

## REVIEW ARTICLE

# Melatonin in regulation of inflammatory pathways in rheumatoid arthritis and osteoarthritis: involvement of circadian clock genes

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Rheumatoid arthritis (RA) and osteoarthritis (OA) are the two most prevalent joint diseases. As such, they are important causes of pain and disability in a substantial proportion of the human population. A common characteristic of these diseases is the erosion of articular cartilage and consequently joint dysfunction. Melatonin has been proposed as a link between circadian rhythms and joint diseases including RA and OA. This hormone exerts a diversity of regulatory actions through binding to specific receptors and intracellular targets as well as having receptor-independent actions as a free radical scavenger. Cytoprotective effects of melatonin involve a myriad of prominent receptor-mediated pathways/molecules associated with inflammation, of which the role of omnipresent NF- $\kappa$ B signalling is crucial. Likewise, disturbance of circadian timekeeping is closely involved in the aetiology of inflammatory arthritis. Melatonin is shown to stimulate cartilage destruction/regeneration through direct/indirect modulation of the expression of the main circadian clock genes, such as BMAL, CRY and/or DEC2. In the current article, we review the effects of melatonin on RA and OA, focusing on its ability to regulate inflammatory pathways and circadian rhythms. We also review the possible protective effects of melatonin on RA and OA pathogenesis.

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### Abbreviations

AA, adjuvant-induced arthritis; Acan, aggrecan; ARNTL2, aryl hydrocarbon receptor nuclear translocator-like; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1; CIA, collagen-induced arthritis; CLOCK, circadian locomotor output cycles kaput; Col2a1, collagen 2A1; Cry 1, cryptochrome 1; DEC1, differentially expressed in chondrocytes 2; iNOS, inducible NOS; LOX, lipoxygenase; MSCs, mesenchymal stem cells; NPAS2, neuronal PAS domain-containing

protein 2; OA, osteoarthritis; Per 1, period 1; RA, rheumatoid arthritis; REV-ERBs, reverse-eritroblastosis viruses; RORa, RAR-related orphan receptor A; RORs, retinoid-related orphan receptors; Sirt1, sirtuine 1; SMAD2/3, mothers against decapentaplegic-homologue 2/3; Sox9, sex-determining region-related protein 9

## Introduction

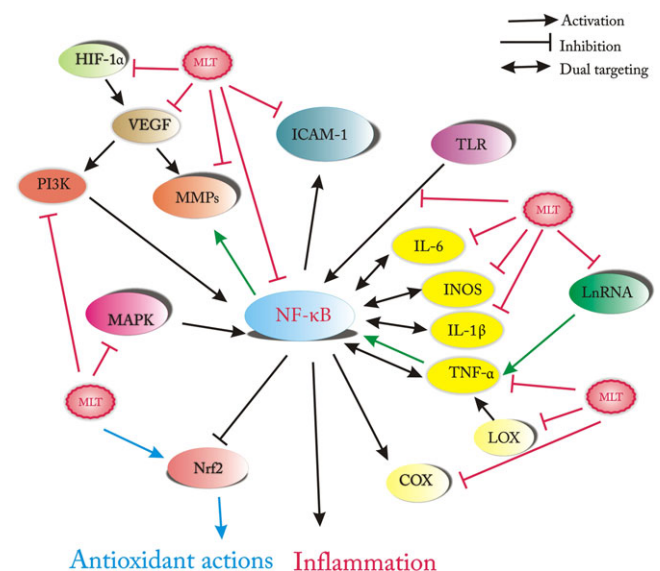
Rheumatoid arthritis (RA) and osteoarthritis (OA) are the two most prevalent diseases of the joints. They are also major causes of pain and disability in a substantial proportion of the human population (Pap and Korb-Pap, 2015). In spite of different etiologies of these disorders, a common characteristic is the erosion of articular cartilage and consequently joint dysfunction (Haas and Straub, 2012). Recent studies have provided evidence for a functional circadian clock in cartilage tissue, which drives downstream expression of clock controlled genes (Kouri *et al.*, 2013). The circadian clock regulates endochondral ossification, a bone synthesis process from a cartilaginous template. Chondrocyte proliferation and the rate of change in the growth plates show a circadian variation, as well (Takarada *et al.*, 2012). Moreover, increased rates of calcium and phospho-mineralization have been detected during the dark phase of the light-dark cycle (Gossan *et al.*, 2015).

