

Circadian Clock: Potential Role in Cartilage Integrity and Disruption

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ABSTRACT

Circadian rhythms and clocks driven by light/dark cycles over a 24-hour period control many bodily functions. Likewise peripheral clocks generate circadian cycles, and at the cellular level circadian clock genes are involved in tissue specific functions. This report highlights articular cartilage clock genes and how their dysfunction may be implicated in osteoarthritis. To address the question of whether disruptions in cartilage clock genes are possible risk

factors for osteoarthritis, research studies were examined and their conclusions noted. While more work is needed the role of cartilage clock genes and their interaction with local cartilage tissue metabolic processes in human subjects should prove highly insightful.

Key words: Cartilage; Circadian rhythms; Clock Genes; Osteoarthritis

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BACKGROUND

Osteoarthritis, a focal or oftentimes a widespread joint disease affecting two or more joints continues to be the number one premature disabling of enormous numbers of young, middle-aged and older adults across the globe. A disease with no known unique cause, and no clear pathological course, osteoarthritis, mostly assumed to be due to degeneration of the articular cartilage lining of one or more freely moving joints is a tissue isolated from any blood or nerve supply and is dependent on movement for maintenance of its structural composition. Very sensitive to anabolic and catabolic processes and disruptions in these, very little attention has been given to research directed towards understanding all aspects of this important tissue, especially factors affecting its homeostasis. In line with catastrophe and chaos theory, implicating small changes that lead to larger distinct changes, as well as the importance of the chondrocyte for fostering cartilage health, plus evidence of a role for the clock genes in the dysregulation of cartilage modeling and viability, this brief examines whether small changes in cartilage clock gene function or structure can predictably produce or induce one or more of those pathological features commonly observed over time in an osteoarthritic joint, such as destruction of matrix, abnormal cell differentiation, and the production of inflammatory mediators^[1]. Limitations in interpreting the present findings and promising future

areas of study are highlighted as well.

The key question driving this review was whether signaling mechanisms that reside in articular cartilage chondrocytes are impacted by the genes that show circadian rhythmic activity if these are inhibited, removed, rendered ineffectual, or altered in some way, and whether small changes or disturbances can account for the profound far reaching effects associated with cartilage destruction.

METHODS

Using the key word *Clock Genes* and *Articular Cartilage*, or *Osteoarthritis*, full length peer reviewed English language articles covering the time periods January 1, 2013-June 18, 2018 were sought using Scopus, PUBMED, Web of Science 5 data bases site, and Science Direct, along with other supporting documents. Those papers deemed most salient in addressing the study questions were downloaded, scrutinized and taken as representative of the work done in this area. Excluded were papers discussing joint structures other than articular cartilage, such as muscle or bone, as well as diseases other than osteoarthritis. All forms of research as well as relevant research reviews were deemed acceptable.

RESULTS

As a whole, the research results show the study of cartilage clock genes in the context of osteoarthritis is an area where limited research prevails, and where most work has been done on non-human primate animal models such as the rat, transgenic, knockout or normal mouse or mouse mutant models, cartilage explants cultures, human chondrocyte derived cell lines, samples of joint tissues taken from pathologically deranged joints, or non human xiphoid cartilage^[2] rather than from assays of in situ human tissues or systems or joints involved in osteoarthritis. The substrate examined in the various studies has similarly emanated from diverse sites, such as the growth plate, adult costosternal cartilage, femoral head cartilage of newborn mice^[3], the rib cage, and other diverse forms of human articular cartilage and bone tissues. Many of the available reports, while reporting similar and/or supplementary findings, emanate from the same laboratory, and require duplication or replication elsewhere. What was deemed of high relevance in the body of literature is however, articulated below.

Generic Observations

According to the prevailing literature, circadian rhythms, which are centrally-controlled cyclical processes that predominantly respond to light and dark phases in a rhythmic manner over a 24 hour time period, control key body functions. As well, peripheral tissues and various key body organs contain circadian type clocks that may be responsive to a variety of intrinsic as well as extrinsic circadian stimuli^[4], which, help to regulate the expression of output genes-termed clock-controlled genes^[5,6].

