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Review

Osteoarthritis year in review 2016: biology

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SUMMARY

This review highlights a selection of literature in the area of osteoarthritis biology published between the 2015 and 2016 Osteoarthritis Research Society International (OARSI) World Congress. Highlights were selected from a pubmed search covering cartilage, bone, inflammation and pain. A personal selection was made based, amongst other things, on topics presented during the 2015 conference. This covers circadian rhythm, TGF-β signaling, autophagy, SIRT6, exercise, lubricin, TLR's, pain and NGF. Furthermore, in this review we have made an effort to connect these seemingly distant topics into one scheme of connections between them, revealing a theoretical big picture underneath.

Introduction

For this year in review in osteoarthritis biology we made a personal selection of the articles from pubmed searches on “osteoarthritis and cartilage”, “osteoarthritis and inflammation”, and “osteoarthritis and subchondral bone”. The selection was further based on topics that were selected for presentation during the Osteoarthritis Research Society International (OARSI) 2015 conference as well as on topics that were researched by multiple groups in this year. We made an effort to interconnect the mechanisms these articles describe to osteoarthritis pathophysiology. This led to addition of a few articles that revealed the connecting elements. This grand scheme of interactions formed the foundation for this review (Fig. 1).

Circadian rhythm

Cartilage degradation is a hallmark of OA. This year, great progress has been made in understanding the role of the circadian clock in cartilage homeostasis, and how dysregulation hereof can result in cartilage degradation and OA. Circadian rhythms are physical, mental and behavioral changes that respond primarily to light and dark and hence roughly follow the 24-h light–dark cycle. It has become clear that this cycle is also present in cells that do have the ability to perceive light and dark themselves, but rely on so-called clock genes to synchronize cellular processes. Recently, it has become clear that these synchronizing processes are important in chondrocyte behavior.

Kc *et al.* compared mice with a standard 12 h light and dark cycles, with mice where this cycle was reversed at the end of each week for the duration of 22 weeks[1]. This shift in cycle resulted in a reduced proteoglycan content, increased fibrillation and a higher OARSI histopathology score, indicating increased OA severity. Remarkably, when a high fat diet (HFD) was added during the final 10 weeks of the protocol, the differences between non-shifted and shifted were further increased, illustrating a link between circadian rhythm and metabolism. When this group investigated which clock genes could be involved by mutating either *Clock* or CSK1 epsilon tau in mice, remarkably, neither of these mutations resulted in joint pathology[2]. In contrast, Dudek *et al.* showed that disruption of another core clock gene, BMAL1, does result in joint damage. BMAL1 was reduced in OA cartilage with increasing severity as well as with aging[3]. By generating a chondrocyte-specific *Bmal1*-KO mouse they disrupted the circadian clock activities in cartilage tissues and as a result found progressive degeneration and lesions in knee articular cartilage from age 2 months on and by 3–6 months this had resulted in severe lesions. Furthermore, with RNA-Seq they screened for possible mechanisms to explain how clock disruption results in OA. With this technique they found that TGF-β receptor ALK1 (Acvrl1) levels were rhythmic in WT mice, but constantly upregulated in the conditional Bmal1-KO mouse at night, while ALK5 (Tgfbr1) levels remained unchanged. This suggested a change to increase in the Alk1/Alk5 ratio which has previously been identified as catabolic for cartilage and associated

