppressing anabolic activity [24▪▪]. Cartilage-specific deletion of mitogen- inducible gene 6 (MIG6), an inhibitor of the EGFR pathway, resulted in induction of some osteoarthritis-like features in the knee joint including chondrocyte proliferation, osteophyte formation, articular cartilage degradation, and subchondral bone cyst formation [25–27]. However, marked anabolic increase of the articular cartilage thickness was also observed in these mice at an early age, and some models have shown that EGFR suppression exacerbates cartilage destruction, further supporting the context-dependent nature of the EGFR signaling pathway [26–28]. Using rodent models of PTOA, recent studies have suggested C-C motif chemokine ligand 2 (CCL2) and integrin α1β1 to be downstream and upstream mediators of EGFR signaling, respectively, further elucidating this complex pathway for better development of therapeutic targets [29▪,30▪▪].

The fibroblast growth factor (FGF) family consists of 22 pleiotropic growth factors that exert their effect by binding to one of four FGF receptors (FGFRs) [31]. Of the four FGFRs, FGFR1 and FGFR3 are most abundantly expressed in articular cartilage, and FGFR3 activation through FGF2 and FGF18 signaling has been proposed to result in anabolic activities in cartilage [32,33]. This is supported by Tang *et al.* [34▪▪], whose work examining conditional *Fgfr3* deletion in adult chondrocytes showed accelerated DMM-induced osteoarthritis development, with increased proteoglycan loss and chondrocyte hypertrophy. Tang *et al.* [34▪▪] further showed conditional *Fgfr3* activation to cause a chondroprotective effect by delaying osteoarthritis development, suggesting FGFR3 to play an important protective role in osteoarthritis. Development of therapeutic strategies targeting FGFR3 activation, however, requires careful optimization due to the pleiotropic nature of the FGF-signaling pathway. This is seen with intra-articular administration of FGF9 to DMM-induced osteoarthritis in mice. Despite FGF9 being another specific inducer of FGFR3 signaling in chondrocytes, exogenous FGF9 administration aggravated osteophyte formation in addition to attenuating cartilage degradation,

cautioning against potential adverse side effects that may arise with FGF signaling modulation [35▪▪].

The Wnt/β-catenin signaling pathway has been shown to play major roles in joint development as well as maintenance of skeletal tissues [36,37]. Balanced Wnt-signaling is essential in cartilage health as both activation and inhibition of β-catenin in cartilage results in cartilage degradation and osteoarthritis [38,39]. Antagonists of Wnt, including frizzled-related protein (FRZB/sFRP3) and dickkopf-related protein 1 (DKK-1), have been shown to possess protective effects against articular cartilage degradation and osteoarthritis [40,41]. However, Wnt-antagonists also play important roles in bone biology, and modulations have been shown to affect subchondral bone changes observed in osteoarthritis pathology [41–43], suggesting further elucidation of Wnt-signaling in different joint tissues to be necessary for optimal targeting of this pathway.

Recent studies are shedding light on novel Wnt-inhibitors that demonstrate potential as therapeutic targets of osteoarthritis. Specifically, hypoxia-inducible factor 1α (HIF1α) has been shown to lower transcription factor 4 (TCF4)/β-catenin transcriptional activity and inhibit MMP13 levels. Intra-articular injection of PKF118–310, an inhibitor of TCF4/β-catenin interaction, resulted in decreased cartilage degradation in surgically induced osteoarthritis of cartilage-specific inducible HIF1α-null mice [44▪▪]. Similarly, intra-articular injections of an inhibitor of histone methyltransferase enhancer of zeste homolog 2 (EZH2), a chromatin modifier that activates Wnt/β-catenin signaling by suppressing sFRP1, also decreased articular cartilage degradation in surgically induced osteoarthritis [45▪▪]. Furthermore, recent preclinical and clinical trials have demonstrated that intra-articular administration of SM04690, a small-molecule inhibitor of the Wnt pathway, prevents cartilage degradation and promotes cartilage health [46▪▪,47▪▪]. This suggests Wnt-inhibitors to possess potential as disease-modifying therapies for osteoarthritis, however further studies examining potential toxicities of these inhibitors as well as their effects on other joint tissues is necessary to optimize its development and use.

INFLAMMATION, OXIDATIVE STRESS, AND DYSLIPIDEMIA

In addition to traditional proinflammatory mediators such as interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), and other chemokines, recent studies have implicated damage-associated molecular patterns (DAMPs), or alarmins, in activating osteoarthritis inflammation pathophysiology [48,49]. DAMPs activate various pattern recognition receptors (PRRs) found on osteoarthritis chondrocytes and synovium, such as toll-like receptor 2, 4 (TLR2, TLR4) and receptor for advanced glycation end-products (RAGE), to induce cytokine production that cause further release of DAMPs and perpetuation of inflammatory responses and catabolic activity in the joint [49,50].

TLR signaling has been heavily studied in osteoarthritis pathology, with TLR expression found in various joint tissues including articular chondrocytes, synovium, subchondral bone, and infrapatellar fat pad [51,52]. Therapeutic strategies targeting TLR signaling has largely centered on agonist blockade and inhibition of TLR activation and signaling [52,53]. Proteoglycan 4 (PRG4/lubricin) has recently been identified as a novel regulator of TLR2, 4 and 5, where intra-articular PRG4 injections in a rat model of DMM-induced osteoarthritis showed decreased expression of inflammatory cytokines and NF-κB, as well as decreased pain response [54▪▪]. Furthermore, PRG4 has been shown to bind to both TLR2 and TLR4 in human osteoarthritis synovial fluid to significantly reduce subsequent TLR2 and TLR4 activation, suggesting PRG4 to possess a novel anti-inflammatory role and act as a potential therapeutic target for osteoarthritis [55▪▪].