Research further shows that at the cellular and molecular levels, circadian tissue based clocks and clock genes are clearly involved in the temporal control of selected tissue specific physiological processes. It also appears that these internal molecular clocks can interact functionally with other molecules to produce cues that foster the generation or maintenance of the surrounding extracellular tissues as well as mediating other cell-specific regulatory functions^[7]. Conversely their malfunctioning or deletion has been linked to a variety of tissue specific pathologies, or an increased risk for specific pathologies such as osteoarthritis^[2]. In the context of

articular cartilage, these abnormalities include, but are not limited to, alterations in chondrocyte proteoglycan content, matrix-degrading enzymes, anabolic mediators, and joint synovial tissues^[4]. Further recent and important findings in this regard show disruptions of the circadian rhythm are linked to increases in osteoarthritis pain^[8] and that two core components of the circadian clock found in the brain and muscle contribute to the homeostasis of articular cartilage, namely the transcription brain and muscle Arnt-like protein 1 (BMAL1 or aryl hydrocarbon receptor nuclear translocator-like protein)^[9,10].

In this regard, both subnormal extrinsic factors such as locomotor activity cycles or phases of activity and their magnitude, as well as internal factors such as circulating hormones, cytokines or metabolites^[7], often associated with osteoarthritis, may impact cartilage clock circadian rhythm processes adversely, thus heightening the risk for the development of mouse knee joint osteoarthritis^[4]. Kanbe *et al*^[11] who explored key mechanical sensitivity of selected genes residing in articular cartilage found that clock and other genes functionally associated with mechanical stress do appear to play an essential role in regulating the biological metabolic rhythms of osteoarthritic chondrocytes. Thus chondrocytes of cartilage do appear to contain autonomous circadian clocks and exhibit circadian rhythms^[3] that are responsive to a variety of intrinsic and extrinsic factors and these appear highly linked to the extent of the prevailing cartilage structure and its functional properties.

Based on a variety of in vivo studies, it also appears that subtle age-associated changes and others in clock gene expression could prove disruptive to the chondrocyte clock genes or their regulatory elements, for example they could alter the period, phase, or amplitude of circadian oscillations and their role in mediating catabolic and anabolic pathways^[2]. Functionally, the local cartilage clock may also become weaker in the presence of chronic inflammation^[5], or under conditions of stress^[7].

Clock Genes

A number of reports indicate that autonomous peripheral clocks containing clock genes can be entrained by a variety of external and internal cues and that regardless of the model studied, these clock genes can be observed in samples of articular cartilage, as well as in other surrounding joint tissues^[2]. Gossan *et al*^[3] who aimed to establish the presence of a circadian clock in murine cartilage tissue along with tissue-specific clock target genes using a mouse osteoarthritis model, as well as a human chondrocyte cell line, did find both these cell structures to contain evidence of circadian clocks. It was also noted that these cartilage clocks could be reset by temperature signals. A PER2: luc bioluminescence assay further confirmed that the amplitude of aging chondrocyte circadian oscillations was significantly lower than that of younger animals. A further finding from a time-series microarray analysis of the mouse tissue used to identify the first circadian transcriptome in these cartilage samples revealed 615 genes (or approximately 3.9% of the expressed genes) did in fact display a circadian pattern of expression. While this percentage was not high, the oscillations of these circadian genes appeared highly involved in mediating cartilage homeostasis and its survival, and were hence implicated to be of potential import in the development of osteoarthritis. In support of this idea, Gosson *et al*^[3] further showed that several clock genes were indeed more likely than not to be disrupted in the early stages of cartilage degeneration in the presence of any circadian rhythm disruption, for example during aging.