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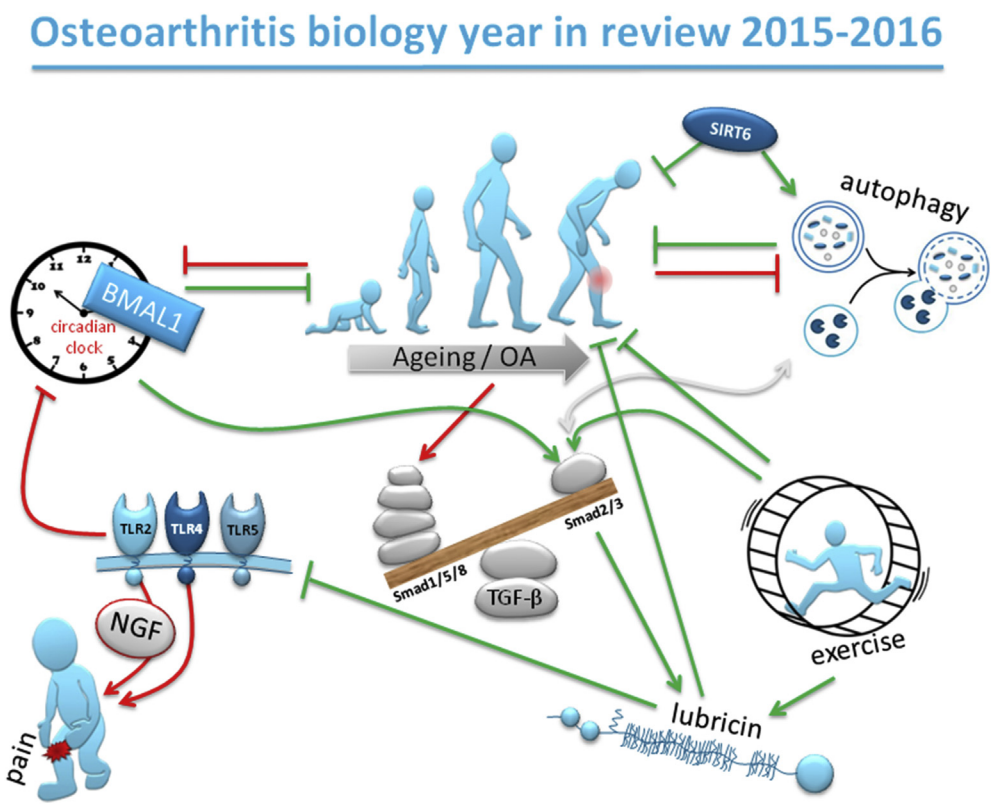

Fig. 1. Scheme of the osteoarthritis biology year in review 2015–2016. This is a simplified scheme of the connections between findings reported in studies selected for this year in review. Connections indicated in green have a positive outcome on OA, whereas those in red have a negative outcome. Arrows indicate stimulation, blocked lines indicate inhibition. This scheme is simplified in such a way that aging and OA are in one frame, but not necessarily connected. Content of this scheme is summarized in the conclusion section of this review.

with OA[4]. This was further confirmed with corresponding downstream alterations: decreased Smad2 but increased Smad1/5 phosphorylation as well as altered expression of downstream markers in conditional Bmal1-KO. In addition, Nfat7c2, which was recently identified as a key chondrocyte transcriptional marker for healthy cartilage, was also reduced in the conditional Bmal1-KO.

Guo *et al.* studied the function of the core Clock/BMAL complex in chondrocyte-like cells and cartilage from a Cry1-luc mouse, which is a reporter for circadian rhythm. They found that addition of the pro-inflammatory factors IL1β or LPS, but not the addition of TNFα, disturbed the circadian rhythm by dampening it. This was reversible by adding the anti-inflammatory and synchronizing agent dexamethasone, but not by other synchronizing agents. Mechanistically it was shown that IL1β disrupted the Clock/Bmal1 complex in an NFκB dependent manner. Strikingly, the inhibitory effect of IL1 and LPS was not observed in tissue explants from lung or esophagus, suggesting perhaps a cartilage-specific mechanism.

Together these studies show that the genes involved in the circadian rhythm, in particular Bmal1, is important for cartilage maintenance and that disruption thereof, which can be achieved by a pro-inflammatory environment, could be involved in OA. Downstream of this circadian rhythm disruption lies the disturbance of cartilage-maintenance factors Nfatc2 and TGF-β signaling.