Reactive oxygen species (ROS) present another type of inflammatory mediators found in osteoarthritis joints, whereby advanced glycation end products (AGEs) can activate RAGE to induce upregulation of inflammatory cytokines [56]. Superoxide dismutase 2 (SOD2) is an enzyme that metabolizes superoxides in the mitochondria and its downregulation has recently been implicated in osteoarthritis pathology [57▪▪]. Specific deletion of *Sod2* in chondrocytes resulted in both accelerated cartilage degeneration during ageing and after DMM-induced osteoarthritis, further confirming the detrimental effect of mitochondrial superoxide production in osteoarthritis [57▪▪]. Alternatively, ablation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcription factor that regulates the expression of antioxidant proteins to defend against oxidative damage, resulted in increased cartilage damage after DMM surgery in mice, suggesting that Nrf2 may possess protective functions in osteoarthritis [58▪▪]. Collectively, these studies highlight the importance of fine-tuning ROS levels in chondrocytes, and suggest regulators of cellular redox states to be potential therapeutic targets in osteoarthritis.

Features of metabolic syndrome, such as obesity and dyslipidemia, have been linked to osteoarthritis pathology in part through proinflammatory conditions and oxidative stress [59]. Alterations in lipid metabolism have been shown to compromise

cartilage homeostasis through ectopic lipid accumulation in chondrocytes [59,60]. Indeed, attenuation of intracellular cholesterol accumulation in chondrocytes using pharmacologic cholesterol inhibition showed reduced severity in surgically induced osteoarthritis [61▪▪]. Additionally, cartilage-specific deletion of PPARδ, a nuclear receptor activated upon fatty acid binding to regulate downstream target genes, resulted in a marked chondroprotective effect against DMM-induced osteoarthritis [62▪▪]. This protective effect could be in part due to ablation of PPARδs induction of fatty acid oxidation, which may lead to greater production of ROS that is detrimental to cartilage health [63,64].

PAIN ASSESSMENT AND NERVE GROWTH FACTOR

The studies previously highlighted focus largely on the structural pathology associated with osteoarthritis; however, one of the most frequently cited reasons for osteoarthritis patients to seek medical attention is joint pain [65]. The mechanisms defining the sustained generation of joint pain in osteoarthritis are currently poorly understood. Indeed, radiographically visible signs of osteoarthritis such as joint space narrowing in humans may not be accompanied by symptoms of pain and vice versa [66]. Furthermore, assessment of pain-like behaviors in animal models is made more complicated as the rodents frequently used in osteoarthritis models appear to mask many outward signs of discomfort and disability [66].

Recent work in a rat ACLT/partial meniscectomy model of osteoarthritis has validated that measurements of joint sparing (e.g., asymmetry in hind limb weight bearing) and animal rearing behaviors progress in parallel to histological signs of cartilage degeneration [67▪▪]. Similar assessments have been frequently made in chemical models of osteoarthritis such as those induced by intra-articular monoiodoacetate (MIA) injection; however, the disease progression in these models develops far more rapidly than surgical models [68▪▪]. A recent study examining pain-related behaviors in rats with bilateral intra-articular injection of MIA demonstrated that decreased spontaneous animal burrowing is correlated with spontaneous animal activity and rearing indicative of pain [69▪▪]. These indications of pain were reversible with various analgesics including the controversial novel anti-nerve growth factor (NGF) therapies [69▪▪]. These techniques may also be valuable in assessing osteoarthritis related pain-like behaviors in comparable surgical models.

NGF binds to the tropomyosin receptor kinase A (TrkA) and is important during development for the formation of nociceptive sensory neurons [70▪▪]. NGF and TrkA are expressed by both chondrocytes and fibroblast-like synoviocytes, and appear to have a role in pain sensitization in osteoarthritis [69▪▪–72▪▪]. Recent clinical trials with anti-NGF monoclonal antibodies have produced results supporting the use of these therapies as antinociceptive in osteoarthritis patients; however, a small number of serious adverse events related to osteonecrosis have raised concern over these treatments [73]. Upregulation of NGF mRNA in articular chondrocytes has been shown to follow surgical induction of osteoarthritis by partial meniscectomy in mice, indicating a dynamic role for NGF in pain generation and sensitization following insult to articular cartilage [71▪▪]. Similarly, decreased pain-like behaviors (gait analysis) have been shown following treatment with the anti-NGF antibody tanezumab in a rat meniscal tear model [72▪▪]. Although the tanezumab treated animals in this study appeared to develop worsening cartilage degeneration, unloading of the joint by mid-tibial amputation largely prevented cartilage destruction [72▪▪]. This study may indicate that excessive loading due to loss of protective nociceptive signaling with anti-NGF treatment following surgical osteoarthritis induction may exacerbate disease progression. However, a comprehensive study using both the MIA and meniscal transection mouse osteoarthritis models in concert with oral delivery of a small molecule inhibitor of TrkA demonstrated antinociceptive effects in animal behaviors without increased cartilage damage [70▪▪]. It is important to note that the power of these studies may not be high enough to examine rare adverse events as seen in the human clinical trials [73], although continuing studies are promising for the use of these treatments for addressing pain in osteoarthritis patients.

CONCLUSION

Pathology in surgical animal models of osteoarthritis is similar to the disease progression seen in humans, and gives us great insight into the mechanisms involved. This review focuses on only a few candidate pathways and molecules that have shown recent promise for further understanding of osteoarthritis pathophysiology and development of therapeutics. However, it is clear that the various joint tissues each play a distinct and important role in disease progression, and symptom generation. With this in mind it is important that future studies continue to address the joint as a whole in a physiological context, and for these reasons animal models will continue to be essential in ongoing osteoarthritis research.

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Conflicts of interest

There are no conflicts of interest.

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