According to further research by Kc *et al*^[4], a reciprocal

relationship can exist between asymmetrical or abnormal circadian responses that govern peripheral clocks in major body organs or tissues, and this may foster joint vulnerability, changes in tissue physiology, and disease status. Indeed, Haas and Straub^[12] who examined the degree of clock *Period 1* and *2* proteins and the presence of mRNA of molecular clocks from synovial fibroblasts of selected cases with osteoarthritis found the expected rhythmicity of the molecular clock protein or mRNA was not present in this disease. In support of chaos theory, the authors speculated that this situation might lead to disruption of other key cellular pathways involved in osteoarthritis such as articular chondrocyte clock genes.

In related work, Guo *et al*^[13] who set out to examine if the catabolic cytokines (Interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF α), important inflammatory mediators in osteoarthritis, affect the circadian clock mechanism and the expression of clock-controlled catabolic genes within cartilage, did find that the exposure of the cartilage clock genes to IL-1 β severely disrupted the cartilage-based circadian gene expression rhythms. This effect was reversed by an anti-inflammatory drug known as dexamethasone, but not by other clock synchronizing agents, suggesting that inflammatory cytokines, specifically the interleukins, are unique determinants of circadian clock functions that can regulate the expression of both endogenous clock genes as well as clock-controlled catabolic pathways because they can interfere functionally with the core Clock/BMAL1 complex. This effect abolishes circadian rhythms in *Cry1-luc* and *PER2: LUC* gene expression that may contribute to osteoarthritis and implies the interleukin inflammatory pathways may be an effective focus for osteoarthritis intervention or disease prevention efforts.

Work by Dudek *et al*^[9] similarly showed the expression of the core clock transcriptional activator, Bmal1, which activates the *Per* and *Cry* genes, and their transcription, among others, is significantly disrupted in human osteoarthritic cartilage as well as in aged mouse cartilage. Furthermore, targeted Bmal1 ablation in mouse chondrocytes abolished their circadian rhythm and caused progressive degeneration of the articular cartilage. It was further shown that Bmal1 directs the circadian expression of many genes implicated in cartilage homeostasis, including those involved in catabolic, anabolic, and apoptotic pathways. As well, loss of Bmal1 reduced the levels of phosphorylated SMAD2/3 (p-SMAD2/3) and NFATC2 and decreased the expression of the major matrix-related genes Sox9, Acan, and Col2a1, but increased p-SMAD1/5 levels. In addition to findings that cartilage explants cultures do produce robust circadian oscillations and that cartilage clock genes are mechanosensitive^[2], levels of Bmal1 along with numbers of BMAL1-positive chondrocytes were found to be significantly lower in human articular cartilage. These results of Dudek *et al*^[9] strongly imply there is an inherent regulatory mechanism linking chondrocyte Bmal1 to the growth and maintenance of articular cartilage, and that a prevailing circadian rhythm disruption in the cartilage core clock mechanisms is a potentially important risk factor for osteoarthritis.

As observed by Yang *et al*^[14] who studied the mRNA and protein levels of circadian rhythm genes, including nicotinamide adenine dinucleotide oxidase (NAD(+)) and the sirtuin 1 (Sirt1)-a pathway associated with aging, found the levels of both Bmal1 and NAD(+) in human osteoarthritic cartilage were significantly decreased. This apparently resulted in the inhibition of nicotinamide phosphoribosyltransferase activity and Sirt1 expression, and the knockdown of Bmal1 was sufficient to decrease the level of NAD(+) and aggravate osteoarthritis-like gene expression changes under the stimulation of IL-1 β . The overexpression of Bmal1 on the other hand, relieved the alteration induced by IL-1 β , and the transfection of Sirt1

small interfering RNA not only resulted in a reduction of the protein expression of Bmal1 and a moderate increase of period 2 (*per2*) and *Rev-Erb α* , but also further exacerbated the survival of cells and the expression of cartilage matrix-degrading enzymes induced by IL-1 β . These findings are consistent with the finding that Bmal1/Clock complex activates E-box gene sequences, and other clock regulatory elements, and can affect growth plate structure negatively, as well as endochondral ossification^[2].