TGF-β signaling

This year many researchers have focused on modulating TGFβ signaling as a potential therapeutic application in OA. As stated before, TGF-β can signal via multiple pathways. The receptors bound by TGF-β govern the downstream signals, hence ALK5 binding leads to Smad2/3 signaling which is associated with a beneficial, maintenance effect on cartilage, whereas ALK1 binding leads to Smad1/5/8 signaling ultimately driving factors associated with chondrocyte hypertrophy. Jeffries *et al.* investigated which pathways and upstream regulators are shared by differentially methylated genes in both OA subchondral bone and cartilage[5]. Strikingly, TGF-β was on top of that list, followed by TNF and p53, further establishing TGF-β as a key player in OA. Xie *et al.* blocked TGF-β systemically by i.p. injection with a neutralizing antibody (1D11) and found that it attenuated ACLT-induced OA in mice[6]. The antibody treatment resulted in decreased thickness of calcified cartilage, reduced proteoglycan loss, slowed degeneration of articular cartilage and improved subchondral bone structure in ACLT mice as compared to vehicle-treated ACLT mice. It is important to note the author statement that the high dose of the antibody resulted in thinner hyaline cartilage and proteoglycan loss, indicating that a certain level of TGF-β should remain in order to preserve bone and cartilage integrity. Interestingly, the levels of phosphorylated Smad2/3, which are indicative of active TGF-β signaling, were reduced in ACLT cartilage and increased in subchondral bone. Treatment with the antibody resulted in reduced phosphorylated Smad2/3 in the subchondral bone, whereas in ACLT cartilage it resulted in less pronounced reduction than ACLT alone. Unfortunately, the authors did not comment on a potential mechanism.

Zhao *et al.* focused on cartilage degeneration and excessive subchondral bone formation in spontaneous OA in Dunkin-Hartley guinea pigs[7]. Doing so, they confirmed earlier findings: a switch in the expression of phosphorylated Smad2/3 to Smad1/5/8 in degenerating cartilage during chondrocyte terminal differentiation.

They also linked the excessive osteogenesis in subchondral bone to activity of TGF-β.

Mori *et al.* observed that knocking out Smad3 resulted in abnormalities in condylar subchondral bone in mandibular condyles[8]. In the cartilage they found Smad3 knock out resulted in loss of cells and proteoglycans, accompanied by a decrease in collagen type 2 and aggrecan and increased MMP13 and MMP9 levels.

Not only in mature cartilage, but also during chondrogenesis blocking of TGFβ/Smad3 proved to have deleterious effects. Huo *et al.* investigated miR-193b and found that it repressed TGFβ2, TGFβR3 and Smad3 phosphorylation and might inhibit early chondrogenesis via this mechanism as well as regulate inflammation via repression of TNFα[9]. The publication of Li *et al.* is in line with the above mentioned studies, where they found that miR-16-5p was upregulated in human OA cartilage samples when compared with healthy controls[10]. Functional analysis showed that miR-16-5p inhibited Smad3 expression, while reducing expression of collagen type II and aggrecan and inducing MMPs and ADAMTS. Huang *et al.* showed that a Smurf2 deficient mouse has milder spontaneous OA as well as DMM-induced OA[11]. Smurf2 is an E3 ubiquitin ligase that inhibits TGF-β signaling by promoting degradation of receptor-Smads1, -2 and -3 as well as of TGF-β receptors. Using immature murine articular chondrocytes (iMAC) from the Smurf2 deficient mice, they found an enhanced expression of chondrogenic genes Sox9, Col2, and Acan, both with and without TGF-β treatment. When stimulating with IL1β, these genes were suppressed, but Sox9 remained high in the Smurf2 deficient cells. Thus lack of inhibition of TGF-β signaling with Smurf2 seems beneficial for chondrocytes. Important to consider here is the fact that the authors also found higher levels of Smurf1 upon TGF-β treatment of the smurf2 deficient iMACs compared with controls. This could compensate for Smurf2 and thereby potentially modulate rather than inhibit the signal: Smurf2 mainly inhibits Smad2, whereas Smurf1 inhibits Smad1/5.

Combined these studies show that interfering with certain parts of TGF-β signaling could be a target for therapy in OA, but it should be tightly controlled as too little or too much can result in opposing effects.

