

Circadian rhythm and atherosclerosis (Review)

ZAIQIANG ZHANG^{1,2*}, BIN YU^{1,2*}, XINAN WANG^{1,2}, CAIYUN LUO^{1,2},
TIAN ZHOU^{1,2}, XIAXIA ZHENG^{1,2} and JIAWANG DING^{1,2}

¹Department of Cardiology, The First College of Clinical Medical Sciences;

²Institute of Cardiovascular Diseases, China Three Gorges University, Yichang, Hubei 443000, P.R. China

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Abstract. Atherosclerosis is the leading cause of morbidity and mortality worldwide. The underlying pathogenesis involves multiple metabolic disorders, endothelial dysfunction and a maladaptive immune response, and leads to chronic arterial wall inflammation. Numerous normal physiological activities exhibit daily rhythmicity, including energy metabolism, vascular function and inflammatory immunoreactions, and disrupted or misaligned circadian rhythms may promote the progression of atherosclerosis. However, the association between the circadian rhythm and atherosclerosis remains to be fully elucidated. In the present review, the effects of the circadian rhythm on atherosclerosis progression are discussed.

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1. Introduction

Atherosclerosis is an inflammatory disease and previous studies have demonstrated that the underlying pathology of atherosclerosis is mainly the damage to vessel walls caused by lipid metabolism disorders and the inflammatory immune

response, leading to abnormal lipid deposition in the intima and its underlying smooth muscle, plaque formation and vascular stenosis (1). Unstable atherosclerotic plaques are likely to rupture and cause thromboembolism, which may result in serious clinical events including acute coronary syndrome and myocardial infarction. Atherosclerosis is a chronic inflammatory condition in which a variety of cell types and physical and chemical factors are involved (2). A mounting body of evidence suggests that these cytokines involved in atherosclerosis exhibit circadian oscillations (3).

2. Biological characteristics of the circadian rhythm

All organisms on Earth, from bacteria to plants and mammals, have intrinsic body clocks that respond to environmental changes by controlling the major physiological activities. In mammals, the central pacemaker of the circadian rhythm exists in the suprachiasmatic nucleus (SCN) of the hypothalamus and consists of 24-h oscillations present in most cells of the body (4). These oscillations are of particular relevance to physiological and biochemical functions, including sleep/wake cycles, feeding behavior and activity rhythms (4). The core of the molecular clock is the transcription factor heterodimer circadian locomotor output cycle kaput (Clock)/brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1), which is driven by two key negative feedback loops that generate 24-h oscillations of daily activity (5), as presented in Fig. 1. The first important feedback loop consists of Clock, Bmal1, period circadian clock (Per) and Cryptochrome/circadian clock (Cry). In this loop, Clock/Bmal1 binds to E-box DNA elements to upregulate Per and Cry levels (3). Per and Cry then accumulate in the cytoplasm and form a complex with the serine-threonine kinase casein kinase 1 ϵ/δ (CK1 ϵ/δ), which translocates to the nucleus to repress its own transcription and Clock/Bmal1 activity, thereby forming a negative feedback loop (5). CK1 ϵ/δ (6,7) and AMP-activated protein kinase (AMPK) (8) phosphorylate unbound Per and Cry, respectively, to promote their degradation. Numerous studies have also demonstrated that other kinase networks, including glycogen synthase kinase-3, PI3K/Akt and MAPKs, are able to phosphorylate Per and Cry to promote their degradation (9). The second feedback loop consists of Clock, Bmal1, nuclear receptor subfamily 1, group D, member 1 (REV-ERB) α and $-\beta$, and orphan nuclear receptor (ROR) α , $-\beta$ or $-\gamma$. Clock/Bmal1 activate the negative regulators REV-ERB α and $-\beta$ and the

Correspondence to: Dr Jiawang Ding, Department of Cardiology, The First College of Clinical Medical Sciences, China Three Gorges University, 183 Yiling Road, Yichang, Hubei 443000, P.R. China
E-mail: dingjiawang@163.com

*Contributed equally

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positive regulators ROR α , - β or - γ (10,11). REV-ERBs bind to the retinoic acid-related orphan receptor response element located in the Bmal1 promoter to inhibit transcription (12). An increasing number of studies have indicated that the molecular clock has an important role in almost all metabolic processes in organisms. Furthermore, disruptions to the circadian rhythm may lead to cardiovascular diseases (13), type 2 diabetes (14) and immune system diseases (15).