Also consistent with the above findings, is data from Akagi *et al*^[11] who further examined circadian rhythmicity in cultured chondrocytes and the specific role of the circadian rhythm genes NR1D1 and Bmal1 in regulating chondrocyte functions. This group showed NR1D1 and Bmal1 mRNA and protein levels were significantly reduced in osteoarthritic cartilage. As well, a clear circadian rhythmicity was observed for NR1D1 and Bmal1 in cultured human chondrocytes, while the sequencing of RNA from chondrocytes treated with NR1D1 or Bmal1 identified 330 and 68 significantly different genes, respectively, that predominantly affected the TGF- β signaling pathway. It was concluded that the circadian rhythm pathway is dysregulated in osteoarthritic cartilage and its status affects TGF- β signaling, a central pathway in cartilage homeostasis. Clearly, one or more of these circadian disruptions could produce local molecular clock and its downstream target changes, and/or the expression of key cartilage regulators^[3]. However, why BMAL1 suppression reduces osteoarthritis related pain^[8], and whether other factors produce a similar result, and to what degree global dysregulation versus local circadian rhythm disruptions influence human articular cartilage chondrocyte functions, and why Bmal1 knockout mice show progressive articular cartilage damage, but no bone or synovial tissue damage^[9], awaits further study.

DISCUSSION

Many different explanations and theories as to the origins of osteoarthritis disability prevail, with few accounting for the observed changes in the entire joint, as well as systemic correlates, or arriving at any firm or testable conclusion. The present review concerning articular cartilage clocks and osteoarthritis interactions represents a relatively new body of thought and research that focuses on the role of clock genes in mediating articular cartilage physiology and metabolism, as a potential disease correlate. This small but growing body of literature extends what we know about osteoarthritis risk factors by revealing that 24-hour circadian rhythms that control human biology through central and peripheral clock mechanisms, and which are sensitive to various rhythmic behaviors, including locomotion, appear to be involved peripherally in controlling articular cartilage physiology, and may hence play a key or significant role in mediating or moderating this disease^[5]. In addition clock genes other than those resident in the chondrocytes of articular cartilage, the tissue most affected by osteoarthritis, such as the bone, muscle, and surrounding tendons^[2], may be disrupted for various reasons with potentially dire consequences for the articular cartilage tissue^[4], and this accords with the view that osteoarthritis is a disease of the entire joint, rather than only the articular cartilage.

Inflammation, and subnormal mechanical and environmental stimuli, as well as cytokines such as TNF- α associated with osteoarthritis can similarly impact cartilage clock gene functions and cartilage homeostasis with immense adverse ramifications^[1]. In particular, loss of the chondrocyte clock gene, BMAL1 or alterations in its circuitry reportedly invokes severe internal derangement of cartilage tissue consistent with more generalized osteoarthritis

models^[9]. This clock gene does seem very important in efforts to understand osteoarthritis pathology, because research shows its disruption can affect both the positive as well as the negative limbs of the cartilage circadian rhythm pathway^[1], with both changes having the ability to induce an adverse disease state and a limited tissue regenerative capacity. Moreover, its malfunctioning is said to be able to produce a globally negative functional impact that can significantly influence the functioning of the entire circadian rhythm pathway^[1], which is responsible for maintenance of many bodily functions. Importantly, the down regulation of the cartilage clock gene *Bmal1* may be due to both intrinsic as well as extrinsic causes outside the circadian loop. As well, its positive local regulators known as RORC and RORA can induce down regulation of the entire circadian rhythm pathway, and additional *Bmal1* down regulation^[1], which suggests this is an important focal point worthy of future investigation.

Thus, while many potential risk factors for osteoarthritis have been discussed and examined for some time to no conclusive avail, and contrary findings by Agaki *et al*^[1] and Chaturverdi *et al*^[15] await resolution, the presently observed connection between circadian rhythm disruptions of one or more critical biorhythms and between peripheral clocks^[16] and the molecular clock system and osteoarthritis, especially cartilage clock gene *Bmal1* levels do seem to provide a relevant perspective, and one that aligns with prior research observations, and should not be overlooked in future efforts to unravel osteoarthritis disease origins.