Autophagy

Autophagy is a protective mechanism of a cell to survive in times of nutrient starvation, and is characterized by a cellular lysosomal degradation pathway. It enables a cell to adapt to stress by degradation of cellular proteins and organelles to suppress damage, maintain metabolism and promote cellular viability and fitness[12].In turn, autophagy has been found to play an important role in chondrocyte homeostasis and osteoarthritis. Using GFP-LC3 transgenic mice, Carames *et al.* could quantify autophagosomes in chondrocytes and found basal autophagy activation in young mice, but with age a reduction in the total number of autophagic vesicles per cell was found as well as a reduction in the total area of vesicles per cell, indicating that the vesicles were also smaller[13]. Major autophagy control genes *Atg5* and *Lc3* were also drastically reduced with age in cartilage in mice, and expression of these genes was negatively correlated to a reduction in cartilage cellularity and an increase in apoptosis. Remarkably, loss of atg5 and lc3 preceded the age-dependent structural damage to cartilage. Taking this one step further, Bouderlique *et al.* conditionally knocked out one of these genes, Atg5, in cartilage, in a col2-cre dependent manner[14]. This resulted in an increase in TUNEL staining, indicating increased cell death as well as an increase in caspase3 and caspase9 positive cells in cartilage which are markers of apoptosis. Moreover, loss of atg5 resulted in an increased OARSI score in age-related OA. Strikingly, this increase in OA was not observed in the mechanically induced MMT model, indicating that autophagy especially plays a role in an age-specific, not trauma-specific mechanism of OA. Taking the reverse approach, Zhang investigated a repressor of autophagy, namely mTOR[15]. They showed that mTOR was increased in human OA cartilage compared with normal cartilage, and also in surgically-induced OA in dogs (model) and mice (DMM). When mTOR was knocked out in a cartilage-specific manner, this resulted in a reduction in the OARSI OA score. The cartilage had an increase in autophagy genes including *ULK1*, *LC3B* and *ATG5* 10 weeks post surgery and *MMP13* expression was reduced. The protective effect of mTOR knockout mice was not limited to cartilage, but also resulted in a reduction in fibrosis. As TGF-β is involved in fibrosis, the authors investigated phosphorylated Smad3 in synovium and found that it was reduced in mTOR knockout mice, underlining the interplay between TGF-β signaling and autophagy.

SIRT6

Autophagy and senescence are distinct cellular processes in response to stress, but are functionally intertwined[12]. Senescence is the irreversible exit out of the cell cycle by a cell and is increased during OA. Nagai *et al.* investigated the role of sirtuin 6 (SIRT6) in human chondrocytes and found that depletion of SIRT6 causes cellular senescence as well as DNA damage and telomere dysfunction[16]. SIRT6 was found in normal as well as OA human chondrocytes and using siRNA for SIRT6 reduced SIRT6-dependent upregulation of MMP1 and MMP13, reduced proliferation and induced an accumulation of ϒH2AX foci and TIFs, which are measures for DNA and telomere damage respectively. Whereas Nagai *et al.* inhibited SIRT6, Wu *et al.* overexpressed SIRT6 and found that this suppressed cellular senescence, supporting the findings of Nagai *et al.* that SIRT6 plays an important role in cartilage homeostasis[17]. Wu *et al.* showed that SIRT6 was reduced in knee cartilage from OA patients compared to normal, and that SIRT6 expression declined with advancing age in murine cartilage. This decline in SIRT6 protein correlated with cellular senescence and by overexpression of SIRT6 replicative senescence of chondrocytes could be suppressed. Interestingly, exposing chondrocytes to IL1β reduced SIRT6 expression, indicating that inflammation regulates SIRT6 expression, and resulted in MMP13 upregulation. Strikingly, this IL1β-induced MMP13 upregulation could be reversed by overexpression of SIRT6, however, via a yet unclear mechanism; SIRT6 significantly attenuated the expression of NFκB dependent genes but did not affect NFκB-p65 nuclear translocation. Also *in vivo*, sirt6 overexpression appeared protective against OA. When inducing mechanical OA by transsection of the medial collateral ligament and medial meniscectomy, overexpression of SIRT6 resulted in reduced OARSI scores indicating a protective effect of SIRT6.