**Melatonin**, a circadian-controlled product secreted by the pineal gland, has been suggested as a potential link between circadian rhythms and joint diseases, including RA and OA (Yoshida *et al.*, 2014a,b; Berenbaum and Meng, 2016). Melatonin and its derivatives are wide-ranging free radical detoxifiers and antioxidants, because of their potential scavenging of free radicals of oxygen species (ROS) and nitrogen species (RNS) and to boost the expression and activity of glutathione and antioxidant enzymes (Reiter *et al.*, 2016). Through these events and their receptor-mediated actions, melatonin and its metabolites modulate a variety of molecular signalling pathways including proliferation, apoptosis, metastasis and inflammation, in a wide range of pathophysiological situations (Hosseinzadeh *et al.*, 2016). Here, we review the effects of melatonin on RA and OA, focusing on its ability to regulate the inflammatory pathways and the circadian rhythms. We also review the possibilities of protective effects of melatonin on RA and OA pathogenesis.

## Melatonin in regulation of inflammatory pathways

The circadian multi-tasking hormone, melatonin, is mainly synthesized by the pineal gland and also a wide range of other tissues (Reiter *et al.*, 2016). Melatonin is evolutionarily conserved and exerts many different regulatory actions through binding to specific receptors and intracellular targets (Reiter *et al.*, 2010). Depending upon a specific cell or species, melatonin activates a variety of different second messenger cascades after its binding to the high-affinity G-protein-coupled melatonin receptors, **MT<sub>1</sub>/MT<sub>2</sub>**, on the plasma membrane of the target cells (Majidinia *et al.*, 2017). **MT<sub>1</sub>** receptors are expressed throughout the body but predominantly in the brain. In suprachiasmatic nucleus, both **MT<sub>1</sub>** and **MT<sub>2</sub>** receptors are involved in regulating circadian rhythms (Wiesenberg *et al.*, 1998). Also, high-affinity nuclear binding sites for melatonin are members of the nuclear

receptor family. These may be involved in the antiproliferative activity of melatonin on cancer cells and its immunomodulatory actions (Hosseinzadeh *et al.*, 2016). Besides plasma membrane and nuclear receptors, receptor-independent signalling accounts for radical scavenging activities of melatonin (Carlberg, 2000). Melatonin is known to elicit both pro and anti-inflammatory effects depending on the cellular state (Radogna *et al.*, 2010). Cytoprotective effects of melatonin involve several prominent pathways/molecules associated with inflammation, such as the transcription factor NF- $\kappa$ B (15, 16), **toll-like receptors** (Chuffa *et al.*, 2015), MAPK (Lin *et al.*, 2016), inflammatory mediators of **arachidonic acid** (AA) pathway including **COX** and lipoxygenase (LOX) (Steinhilber *et al.*, 1995; Li *et al.*, 2000; Lu *et al.*, 2016), long non-coding RNAs (Akhtar *et al.*, 2016), (Mishra *et al.*, 2011), hypoxia inducible factor(HIF)-1 $\alpha$  (Park *et al.*, 2009; Jahanban-Esfahlan *et al.*, 2017) and nuclear erythroid 2-related factor 2 (Nrf2) (Negi *et al.*, 2011). With a closer look, it appears that NF- $\kappa$ B signalling is central to all these inflammation-related pathways, and more importantly, the wide-spectrum antioxidant and anti-inflammatory effects of melatonin eventually lead to the blockade of NF- $\kappa$ B signalling (Figure 1). Melatonin protects cells from the devastating effects of oxidative stress, through modulation of NF- $\kappa$ B pathway, as well (Reiter *et al.*, 2016).



**Figure 1**

Cellular insults such as ROS, LPS, cytokines (e.g. TNF- $\alpha$ ), several lncRNAs and signalling pathways (e.g. MAPKs, hypoxia inducible factor-1 $\alpha$  and LOX) induce activation of NF $\kappa$ B signalling, which in turn leads to the expression of downstream genes involved in the inflammatory responses, including pro-inflammatory cytokines (IL1 $\beta$ , IL-6, TNF- $\alpha$ ), iNOS, adhesion molecules, COX-2 and MMPs. The anti-inflammatory effects of melatonin involve inhibiting a wide range of these inflammatory molecules, and the final result is to mitigate inflammation through blockade of NF- $\kappa$ B signalling.