3. Circadian rhythm and atherosclerosis

The progression of atherosclerosis is characterized by the accumulation of fatty deposits in the inner layer of arteries. It is well known that the development of atherosclerosis is related to lipid metabolism, inflammatory reactions, endothelial cell dysfunction and immune function, and there is growing evidence that circadian rhythms have a critical role in the development and progression of the condition. For instance, it was reported that low density lipoprotein receptor (Ldlr)^{-/-} and apolipoprotein E (ApoE)^{-/-} mice with global Clock knockout fed a standard chow diet developed more lesions at the aortic arches and aortic root (16), and that upregulated Cry1 expression (17) or REV-ERB β agonist delivery (18) reduced atherogenesis in Ldlr^{-/-} and ApoE^{-/-} mice.

4. Circadian rhythm and glycolipid metabolism

Circadian rhythm and glycometabolism. Glycometabolism is a complex physiological process. In humans, the daily variation in insulin secretion and insulin sensitivity over 24 h displays an obvious diurnal rhythm (19). Buxton *et al* (20) demonstrated that the risk of insulin resistance/type 2 diabetes may be reduced if shift workers focus on improving sleep duration and implementing circadian readjustment strategies (such as sleeping during the biological night and eating during the biological day). Glucose tolerance is higher in the morning than in the evening and at night (21). Impaired glucose tolerance of type 2 diabetes (22) appears when the circadian oscillation of the glycometabolism is disrupted. The neural and peripheral clocks regulate the enzymes of glycolysis, fatty acid oxidation and oxidative phosphorylation during the 24-h day to guarantee that these enzymes function at the appropriate times during the process of glycometabolism (23-25), which is associated with the circadian rhythm at the transcriptional level.

A large number of animal experiments and clinical studies have reported that glucose tolerance and diabetes are closely related to circadian rhythm disorders. In a laboratory test, rodents with SCN lesions exhibited whole or partial clock gene disruptions and developed glycometabolic disorders, including impaired glucose tolerance (26), β -cell failure (27), decreased insulin sensitivity (28), hyperglycemia (29) and hyperinsulinemia (29). Clock^{-/-} (29) or Bmal1^{-/-} (30) mutant mice exhibit impaired glucose tolerance, reduced insulin secretion and decreased pancreatic islet proliferation. Furthermore, a study involving mice with Clock gene mutations reported dampening of the oscillations of hepatic glycogen and glycogen synthase 2 and expression of the limiting enzyme of glycogenesis (31). Pancreas- or β -cell-specific Bmal1-knockout mice had elevated plasma glucose levels, impaired glucose tolerance and decreased insulin secretion. In addition, repression of Per2

expression resulted in reduced plasma glucose levels, enhanced insulin secretion and impaired gluconeogenesis (32). However, in mice lacking Cry1 and Cry2, plasma glucose levels were elevated in response to acute feeding after a 12-h overnight fast (33). REV-ERB α is able to regulate plasma glucose homeostasis by controlling the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxylase. REV-ERB α -mutant mice on a high-fat diet had increased adiposity and mild hyperglycemia without insulin resistance (34). In general, these studies demonstrate that the circadian clock system possibly maintains the homeostasis of glycometabolism by regulating the activities of the key enzymes of glycometabolism (35).