Ailixiding *et al.* sought to investigate the role of SIRT6 in the crosstalk between aging and metabolic syndrome/OA and therefore used a SIRT6 haploinsufficient mouse[18]. Feeding these mice a HFD resulted in a significantly higher blood glucose level in the SIRT6 haploinsufficient mice compared to control mice, indicating susceptibility to metabolic syndrome. SIRT6 haploinsufficiency resulted in cartilage clefts and proteoglycan loss by 9 months of age, enhanced Collagen type X expression (deep zone), increased MMP13 expression (superficial zone) and osteophytes. In addition, synovial hypertrophy accompanied by infiltrating inflammatory cells was observed. HFD resulted in OA features in both WT and SIRT6 haploinsufficient mice in which the SIRT6 haploinsufficient mice showed a further enhanced osteophyte formation in HFD conditions, which was associated with enhanced synovial hypertrophy. When using a *Prx1*-Cre; Sirt6$^{f/f}$ mouse, which lack SIRT6 only in their limbs, a marked reduction of cartilage proteoglycan

content was observed at 6 months of age compared to controls. Strikingly, these mice did not show the enhanced Collagen type X or MMP13 expression that was observed in the haploinsufficient mice. When investigating TUNEL staining, the SIRT6 haploinsufficient mice showed an increased apoptotic cell number, but the *Prx1*-Cre; $Sirt6^{f/f}$ mice did not.

Overall these studies show that SIRT6 can have protective effects during OA which might be due to SIRT6 effects on inhibition of senescence.

The extracellular matrix matters

Chondrocytes are pivotal in maintaining a healthy cartilage extracellular matrix, but the vice versa also holds true. Kim *et al.* showed that during OA there is an increase in matrix/collagen cross linking enzymes[19]. These cross linking enzymes ultimately resulted in a stiffer matrix and this stiffer matrix greatly affected mechanotransduction. They showed that in a stiffer matrix, chondrocytes expressed more MMP's and ADAMTS' whereas SOX9, COL2a1 and Aggrecan expression was reduced. This indicated that a stiffer matrix could actually contribute to OA, but the proof came from an experiment where the cross linking enzyme LOX was inhibited during a DMM experiment, which resulted in an improvement of cartilage integrity.

Exercise

Besides investigating molecular and cellular processes that can benefit OA patients by pharmacotherapeutic interventions, there are interventions patients can do for themselves like exercise. Even though exercise might be beneficial, patients that have OA and are in pain might be afraid to load their joints, believing that it may cause damage. This year substantial evidence was added to show moderate exercise can prevent loss of crucial joint tissues due to OA.

Iijima *et al.* used rats that underwent DMM surgery and assigned them to a sedentary or walking group that had access to a treadmill[20]. The walking group was divided into groups with a different duration of access to a treadmill: treadmill access throughout the entire 8 week experiment, treadmill access during the first 4 weeks out of a total of 8 weeks, or treadmill access during the last 4 out of a total of 8 weeks. All rats that underwent DMM and were assigned to any of the walking groups showed an improvement on all outcomes as compared to the sedentary DMM group. In cartilage the walking groups had a lower OARSI score, more Toluidine blue staining, and more collagen type II staining. On bone parameters the walking groups showed reduced subchondral bone cysts, prevention of subchondral bone sclerosis, reduced osteocyte death and a decreased number of TRAP and an increased number of ALP positive cells. The group that had access to the treadmill during the last 4 weeks out of a total of 8 weeks performed slightly better than the other walking groups. Rats in all walking groups showed enhanced BMP2 and -6 staining in cartilage and bone, which the authors proposed as a mechanism behind the protective effects.