As per chaos theory, the emerging data concerning the salience of autonomous circadian clocks and inflammatory factors^[12] and/or the environmental disruption of circadian rhythms that appear to predispose to osteoarthritic-like damage^[9] strongly suggest that even minimal disruptions in any related pathway can lead to significant long term irreversible pathology that may spread to other joints. The presently reviewed preclinical as well as human data further reveal related data that is very consistent with an aging, mechanical, and inflammatory set of key risk factors for articular damage as a result of circadian clock disruptions, even though this data is clearly limited in scope, depth, human substrates, and lacks replication.

Of possible very high relevance is additional emergent data from several studies that shows the core clock gene *Bmal1* found in the chondrocyte of articular cartilage, among others cells, is strongly implicated in a variety of those cartilage regulatory processes, as well as regulatory processes in bone and muscle. *Bmal1* is also susceptible to pro-inflammatory factors that can induce chondrocyte DNA damage or dysfunction that is not isolated to any specific zone or articular cartilage site. The reversal of this inflammatory effect, not evident in other cells, specifically suggests this is a unique cartilage-specific mechanism, and a possible important therapeutic target that may help to offset chondrocyte inflammatory damage that can in turn, alter central and peripheral autonomous oscillations^[16], as well as inducing a spiral of chondrocyte DNA damage or dysfunction^[10] or an imbalance between negative and positive oscillations of cartilage target genes^[16] that is hard to reverse generally.

Other more recent studies have shown over-expression of both the *Bmal1* chondrocyte gene as well as *Sirt1* can restore the metabolic imbalance of chondrocytes caused by IL-1 β , observations that further suggest *Bmal1* is a key clock gene involved in cartilage homeostasis and is mediated through *sirt1*- a recognized osteoarthritis correlate. Related observations by Cho *et al*^[17] showed a profound existing connection between *BMAL1* and the REV-ERB- α and REV-ERB- β genomic regulatory circuits that was taken to show these genes to be highly linked in the context of both clock and metabolic functions, and which could be readily disrupted as verified in double-

knockout mice. These double-knockout mice showed markedly altered circadian wheel-running behaviour and deregulated lipid metabolism, both factors speculated to increase risk for osteoarthritis and articular cartilage damage. These data linking REV-ERB- α and REV-ERB- β with PER, CRY and other components of the principal feedback loop that drives circadian expression were taken to indicate the existence of a general highly integrated mechanism for the coordination of circadian rhythm and metabolism that can potentially mediate or moderate cartilage metabolism. In addition, the data further implied that efforts to manipulate circadian rhythm gene expression or to examine the nature of these feedback loops more closely in health and disease may yield novel insights into strategies to offset osteoarthritis, as well as highlighting unexplored aspects of the nature of cartilage diseases such as osteoarthritis^[14].

Additional novel observations by Chaturverdi *et al*^[15] should also be acknowledged and followed up in this respect. This group found that among the 48 known nuclear receptors, REV-ERB- α , a transcription repressor, was the most highly expressed in osteoarthritic cartilage, but that treatment of articular chondrocytes with known catabolic agents resulted in its induction, whereas stimulation with anabolic agents led to a decrease in its expression. The overexpression of the nuclear receptor was associated with an increase in the expression of matrix-degrading enzymes such as matrix metalloproteinase 13 and aggrecanase. In contrast, a decrease in REV-ERB- α expression led to a concomitant reduction in the activity of matrix-degrading enzymes.

Hence, while this review is not a systematic one and may have omitted some contrary research, the bulk of the carefully selected literature implies that the status of a synovial joint can be mediated or moderated by an array of factors including cellular clocks and clock genes within articular cartilage chondrocytes, which seem tissue specific^[18]. In turn, circadian rhythms produced by articular cartilage core clock genes can impact the turnover of local as well as adjacent musculoskeletal tissue constituents, while simultaneously impacting the extent and rhythmicity of the master circadian clock, as well as fostering intractable bouts of pain and inflammation (See Table 1 and Figure 1).