NF- $\kappa$ B is an omnipresent oxidative stress-sensitive transcription factor, which has a primary role in cell survival, immune and inflammatory responses. These actions involve a variety of genes essential in cellular responses (Hoesel and Schmid, 2013). The classic form of NF- $\kappa$ B is a dimer, consisting of a p50 subunit and a trans-activating subunit p65 including RelA, RelB and c-Rel. The dormant form of NF- $\kappa$ B appears in the cytoplasm of resting cells, forming a complex with its inhibitors, the I $\kappa$ Bs (I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$ ) (Gilmore, 2006). Upon stimulation, NF- $\kappa$ B is activated through phosphorylation and degradation of the I $\kappa$ Bs by **IKK**. After its liberation, NF- $\kappa$ B translocates to the nucleus and binds to enhancer elements within the promoters of responsive genes. NF- $\kappa$ B/DNA complex recruit coactivators (e.g. p300) and RNA polymerase to activate transcription of target genes (Hoffmann *et al.*, 2006). As shown in Figure 1, NF- $\kappa$ B activates in response to different inducers, such as ROS, bacterial **LPS** and several cytokines, and induces the expression of genes involved in the inflammatory responses, including pro-inflammatory cytokines (**IL-1 $\beta$** , **IL-6**, **TNF- $\alpha$** ), **inducible NOS (iNOS)**, adhesion molecules, **COX-2** and MMPs (Li and Verma, 2002).

It should be interesting to note that melatonin can be synthesized by the macrophages present in the arthritis lesions. Indeed, NF- $\kappa$ B is capable of inducing the synthesis of melatonin by immune-competent cells. NF- $\kappa$ B activation triggers the synthesis of melatonin in macrophages in the regulatory phase and also in chronic inflammatory processes. This is a long-term response as it is mediated by the cRel NF- $\kappa$ B subunit, induced by the p50/RelA NF- $\kappa$ B (Muxel *et al.*, 2016). The same subunit is relevant for RA and other autoimmune responses. p50/cRel and cRel/cRel interact with Aa-nat promoter inducing melatonin synthesis, and on the other hand, CD40/cRel is the signalling pathway for RA and p50/cRel induces antibodies and co-stimulatory molecules (Gregersen and Olsson, 2009; Criswell, 2010). The fact that immune competent cells are able to produce melatonin is important information for understanding the disease and for evaluating the use of melatonin.

## Melatonin actions in RA

While the anti-inflammatory and cytoprotective effects of melatonin are well described in many diseases (Carrillo-Vico *et al.*, 2013), there are conflicting data regarding beneficial or worsening effects of melatonin in RA patients. A report by Hansson *et al.* (1990) claimed that constant darkness, by disturbing the rhythm of production of melatonin, promoted autoimmunity to type II collagen and augmented the onset of collagen-induced arthritis (CIA) in DBA/1 mice. The effect of constant darkness (which causes the melatonin rhythm to run) and constant light (which eliminates pineal melatonin production) was investigated on the disease course of an experimental autoimmune model, type II CIA in DBA/1 female mice. Their results indicated that under constant darkness, mice tend to develop more severe CIA and show a higher level of serum anti-collagen antibodies than mice maintained under a regular photoperiod or constant light (Hansson *et al.*, 1990). Furthermore, pinealectomy reversed the effects of constant darkness (Hansson *et al.*, 1993). Daily injections of melatonin to DBA/1 mice enhance T cell priming and may aggravate CIA development (Hansson *et al.*, 1992). The dual effect of melatonin as a pro-inflammatory

agent and antioxidant has also been seen in CIA models in rats. Jimenez-Caliani *et al.* (2005) claimed that melatonin increased anti-collagen antibodies, IL-1 $\beta$  and IL-6 levels in the serum and joints of arthritic rats, while it lowered oxidative markers nitrite/nitrate and lipid peroxidation in serum but not in joints. Pinealectomy abolished the effects of melatonin and reduced oxidative stress as well as concentrations of cytokines and antibodies in joint but increased the level of serum oxidative markers.

The beneficial anti-inflammatory effects of melatonin have been reported in a model of adjuvant-induced arthritis (AA) in rat (Chen and Wei, 2002). In this study, administration of the prophylactic and therapeutic doses of melatonin repressed the inflammatory response and produced enhanced proliferation of thymocytes and secretion of **IL-2** in AA rats. In addition, melatonin decreased the elevated level of **cAMP** induced by **forskolin**. In AA rats, the drop in thymocyte proliferation correlated highly with the decrease in the levels of **Met-enkephalin (Met-Enk)** in the thymocytes, and melatonin strikingly augmented the level of Met-Enk, which were hindered by a Ca<sup>2+</sup> channel antagonist, **nifedipine**. The anti-inflammatory and immunoregulatory actions of melatonin involved a G protein-adenyl cyclase-cAMP transmembrane signal and Met-Enk release by thymocytes (Chen and Wei, 2002).