Exercise and lubricin

One way exercise can improve cartilage function, is by the induction of lubricin, also known as proteoglycan 4 (PRG4). Musumeci *et al.* investigated the effect of physical exercise in rats in healthy young, adult and aged rats and whether lubricin could be used as a new marker for chondrocyte senescence[21]. They found that lubricin was highly expressed in chondrocytes of healthy young rats and that this declined with age. However, if the rats were subject to exercise, the reduced lubricin expression was at least in part restored. Another study investigated the potential therapeutic application of lubricin by using recombinant lubricin in ovariectomized (OVX) rats as a model for osteoporosis-related OA[22]. They gave the lubricin either early, at the day of OVX, or late at 2 weeks after OVX. The OVX resulted in loss of proteoglycans and increased the calcified cartilage zone, increased MMP13 and collagen type X expression as well as enhanced TRAP and OSX staining. All of this was counteracted by treatment with recombinant lubricin. This was also reflected by the data on bone as the lubricin treatment normalized the subchondral bone architecture, which was especially clear on the bone volume/tissue volume (BV/TV value), which was restored to normal. Also CD31 immunohistochemical staining, indicative of angiogenesis, was reduced to normal levels by early lubricin treatment and drastically decreased in late treatment groups. Similar to the paper of Iijima *et al.* late treatment of OVX rats with lubricin seemed to perform slightly better than early treatment reflected by improved OARSI scores. However, this did not hold true for all parameters. For example, early lubricin treatment performed better in decreasing MMP13 and ColX expression in cartilage, decreasing TRAP and OSX in bone and reducing vessel area in the calcified cartilage.

Of note, recombinant lubricin induced lubricin expression suggesting a positive feedback loop.

Lubricin and TLR's

Apart from its lubricating function, Lubricin has been found to have anti-inflammatory effects. Alquraini *et al.* investigated the mechanism behind this, and found that lubricin can bind to both the pro-inflammatory receptors TLR2 and TLR4 in HEK cells and by doing so could dose-dependently inhibit activation of these receptors, even in conditions with OA and RA synovial fluid[23]. Iqbal *et al.* investigated a similar mechanism, but added an *in vivo* study on OA and pain[24]. They found that lubricin could bind to TLR2, 4 and 5, resulting in NFκB activation, which ultimately led to a change in signaling and therefore an altered cytokine/chemokine secretion, for example Lubricin decreased NFκB translocation in the presence of LPS or flagellin. In a rat DMM model Iqbal *et al.* showed that lubricin treatment maintained cartilage integrity, reduced osteophyte formation and reduced synovial inflammation as well as expression of inflammatory cytokines. Strikingly, with the rat grimace scale the authors observed a reduction in pain in the lubricin-treated mice as well. To investigate whether the pain reduction could be TLR-lubricin dependent, the authors investigated NFκB expression and found that it was limited in a normal joint, but upon injury NFκB expression and translocation was observed. Upon lubricin injection this NFκB expression and translocation was however inhibited to the level of non-injured joints.

TLR's and pain

The link between TLR's and pain has also been made by Miller *et al.* They investigated TLR4 from the angle of DAMPs being involved in pain by performing a DMM in TLR4 null mice[25]. They showed that dorsal root ganglion (DRG) neurons expressed TLR4 and that exposure of DRG cultures to TLR4 ligands S100A8 and α2-macroglobulin resulted in release of the proalgesic chemokine MCP1 and increased intracellular calcium concentrations. Blocking of TLR4 could fully prevent this response. The intact DRG's also responded to the TLR4 agonist LPS. One would expect, given these findings that this would translate to *in vivo* results of TLR4 blockage on pain as well. However, the TLR4 null mice were not protected from mechanical allodynia, nor from joint damage. In the TLR4 null mice there were still a few neurons that responded to S100A8 and α_2-macroglobulin, indicating that other pathways, including RAGE,

may also facilitate responses to DAMPS. The authors also include TLR2 and pathways driven by proalgesic cytokines and chemokines as hypothetical causes of the mechanical allodynia in DMM in the TLR4 null mice. Krock *et al.* found that TLR2 was a regulator for NGF in human intervertebral discs, underlining the possibility of multiple mechanisms leading to pain via TLRs[26].

Pain vs structural damage, and NGF

The link between pain and structural damage in OA has been an enigma. OA leads to pain, but the pain does not seem to correlate with the actual structural damage in many studies. Kc *et al.* investigated PKCδ null mice in OA as PKCδ mediates cartilage damage[27]. They saw a highly significant reduction in structural damage in DMM in the PKCδ null mice, yet the pain was increased compared to WT DMM mice. Here structural damage and pain not only lacked a correlation, but even deviated in opposite directions. The authors found that this could be explained by the PKCδ mice having enhanced expression of both TrkA and NGF and, as a result, augmented axonal outgrowth.