Circadian rhythm and lipid metabolism. Levels of serum lipids also display an obvious circadian rhythm. For instance, plasma levels of lipids exhibit day-night variations independent of food intake, suggesting that the circadian clock is an important regulator of lipid metabolism (36). However, the peak level of plasma high-density lipoprotein (HDL) appears in the early rest phase and decreases during the active phase (37). A prospective clinical study suggested that an unhealthy lifestyle and poor-quality sleep predict the development of hyperlipidemia and obesity with age (38). Another study suggested that reduced sleep duration in children contributes to an increased risk of being overweight (39). It is therefore indicated that circadian rhythm disorders are associated with lipid metabolism in mammals. In a clinical study on a population with obesity, the expression of REV-ERB α exhibited a marked positive correlation with the body mass index and waist circumference, and the expression of ROR α and Clock was correlated with HDL and low-density lipoprotein (LDL) levels, respectively (40). That study also indicated that the clock genes Cry2 and REV-ERB α were upregulated in obesity over a 24-h period (40).

Animal experiments also indicated that clock gene mutations are closely associated with dyslipidemia. Pan *et al* (41) reported that compared to ApoE^{-/-} mice, Bmal1^{-/-} ApoE^{-/-} and Bmal1 in liver (L-Bmal1)^{-/-} ApoE^{-/-} mice had an increased risk of hyperlipidemia and atherosclerosis but that L-Bmal1^{-/-} ApoE^{-/-} mice with adenovirus-mediated liver overexpression of Bmal1 had a reduced risk of hyperlipidemia and atherosclerosis. A recent study suggested that Bmal1 functions as a positive regulator of vascular smooth muscle cell (VSMC) proliferation following vascular injury (42), and liver-specific Bmal1- or REV-ERB α -knockout mice exhibited increased levels of cholesterol, triglycerides and free fatty acids (43). Furthermore, physiological studies have demonstrated that enterocytes expressing the dominant-negative Clock mutant protein (Clock ^{Δ 19/ Δ 19}) protein absorb more cholesterol from the intestinal lumen and secrete cholesterol and chylomicrons (16). This evidence indicates that Clock has a vital role in the regulation of cholesterol metabolism. Additional supporting evidence for circadian clock involvement has revealed that other core clock genes are associated with lipid metabolism. Grimaldi *et al* (44) indicated that Per2 was a natural modulator controlling the proadipogenic activity of peroxisome proliferating activated receptor (PPAR) γ and a major regulator of lipid metabolism. In addition, Per1/2-null mice or Per2-null mice had lower hepatic triglyceride levels than wild-type mice (37). Furthermore, REV-ERB α deficiency may cause marked hepatic steatosis (10), and mice lacking REV-ERB α displayed reduced levels of hepatic triglycerides and cholesterol and elevated levels of plasma lipids (45).

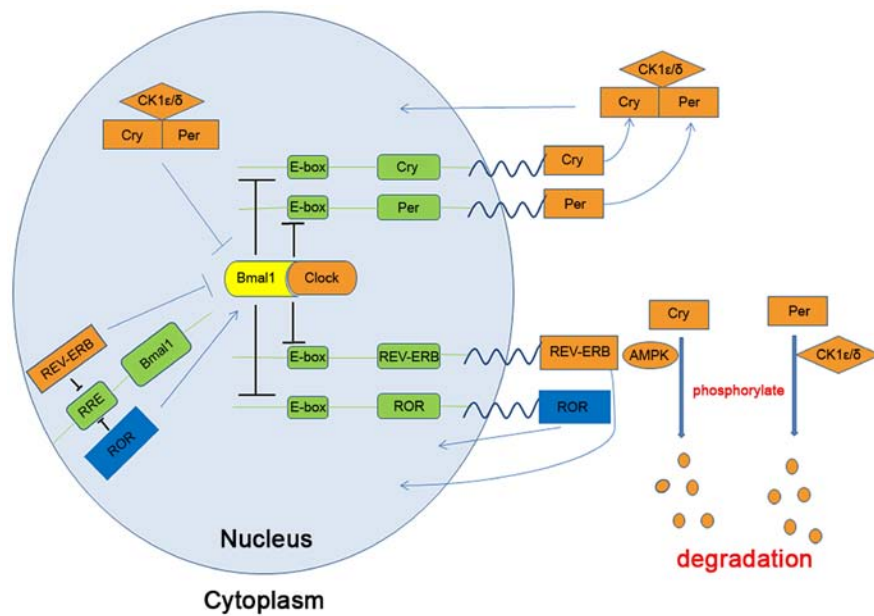


Figure 1. Schematic of the molecular clock consisting of two key negative feedback loops. Bmal1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1; Clock, circadian locomotor output cycles kaput; Cry, Cryptochrome/circadian clock; Per, Period circadian clock; REV-ERB, nuclear receptor subfamily 1, group D, member; ROR, Orphan nuclear receptor; RRE, ROR-binding element; CK1 ϵ/δ , serine-threonine kinase casein kinase 1 ϵ/δ ; AMPK, AMP-activated protein kinase.