In turn, efforts to optimize the day to day functioning of the master clock that controls overall circadian rhythms may both foster more optimal clock gene functions with respect to cartilage maintenance and regeneration, gene transcription and signaling mechanisms, as well providing more appropriate biological oscillations, in general. As well, it seems the careful administration of specific anti-inflammatory agents, sound joint protection strategies, appropriate exercise schedules and intensity, stress reduction, and assistive devices in cases of ligament laxity or tendon damage may prove helpful in combating osteoarthritis pathology. The importance of bone health, and minimizing inflammation should also be stressed. Importantly it does seem that in cases with osteoarthritis, fewer clock cartilage genes are likely to be evidenced, so taking precaution against this disease or its progression as early as possible appears to be a well-founded potential intervention strategy.

Limitations to these prevailing data clearly preclude definitive conclusions and assertions, however. They include issues such as limited sample sizes, unknown impacts of the sampling method, the timing of the sample collection[eg is there a problem if explants are studied after the individual or animal model is deceased], the exclusion of light stimuli, which constitute an important component of the circadian pathway in man, the varied sites studied, and overall varied research questions and methodologies, among others. Thus caution is advised in interpreting the prevailing findings.

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Table 1 Key findings of articular cartilage clock gene studies and linkages to osteoarthritis.

| Authors | Substrate | Methods | Findings | Conclusion |
|------------------------------------|--|---|--|---|
| Agaki <i>et al</i> ^[11] | Cultured chondrocytes from normal and osteoarthritic human knee cartilage | The researchers examined circadian rhythmicity in RNA and protein expression using bioinformatic analyses and quantitative polymerase chain reaction assays | NR1D1 and BMAL1 mRNA and proteins were reduced in the osteoarthritic cartilage | The circadian rhythm is dysregulated in osteoarthritic cartilage. Interference with circadian rhythmicity in cultured chondrocytes affects TGF-Beta signaling-a central component of the cartilage homeostasis pathway. The genes NR1D1, BMLHE40, and BMAL1 in osteoarthritic tissue differed most from that of normal cartilage. |
| Dudek <i>et al</i> ^[9] | Aged and young mouse and human cartilage tissue and chondrocytes | Live tissue explants cultures and bioluminescence were carried out along with histology and IHC, PCR mRNA assays, immunofluorescence, | Core clock transcription factor BMAL1 is disrupted in osteoarthritic cartilage. Reduction of NR1D1 and BMAL1 in occur in osteoarthritic cartilage is a response to osteoarthritic damage, independent of age | BMAL1 directs circadian rhythm of many genes that control cartilage homeostasis. Bmal1 knock out mice cartilage shows an overall shift to a catabolic state. Two key pathways controlling cartilage homeostasis -TGF-Beta nuclear factor of activated T cells, and cytoplasmic 2 were found dysregulated |
| Gossan <i>et al</i> ^[3] | Mouse cartilage tissue explants from aged and young animals and human chondrocytes | Time series micro arrays were used to identify genes expressed in a circadian manner | Mouse cartilage as well as human chondrocytes contain molecular circadian clocks | An autonomous circadian clock in chondrocytes can be implicated in aspects of cartilage biology and pathology |
| Kanbe <i>et al</i> ^[11] | Mouse chondrocytes | Cultured chondrocytes were subjected to mechanical stress | Clock and other genes responding to mechanical stress regulate chondrocyte metabolism in osteoarthritic cartilage | Clock genes have a role to play in maintaining the normal metabolism of chondrocytes |
| Yang <i>et al</i> ^[14] | Osteoarthritic cartilage from femoral condyles of knee osteoarthritis cases awaiting surgery and cartilage from disease free cases | Chondrocyte cultures were analyzed | Levels of BMAL1 and NAD decreased significantly in the diseased cartilage | BMAL1 is a key clock gene involved in cartilage homeostasis |