On the other hand, O'Dricsoll *et al.* investigated cartilage, meniscus and bone of mice undergoing partial meniscectomy or DMM at the point in time where pain was experienced[28]. They found that NGF was consistently upregulated in the articular cartilage. They identified that NGF and tachykinin could be upregulated in articular cartilage by mechanical injury in a TAK1, FGF2 and Src-kinase dependent manner. In contrast to Kc, here structural damage was linked to pain.

It is now well known that NGF is a key player in OA pain. Several researchers have focused on the benefits of blocking either NGF of NGF signaling, via TrkA. Nwosu *et al.* showed that blocking TrkA in rats in either MIA or MNX inhibited pain behavior, as measured by weight bearing asymmetry[29]. In dogs with degenerative joint disease, Lascelles *et al.* showed that anti-NGF treatment resulted in enhanced activity[30]. Also in rats, Ishikawa *et al.* showed blocking NGF with an antibody led to an improvement in gait, even long after a single dose injection[31].

Conclusion

We selected these manuscripts based on the pubmed search, presented abstracts during the 2015 conference and interconnectivity into one scheme (Fig. 1). We started with the circadian rhythm, which is a relatively new subject to the field of OA. Clock genes were disturbed during aging and OA and vice versa disturbing the circadian rhythm induced OA pathology. Mechanistically the data pointed toward Bmal1 as a pivotal factor in this process. The expression of Bmal1 was reduced by IL1. Disruption of Bmal1 leads to disturbance of Nfatc2 and TGF-β signaling. Many authors showed that altering TGF-β signaling affected OA, pointing towards a disruption in the ALK1/ALK5 signaling balance. TGF-β clearly required tight control as too little as well as too much resulted in deleterious effects. There was a link between autophagy and TGF-β as knock out of autophagy inhibitor mTOR reduced Smad3 phosphorylation, which is downstream of ALK5. Autophagy control genes Atg5 and Lc3 were drastically reduced in aging cartilage and knocking out Atg5 induced OA-like changes. Strikingly this only held true in an age-dependent model, not a mechanical DMM model. In the mTOR knock out mouse, however, the DMM did show reduction in OA. Autophagy and senescence are closely linked and functionally intertwined. SIRT6, which was reduced in OA, was protective against cellular senescence and its overexpression led to protection against OA.

A hot topic this year was exercise. From several angles it was shown that exercise had a positive effect on several OA parameters in mice. During the 2016 OARSI conference it was presented that lack of loading results in decrease TGF-β signaling via Smad2/3, which links exercise to the other studies[32]. A mechanism of exercise that was researched by several groups was the increase in lubricin upon exercise. Lubricin was found to bind to TLR's and thereby inhibit their activity. Lubricin treatment itself had a positive effect, not only on pathology in OA, but also on pain, which was TLR-dependent. The link between TLR's and pain also became clear in TLR4 null mice, which clearly showed a reduction in many proalgesic factors in the DRG of DMM-mice. However, this did not lead to a reduction in mechanical allodynia. This could lie in the type of pain that was measured or via other mechanisms, like TLR2, which is currently under investigation. TLR2 was found to upregulate NGF. Blocking of NGF has so far consistently been able to block pain in OA. In a study using PKCδ null mice, which had a significant reduction in DMM-induced pathology, pain still persisted in an NGF-dependent manner. This study shows that the damage-pain disconnect could lie in the levels of NGF. In an OA-unrelated manuscript it was shown that TLR's are involved in disrupting the circadian clock via disruption of Bmal1, which will most likely hold true for OA as well.

This review tied together a selection of seemingly distant manuscripts on the same disease: osteoarthritis. By doing so, this review underlines that we are all working together on solving the big OA-puzzle, one piece at a time (Fig. 1).

Author contributions

ENBD, APMvC and PMvdK performed the literature search for this manuscript. ENBD and APMvC structured the selected manuscripts into an underlying scheme that formed the framework for this manuscript. ENBD wrote the manuscript based on this framework, which was edited by all authors into the final manuscript.

Conflict of interest

The authors have no competing interests.

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