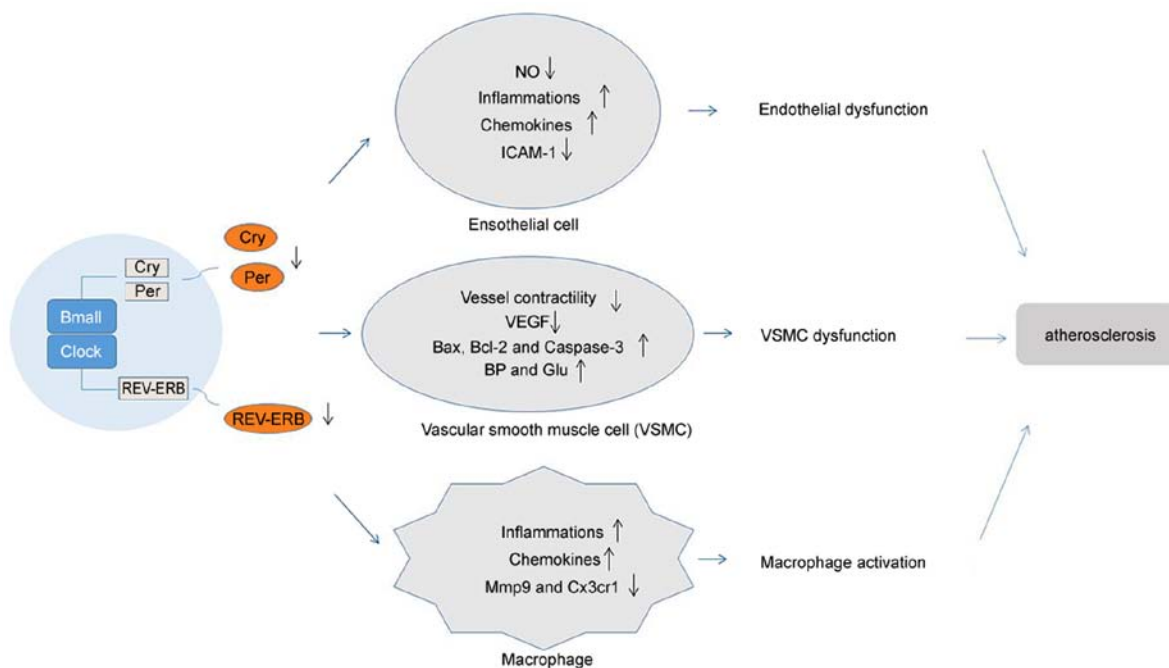


Figure 2. Schematic: The circadian clocks exerts effects on endothelial cells, vascular smooth muscle cells and macrophages and may have potential roles in atherosclerosis. Bmal1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1; Clock, circadian locomotor output cycles kaput; Cry, Cryptochrome/circadian clock; Per, Period circadian clock; REV-ERB, nuclear receptor subfamily 1, group D, member; NO, nitric oxide; ICAM-1, intercellular adhesion molecule-1; VEGF, Vascular endothelial growth factor; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; BP, blood pressure; Glu, glucose; Mmp9, matrix metalloproteinase9; Cx3cr1, CX3C chemokine ligand receptor 1.

5. Circadian rhythm and vascular cells

Emerging evidence indicates that circadian rhythm have a very close connection with vascular cells with regard to vascular function and health (46). Circadian rhythms influence the activities of systemic atherosclerosis mediators, including leukocytes and macrophages, and locally manipulate cells

within the vessel wall. Indeed, studies have indicated that the functional circadian clock exists within the vasculature (47). A growing number of studies (48-55) have identified that circadian clocks regulate the functions of endothelial cells, VSMCs and macrophages, suggesting the possibility of circadian clocks to influence the progression of atherosclerosis (as presented in Fig. 2). In the present review, a series of clock

Table I. Critical roles of circadian rhythm-associated genes in various types of vascular cell according to previous studies.