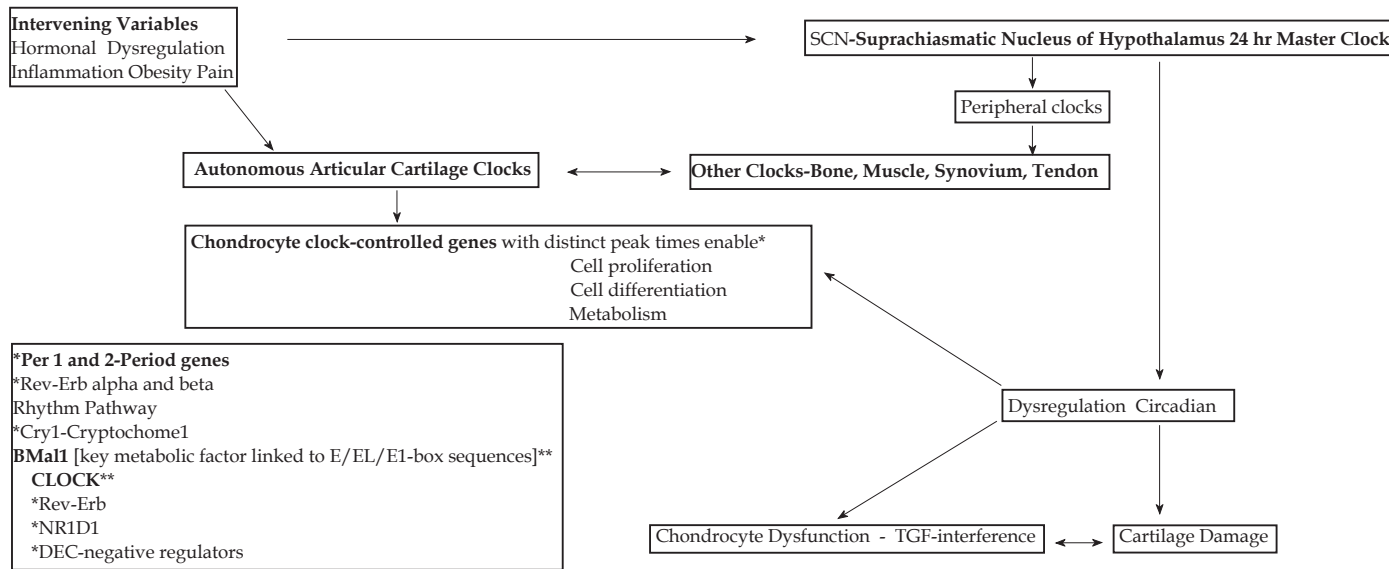


Figure 1 Hypothetical depiction of osteoarthritis disease pathways, and their interactions and key points of clock gene relevance. Adapted from^[1,7,9,14,16,22,24-27,30], ** = most important cartilage clock genes.

In the interim, the bulk of the presently carefully selected literature clearly shows the status of a synovial joint can be readily mediated or moderated by an array of factors including cellular clocks and clock genes within articular cartilage chondrocytes^[22], which seem tissue specific^[18]. In turn, disturbances of circadian rhythms produced by core clock genes can invoke significant health disturbances such as pain and inflammation. However, efforts to maximize the functioning of cartilage clock genes, as well as protecting their complex feedback mechanisms appear to provide potential intervention points for preventing or alleviating osteoarthritis pathology, an hypothesis that warrants further exploration.

CONCLUSION

Osteoarthritis (OA), a prevalent progressively disabling joint disease that is not reversible, nor readily responsive to any known form of treatment, may be influenced negatively by multiple risk factors, such as aging, and its impact on clock genes, progressive damage and loss of articular cartilage, the key involved tissue in this disease. However, despite their putative role in regulating the matrix extracellular constituents that constitute articular cartilage, the role of the chondrocyte clock genes that have been identified in cartilage is not well-established in the mainstream literature, and awaits further study^[19]. In particular, the role of central circadian disruptions as well as alterations in the local inflammatory environment of joints such as the knee and their relationship to the functionality of chondrocyte clock genes and osteoarthritic-like changes^[3,5,12,19,22], along with other factors shown in Box 1 that possibly impact observed daily fluctuations in arthritis symptomology^[24], should be explored to tease out the relevance of their individual and collective influences.