The incidence and severity of RA tend to be exacerbated at high latitudes, such as Estonia (Cutolo *et al.*, 2005a). RA patients at these latitudes may experience a more prolonged elevation of melatonin and TNF- $\alpha$  during the winter months. However, there is no significant difference between the serum level of **cortisol** and **IL-6** in Estonian rheumatoid patients and controls from lower latitudes (Cutolo *et al.*, 2005b). RA symptoms are reported to exacerbate in the early morning as well, when the level of pro-inflammatory cytokines are high and cortisol concentration is low (Sulli *et al.*, 2002). In contrast, West *et al.* measured significantly lower levels of morning plasma melatonin in RA patients (West and Oosthuizen, 1992) and an increased nocturnal production of melatonin induced by Freund's adjuvant in an experimental model of arthritis (Cano *et al.*, 2002). Synovial macrophages from RA patients elicit high-affinity binding sites for melatonin *in vitro* and secrete high levels of **NO** and **IL-12** upon melatonin treatment (Cutolo *et al.*, 1999). Additionally, the amount of melatonin is relatively high in synovial fluid from RA individuals (Maestroni *et al.*, 2002). Conversely, melatonin activates the ERK-stimulated cyclin-dependent kinase inhibitors P21 and P27 to hamper the excessive proliferation of RA-derived fibroblast-like synoviocytes (Nah *et al.*, 2009). Clearly, the relationship between melatonin and RA requires further definition.

## Melatonin actions in OA

In contrast to the controversial effects of melatonin on the prognosis of RA, the cytoprotective and therapeutic potential of melatonin is well established in preclinical and clinical OA settings. Lim *et al.* (2012) disclosed that the cytoprotective and anti-inflammatory effects of melatonin in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-stimulated CHON-001 human chondrocyte cell line and in a rabbit model of OA are mediated through a **sirtuin 1** (Sirt1) pathway. Sirt1 is a NAD<sup>+</sup>-dependent protein deacetylase that controls many physiological pathways, including circadian rhythms in peripheral tissues (Yang *et al.*, 2016). Melatonin

inhibited H<sub>2</sub>O<sub>2</sub>-stimulated cytotoxicity, iNOS and COX-2 protein, and mRNA expression as it dampened the synthesis of NO and PGE<sub>2</sub> and blocked H<sub>2</sub>O<sub>2</sub>-induced TNF- $\alpha$ , IL-1 $\beta$  and IL-8 release. In addition, melatonin treatment significantly inhibited H<sub>2</sub>O<sub>2</sub>-induced phosphorylation of **PI3K/Akt, p38, ERK, JNK** and MAPK, as well as activation of NF- $\kappa$ B. Intra-articular injection of melatonin significantly reduced cartilage destruction in a rabbit model of OA. The effects of melatonin were reversed by sirtinol and Sirt1 siRNA, which blocks Sirt1 activity and down-regulates its expression respectively. Based on these findings, the authors suggested that the anti-inflammatory and cytoprotective effects of melatonin in human chondrocytes involve the dynamic action of the Sirt1 pathway (Lim *et al.*, 2012). Hong *et al.* established an osteoarthritic chondrocyte model characterized by high levels of **MMP13**, shift of cytosolic MMP-13 into the nucleus, metabolic disruption of collagen 2A1 (COL2A1), proteoglycan loss, atypical distribution and excessive Ca<sup>2+</sup> deposition (Hong and Hong, 2015). Administration of melatonin (1 nM) restored aberrant levels of COL2A1 and blocked the cytonuclear translocation of MMP-13. Moreover, melatonin increased the amount of proteoglycan, which was optimally distributed in chondrocytes. Accordingly, melatonin reduced the amount of cells with Ca<sup>2+</sup> crystals. These results suggest the potential of melatonin to turn off the catabolic switch through the regulation of MMP-13 localization (Hong and Hong, 2015). Pei *et al.* (2009) reported that melatonin promotes matrix synthesis by articular chondrocytes *in vitro*. Melatonin also elevated the expression of chondrogenic marker genes such as sex-determining region-related protein 9 (Sox9), collagen II and aggrecan (Acan) while down-regulating collagen X, a hypertrophic marker. These findings document that melatonin not only up-regulates chondrogenic differentiation but also may reduce osteogenic differentiation. Melatonin enhances internal TGF- $\beta$ 1 expression in treated chondrocytes suggesting that it enhances cartilage matrix synthesis through activation of the TGF- $\beta$  signalling pathway (Pei *et al.*, 2009).

Mesenchymal stem cells (MSCs) can differentiate into multiple lineages, including chondrocytes (Zhou *et al.*, 2015). In injured or diseased joints, elevated oxidative stress in the inflamed micro-environment hinders the chondrogenic differentiation of MSCs and leads to degradation of neocartilage (Wang *et al.*, 2015). Liu *et al.* (2014) showed that melatonin successfully restored the inhibitory effect of IL-1 $\beta$  and TNF- $\alpha$  on MSC chondrogenesis, which presumably correlated with melatonin's ability to scavenge free radicals, suppress MMPs levels and preserve SOD activity.