A, Endothelial cells		
Gene	Function and result	(Refs)
Clock	Loss of protective endothelial expression contributes to the vulnerability of human carotid plaque.	(48)
Bmal1	Major role in the regulation of intravascular coagulation.	(49)
Per2	Increased production of nitric oxide and vasodilatory prostaglandin(s) and decreased release of cyclooxygenase-1-derived vasoconstrictor(s).	(50)
B, Macrophages		
Clock gene	Function and result	(Refs)
Clock	Clock-mutant mice have increased circulating IL-12 and IL-17 levels and altered NF- κ B-induced macrophage activation.	(51)
Bmal1	Regulates oxidative stress pathways in macrophages to limit the production of the proinflammatory cytokine IL-1 β .	(52)
REV-ERB α	Activation of REV-ERB α reduces the severity of inflammation in peritoneal macrophages.	(53)
C, VSMCs		
Clock gene	Function and result	(Refs)
Bmal1	Knockdown of Bmal1 promotes VSMC apoptosis by regulating Bax, Bcl-2, cytochrome c and caspase-3 levels.	(54)
Clock	Knockdown of Clock promotes VSMC apoptosis by regulating Bax, Bcl-2, cytochrome c and caspase-3 levels.	(54)
REV-ERB α	REV-ERB α upregulates NF- κ B-responsive genes in VSMCs.	(55)
VSMC, vascular smooth muscle cell; Clock, circadian locomotor output cycle kaput; Bmal1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1; Per; Period circadian clock; REV-ERB, nuclear receptor subfamily 1, group D, member; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.		

genes with roles in vascular cells are presented. Those genes and their functions in different vascular cell types are listed in Table I.

Circadian rhythm and endothelial cells. It is generally known that dysfunction in the vascular endothelium is a pivotal factor in atherogenesis. Endothelial cells may be injured and accordingly activated by numerous stimuli, including oxidized LDLs, hypertension, hyperglycemia, turbulent blood flow and inflammation. Furthermore, endothelial cell activation leads to expression of adhesion molecules, loss of barrier function, migration of leukocytes into the vascular wall and improvement in inflammatory responses (46). Tang *et al* (48) indicated that loss of protective endothelial Clock expression contributes to the progression of atherosclerosis and aggravates plaque vulnerability. It appears that the circadian clock is able to regulate the release of nitric oxide (NO) and disruption of the clock leads to endothelial dysfunction (56). Endothelial dysfunction is associated with decreased bioavailability of endothelial NO, which is produced by endothelial NO synthase

(eNOS) and the relevant regulatory mechanisms of eNOS activity are closely interrelated with endothelial dysfunction in atherosclerosis (57). Human endothelial function is measured by flow-mediated dilation (FMD) and a recent study suggested that FMD was decreased in patients with congestive heart failure and that the circadian variation in endothelial function was deficient (58).

There is much evidence to support that Bmal1-knockout and Clock-mutant mice have endothelial dysfunction. Genetic ablation of the Bmal1 gene in endothelial cells enhances the expression of the chemokines C-C motif ligand (Ccl)8, Ccl20 and chemokine (C-X-C motif) ligand (Cxcl)5, damages endothelial integrity and barrier function (59) and leads to phenotypic features similar to those of diabetes (60). Furthermore, Gao *et al* (61) reported that the circadian clock may drive endothelial cells to express intercellular adhesion molecule-1 and promote the adhesion of monocytes to endothelial cells. Viswambharan *et al* (50) indicated that mutation in the Per2 gene in mice is associated with aortic endothelial dysfunction involving decreased production of NO and