Moreover, to overcome limitations in applying the results of preclinical studies to the clinical realm, the testing of human material to ascertain if the circadian master clock and clock genes located in articular cartilage do comprise an important biological pathway that can account for the well-known daily fluctuations of the symptoms of the disease, plus abnormalities in gene expression, translation and transcription of the key tissue involved in this disease as per Figure 1 should be undertaken.

It the meantime it can be tentatively concluded that even minimal disruption or deletion of any arm of this intricate pathway shown in Figure 1, especially the genes BMAL1 and CLOCK genes can induce a series of highly negative local tissue metabolic and structural effects, as well as the ability to influence central pathways-even if this does not replicate the chronic long term trajectory of the

Box 1 Factors that may influence the biology of the Circadian Master Clock and its circuits.

| |
|--|
| Aging ^[20,22] |
| Daily activity/reduced physical activity patterns ^[22] |
| Compromised tendon, bone, or muscle function ^[22] |
| Genetic factors ^[3] |
| Humeral and neural factors eg glucocorticoid signaling ^[5,22] |
| Mechanical cues and loading ^[11,22] |
| Metabolic factors such as glucose metabolism ^[22] |
| Obesity ^[3,22] |
| Pain |
| Prevailing melatonin levels ^[4,23] |
| Sleep patterns ^[2] |
| Social factors ^[29] |
| Temperature ^[8] |

disease at all well. Consequently, until more research is forthcoming, efforts to minimize or prevent disturbances of circadian timekeeping at the central, as well as at the local level as early as possible, may arguably be more useful than not in the context of fostering joint health and the maintenance of articular cartilage, regardless of joint status. Moreover, although this review has focused solely on articular cartilage clock genes, since both bone as well as epiphyseal cartilage in the growth plate express circadian rhythms^[25], and several clock-controlled genes in surrounding tissues and other mechanisms involved in osteoarthritis have a bearing on clock-related cartilage genes function, interventions to protect the hypothetical pathways shown in Figure 1 early on in the disease process^[3], plus obtaining a comprehensive understanding of all these interacting forces and their physiological implications, appears crucial^[22,26].

Comparative studies of other hypothesized osteoarthritis mechanisms and pathways over extended time periods using biochemical, histological, along with dynamic and structural chondrocyte cell assays, plus intervention studies that apply Bellamy *et al*'s^[24] along with Kato *et al*'s^[27] approaches to measuring circadian rhythms, may similarly help to further solidify the extent to which the presently discussed circadian clock pathways and genes are not only implicated in osteoarthritis pathology, but whether they are amenable to intervention. To this end, efforts to determine a more precise mapping of the temporal and pathological stages of the disease and its linkages to cartilage clock gene oscillations, and their differential interactions with EL-box elements and other circadian gene regulators^[25], and exploring the implications of these influences are strongly recommended.

As well, carefully construed comparative studies of multiple forms of well-defined osteoarthritis in cohorts of different ages using agreed upon approaches across different laboratories, plus the application of common sense in efforts to attenuate osteoarthritis based on circadian clocks that focuses on injury control, a sound daily activity-rest schedule, and the avoidance of excessive psychological and mental distress, while encouraging sound nutrition and moderate exercise practices and careful timing/scheduling thereof^[28] may be extremely helpful. Additionally, possible medication adjustments as well as the use of other forms of pain relieving treatment that correlate optimally with circadian fluctuations, and preclude drugs that reduce important chondrocyte, bone, muscle, or tendon circadian clock functions may be highly salient, as well as cost-effective preventive strategies against the magnitude and severity of this intractable condition. Introducing healthy mobility and sleep practices in younger cohorts, those who are obese, and those with metabolic problems may be especially promising in reducing age and comorbid disease associated impacts of circadian rhythms on cartilage health.

The role of light therapy, sleep interventions, implantable medical devices, neurostimulation, dietary and antidepressant therapy, as well as the role of muscular and osseous circadian clocks and their relationship to a variety of cartilage clock-controlled physiological processes in health and disease should also be especially explored^[21,22].

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