## Circadian clock, melatonin and joint disease

Suprachiasmatic nuclei in the CNS and clocks in peripheral tissues regulate key aspects of physiology by regulating circadian rhythms in tissue-specific sets of downstream genes. These circadian clocks regulate many aspects of the immune system, and their disruption contributes to the onset of various inflammatory-related disease states, as well as joint disease (Takahashi *et al.*, 2008).

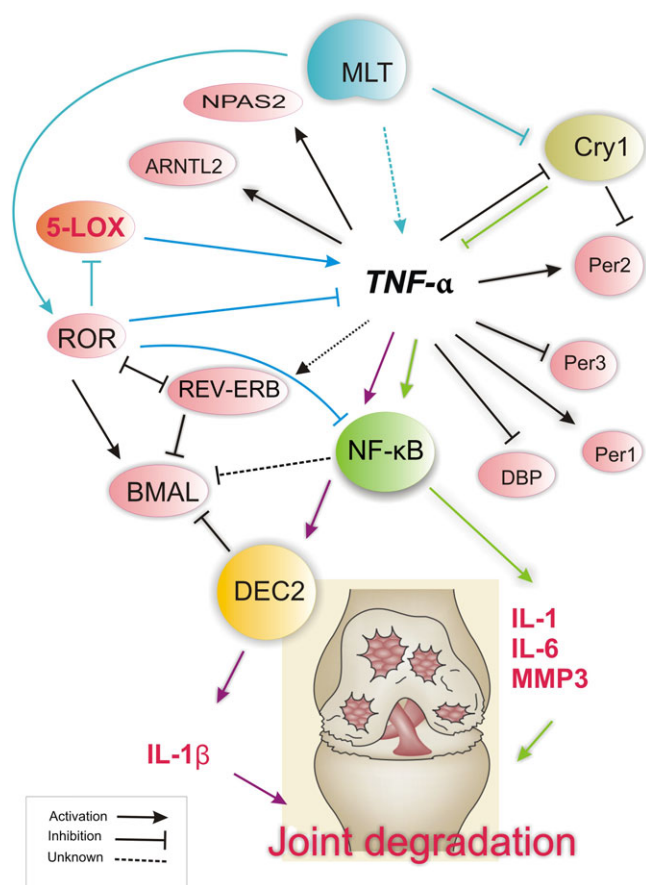
Clock genes constitute auto-regulatory feedback loops with heterodimers formed between brain and muscle aryl hydrocarbon receptor nuclear translocator-like (ARNTL) 1

(BMAL1) and circadian locomotor output cycles kaput (CLOCK) genes. They serve as the main positive transcription factors binding to the E-box cis-regulatory enhancer elements, which are found within target gene promoters or enhancers (Buttgereit *et al.*, 2015). An important downstream transcriptional target for BMAL1/CLOCK encodes for Per (Period 1 and 2) and cryptochrome 1 (Cry 1 and 2), the negative feedback proteins, which by accumulating in the nucleus decrease expression of BMAL1/CLOCK complex and so Per/Cry expression, as well (Gibbs and Ray, 2013). Less studied clock molecules, DEC1 and DEC2, compete with BMAL1/CLOCK for E-box binding (Kouri *et al.*, 2013). Also, ARNTL2/NPAS2 are functional paralogues of BMAL1/CLOCK respectively. Additional downstream targets of CLOCK/BMAL1 are transcriptional activators of **retinoid-related orphan receptors (RORs)** and repressors such as **REV-ERBs** (Roenneberg and Meroz, 2005). Albumin D site-binding protein is an extra BMAL/CLOCK downstream gene, and its expression is under the control of both BMAL and CRY1 (Stratmann *et al.*, 2010). This core clock machinery regulating circadian time exists in virtually all cells of the body, as well as in the joint chondrocytes (Takarada *et al.*, 2012; Gossan *et al.*, 2013; Honda *et al.*, 2013; Guo *et al.*, 2015).

## Melatonin in modulation of circadian clock and inflammation in RA

Disturbance in circadian timekeeping is closely involved in the aetiology of RA (Kouri *et al.*, 2013; Yoshida *et al.*, 2014a,b). Patients with RA exhibit disturbances in the hypothalamic–pituitary–adrenal axis, which is reflected in the altered circadian rhythm of circulating serum level of melatonin, IL-6, cortisol and in chronic fatigue (Cutolo *et al.*, 2005a; Haas and Straub, 2012). These changes are intertwined with the abnormal expression of several clock genes BMAL, CLOCK, ARNTL2, DEC2 and NPAS2 (Figure 2) (Gibbs and Ray, 2013; Kouri *et al.*, 2013). Interestingly, DEC2, which is selectively expressed in Th2 immune cells, increases the expression of IL-1 $\beta$  and is abundantly present in the synovial membranes of RA. TNF- $\alpha$  up-regulates DEC2 at both mRNA and protein level in a NF- $\kappa$ B-dependent manner in cultured human synovial fibroblasts (Oikkonen *et al.*, 2015) and not only DEC2 but also ARNTL2 and NPAS2 are up-regulated by TNF- $\alpha$ . The effects of TNF- $\alpha$  on DEC2 expression are mediated *via* NF- $\kappa$ B in HEK293 cells and primary human fibroblasts. The NF- $\kappa$ B-mediated effects on DEC2 are manifested by up-regulation of IL-1 $\beta$  (Oikkonen *et al.*, 2015).

Narasimamurthy *et al.* (2012) found another clock gene with impaired expression in RA. The authors showed that the ablation of the core clock gene, **Cry1**, leads to a constitutive elevation of pro-inflammatory cytokines in a cell-autonomous manner. **Cry1 protein** is known to bind to adenylyl cyclase and limit cAMP production, suggesting that the absence of Cry protein(s) might decrease the inhibition of cAMP production, resulting in elevated cAMP and increased PKA activation, subsequently leading to NF- $\kappa$ B activation through phosphorylation of p65 at S276 (Narasimamurthy *et al.*, 2012). Melatonin is known to attenuate Cry1 gene expression in RA, leading to elevation of type II collagen antibodies and increasing the severity of CIA. In



**Figure 2**

Implications of melatonin in the regulation of clock gene expression in joints during RA. Disruption of circadian clock gene expression is mainly under the influence of TNF- $\alpha$ . This pro-inflammatory cytokine is produced at high levels by fibroblast-like synoviocytes. The unfavourable effects of clock gene disruption in RA is reflected in the suppression of the negative arm action, of which the loss of CRY expression results in pronounced up-regulation of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and enhanced severity of RA. Another established link is the up-regulation of DEC2 *via* TNF- $\alpha$ , which subsequently leads to an elevated expression of IL-1 $\beta$ , hence enhancing the pro-inflammatory and destructive effects of TNF- $\alpha$  on the RA joint. High levels of melatonin in RA patients may contribute to clock disturbance at least through three means: (i) modulating pro-inflammatory states through increasing the level of pro-inflammatory cytokines, including TNF- $\alpha$ ; (ii) attenuating clock gene cryptochrome1 expression, through the inhibitory mechanism of melatonin; (iii) modulating ROR activation. ROR acts as a major negative regulator of inflammation and is central to both BMAL and melatonin actions. ROR inhibition by REV-ERB may contribute to BMAL suppression and exacerbate RA. Though the role of BMAL1 clock gene is central to regulating the circadian oscillations and RA joints show abnormal cytoplasmic localization of BMAL in response to TNF- $\alpha$  induction, the extent of BMAL1 disruption in RA state along with other core clocks remains to be determined.

this context, Bang *et al.* (2012) demonstrated the marked down-regulation of both mRNA and protein levels of Cry1 in CIA after melatonin treatment, compared with those in the control and CIA-only group. Melatonin therapy also increased paw thickness. In addition, there was an increased

infiltration of inflammatory cells, synovial hyperplasia and the destruction of articular cartilage and bone by melatonin. The concentration of anti-type II collagen antibodies along with elevated serum concentrations of TNF- $\alpha$  and IL-6 in CIA plus melatonin mice was significantly higher than those in the control and CIA-only animals. Melatonin had no effect on transcription of two other important clock genes, BMAL and Per1 in CIA mice. These results suggest that the clock gene Cry1 may be involved in the exacerbation of melatonin-mediated arthritis in mice with anti-type II collagen antibody-induced arthritis (Bang *et al.*, 2012).

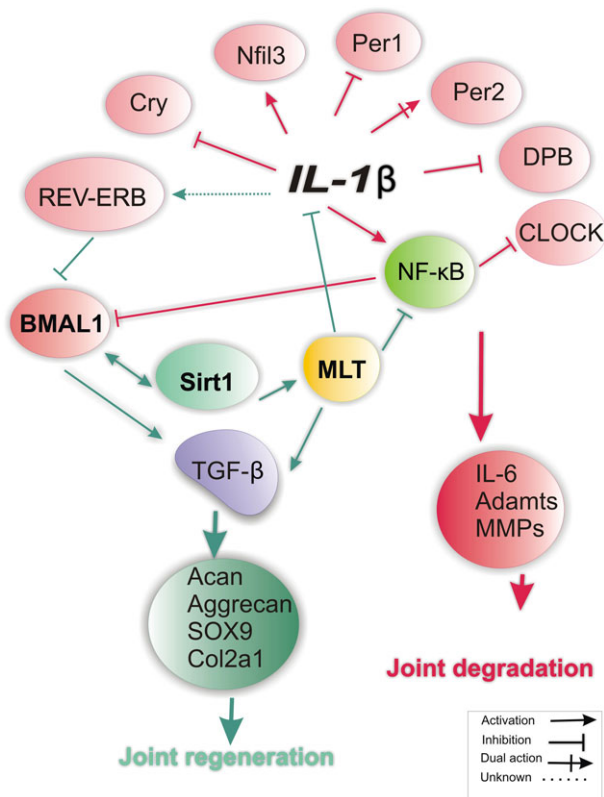
Another likely link can be made between melatonin, clock gene ROR and BMAL. As discussed earlier, melatonin actions involve three pathways: (i) a G-protein-mediated membrane signalling; (ii) a nuclear signalling; and (iii) a receptorless radical scavenging action. Nuclear signalling involves the clock gene transcription factor RZR/ROR complex (Carlberg, 2000). Melatonin elicits anti-allergic and anti-inflammatory actions by inhibiting **5-lipoxygenase (5-LOX)** through transcriptional activation of RZR/ROR (Steinhilber *et al.*, 1995; Carlberg, 2000). The LOX pathway is a main contribution to the RA inflammatory state, and synovial fluid in RA patients shows high levels of **LTB<sub>4</sub>** – a major product of 5-LOX. LTB<sub>4</sub> is a potent pro-inflammatory chemotactic agent and is reputed as an important mediator of joint inflammation in RA. Importantly, **5-LOX** inhibitors block TNF- $\alpha$ -induced NF- $\kappa$ B signalling in rheumatoid fibroblasts *in vitro* and a murine paw oedema model (Lin *et al.*, 2014). What is more, **ROR $\alpha$ 1** inhibits NF- $\kappa$ B-mediated inflammation through activation of its major inhibitor, I $\kappa$ B $\alpha$ . Ectopic expression of ROR $\alpha$  is also reported to inhibit TNF- $\alpha$ -induced IL-6, IL-8 and COX-2 expression in human smooth muscle cells. Thus, ROR $\alpha$  agonists as well as 5-LOX inhibitors are potential targets in the treatment of RA (Lin *et al.*, 2014; Muxel *et al.*, 2016). Clock gene ROR is indispensable for activity of both melatonin and BMAL. ROR actions could be antagonized with REV-ERB, the expression of which significantly increased in RA synovium compared with OA synovium (Kouri *et al.*, 2013).

It is established that Cry-null mice develop maximal exacerbation of joint swelling and up-regulation of essential mediators of arthritis including TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and MMP-3 (Hashiramoto *et al.*, 2010). Given the fact that the stimulation of membrane melatonin receptors inhibits adenylyl cyclase activity, as the CRY transcription factor does, opens the question that how melatonin counteracts the CRY anti-inflammatory activity in RA joints (Carlberg, 2000).

### Melatonin in modulation of circadian clock and inflammation in OA

Recent evidence strongly suggests that the chronic, low-grade inflammation, which develops with age or autoimmune conditions, is involved in both early and late stages of cartilage degeneration during the development of OA (Goldring and Otero, 2011). In aged, injured and OA joints, levels of pro-inflammatory and catabolic cytokines, for example, IL-1 and TNF- $\alpha$ , are elevated in both animal models and in humans, with signs of synovitis (van den Berg, 2001; Goldring and Otero, 2011). Pro-inflammatory cytokines/proteins, such as IL-6, **Adamt4**, and MMPs, not only elicit

catabolic effects and destroy matrix but also suppress the anabolic pathways by inhibiting Sox9 and Col2a1 expression, further impairing tissue repair (Figure 3) (Guo *et al.*, 2015; Berenbaum and Meng, 2016). Guo *et al.* (2015) demonstrated that exposure to catabolic cytokines IL-1 $\beta$ , but not TNF- $\alpha$ , severely disrupted circadian gene expression rhythm in cartilage. The effect of IL-1 $\beta$  on the cartilage clock was partly through functional interference with the core CLOCK/BMAL1 complex, mediated *via* an NF $\kappa$ B-dependent pathway. IL-1 $\beta$  but not TNF- $\alpha$  disrupts the rhythmic expression of circadian clock genes in cartilage. Interestingly, an unexpected lack of effect of TNF- $\alpha$  in regulating circadian



**Figure 3**

Interplay between melatonin and clock genes in joint during OA. Among pro-inflammatory factors implicated in OA, IL-1 $\beta$  accounts for disrupted expression of various clock genes. The role of BMAL1 is dominant in maintaining chondrocyte homeostasis in OA. Here, high levels of IL-1 $\beta$  in the OA joint disturb the expression of most clock genes, leading to the suppression/reduction of BMAL-1 expression, of which the loss of Sirt1 expression is central. Loss of BMAL due to inhibition of Sirt1 or *via* other pathways (up-regulation of REV-ERB- $\alpha$ ) can lead to the activation of MMPs, hence promote joint degradation. Melatonin is implicated in clock gene expression at least *via* two ways: (1) direct inhibitory actions on the release of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-8); and (2) blockade of NF $\kappa$ B signalling, which leads to the inhibition of matrix degrading enzymes (MMPs and Adamts4). Notably, circadian gene expression of Sirt1 is a prerequisite for BMAL activation and for the anti-inflammatory effects of melatonin in the OA joints. Both melatonin and BMAL exploit a common pathway (TGF- $\beta$  signalling) to enhance matrix synthesis by articular chondrocytes.

pathways and NF- $\kappa$ B signalling has been demonstrated in normal cartilage explants (Guo *et al.*, 2015).

It was recently documented that the expression of the core clock gene BMAL1 is disrupted in human OA cartilage and in aged mouse cartilage. Targeted BMAL1 ablation in mouse chondrocytes abolishes the circadian rhythms and promotes advanced destruction of the articular cartilage (van den Berg, 2001). BMAL1 modulate the circadian expression of many anabolic, catabolic and apoptotic genes that implicate in cartilage homeostasis. BMAL1 loss results in reduced levels of phosphorylated SMAD2/3, NFATC2, Sox9, Acan and Col2a1, but increased levels of phosphorylated SMAD1/5 (Dudek *et al.*, 2016).

Yang *et al.* (2016) measured both mRNA and protein levels of circadian genes, NAD oxidase (NAD<sup>(+)</sup>) and Sirt1, in human knee articular cartilage. The results showed that in OA cartilage, the levels of both BMAL1 and NAD<sup>(+)</sup> decreased significantly, which resulted in the inhibition of nicotinamide phosphoribosyl transferase activity and Sirt1 expression. Furthermore, the knockdown of BMAL1 reduced the level of NAD<sup>(+)</sup> and exacerbated OA-like gene expression changes during stimulation with IL-1 $\beta$ . The overexpression of BMAL1 rescued the gene alterations stimulated by IL-1 $\beta$ , which was consistent with the inhibitory effect of REV-ERB $\alpha$ . Sirt1 siRNA treatment not only reduced the protein levels of BMAL1 and moderately elevated the levels of Per2 and REV-ERB $\alpha$  but also further aggravated the survival of cells and the expression of cartilage matrix-degrading enzymes induced by IL-1 $\beta$ . Up-regulation of Sirt1 reversed the IL-1 $\beta$ -induced metabolic imbalance of chondrocytes, suggesting that BMAL1 is a key clock gene implicated in Sirt1-stimulated cartilage homeostasis (Yang *et al.*, 2016). Notably, anti-inflammatory and cytoprotective effect of melatonin in OA and MSCs also depends upon Sirt1 activity (Lim *et al.*, 2012; Zhou *et al.*, 2015). It appears that Sirt1 activation is central to the anti-inflammatory and chondrocyte matrix regeneration potential of BMAL and melatonin (Lim *et al.*, 2012; Zhou *et al.*, 2015; Yang *et al.*, 2016). Needless to say, additional studies are required to shed additional light on the role of the circadian hormone melatonin in the regulation of clock genes in OA.

## Conclusions

Circadian expression of the pleiotropic hormone melatonin regulates a broad spectrum of the critical biological processes, including inflammation. The anti-inflammatory actions of melatonin involve a myriad of inflammation-wired molecules and pathways, to directly or indirectly block NF- $\kappa$ B signalling and terminate the inflammatory responses. High levels of melatonin exert favourable effects on the prognosis of most inflammation-related, self-destructive diseases, except RA. High levels of melatonin could lessen the clinical manifestation of OA, although in the context of its sister joint disease, RA, the results are somewhat conflicting. On the other hand, new data point to the deregulation of certain circadian clock genes in the pathology of the joint inflammatory diseases. Disturbed circadian rhythm is associated with disrupted expression of pro-inflammatory cytokines, as well as deregulated expression of melatonin. It appears that

restoring circadian rhythm may open new avenues to alleviate the symptoms of both inflammatory joint diseases, especially in RA patients, where an unfavourable prognosis is predicted by an unbalanced expression of the circadian hormone melatonin.

### *Nomenclature of targets and ligands*

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b,c,d,e).

## Conflict of interest

The authors declare no conflicts of interest.

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