vasodilatory prostaglandin and increased release of cyclooxygenase-1-derived vasoconstrictor. Carvas *et al* (62) concluded that Per2 gene mutation in aorta reduces insulin-stimulated NO release from endothelial cells. In a mouse model of sleep deprivation, Qin and Deng (63) indicated that sleep deprivation promoted the expression of proinflammatory cytokines and decrease that of Cry1 in vascular endothelial cells. Furthermore, Savalli *et al* (64) studied a naturalistic animal model of depression, concluding that chronic mild stress-induced anhedonic behavior is associated with disturbed diurnal oscillation of Clock, Cry2 and Rev-ERB α expression in the mouse basolateral amygdala and that Clock gene desynchronization appeared to be involved in vascular endothelial growth factor (VEGF) variations. In general, it is well accepted that the circadian clock is important for maintaining normal endothelial cell functions.

Circadian rhythm and VSMCs. VSMCs constitute the vascular media. During atherosclerosis progression, the migration of VSMCs from the middle layer to the intima is of great significance (65). VSMCs are stimulated by a variety of cytokines and inflammatory cytokines; the phenotype of SMCs switches from contractile SMCs to secretory SMCs and VSMCs simultaneously proliferate and migrate to the intima. Specifically, VSMCs and the extracellular matrix are the major components of the neointima and subsequent vascular stenosis (66-68).

Studies have indicated that specific clock gene knockout of Bmal1 in VSMCs impairs vessel contractility and decreases the blood pressure; in addition, smooth muscle-specific Bmal1 has a vital role in normal VSM contraction (69). According to Suyama *et al* (70), Bmal1 knockdown resulted in a decrease in VEGF and VEGF receptor mRNA expression compared with the control. Furthermore, VSMCs cultured from the carotid arteries of healthy donors exhibited regular circadian mRNA expression of Bmal1, Per1, Per2, Per3, Cry1, Cry2 and Rev-ERB α (71). In addition, the circadian rhythm of the major rhythm genes isolated from human plaque-derived VSMCs is significantly attenuated compared to that from cells cultured from the carotid arteries of healthy donors (71). Similarly, hyperglycemia and hyperlipidemia are associated with circadian gene expression in VSMCs (72,73). Su *et al* (72) suggested that expression of Per1/2, Cry1/2, D site albumin promoter-binding protein (Dbp) and PPAR γ in the aorta and mesenteric arteries in db/db mice was suppressed compared with that in control mice. Specifically, Migita *et al* (55) reported that Rev-ERB α causes upregulation of NF- κ B-responsive genes in VSMCs. These observations indicate that the circadian clock is of great significance to VSMC functions.

Circadian rhythm and macrophages. Macrophages are involved in the formation of atherosclerosis and have a different role in atherosclerotic lesion development (46). Macrophage accumulation within the vascular wall is a hallmark of atherosclerosis. In atherosclerotic lesions, macrophages respond to various environmental stimuli, such as modified lipids, cytokines, and senescent erythrocytes, which can modify their functional phenotypes. Furthermore, an increase in the inflammatory reaction decreases plaque stability and results in a thrombotic event. Of note, as with VSMCs and endothelial cells, circadian rhythms are able to regulate macrophage function (74).

In the wall of vessels affected by atherosclerosis, macrophages secrete inflammatory factors, including chemokines and cytokines. Specifically, the cytokine storms of infected mice display the greatest reaction at the beginning of infection (75). In addition, circadian expression of monocyte chemoattractant protein-1 (MCP-1/JE) in macrophages is regulated by Bmal1 via activation of NF- κ B (76). The molecular mechanisms by which cytokine production by macrophages is regulated remain to be fully elucidated. However, evidence indicates that the expression of Bmal1 is regulated by a circadian rhythm in macrophages (76). Rev-ERB α - or Bmal1-knockout macrophages displayed increased production of cytokines and decreased expression of circadian rhythm genes (75). Huo *et al* (77) concluded that Bmal1 deficiency in macrophages may exacerbate atherosclerosis by promoting the recruitment of Ly6Chi monocytes to atherosclerotic lesions. Similarly, compared to wildtype control mice, Clock-mutant mice have increased circulating IL-12 and IL-17 levels and altered NF- κ B-induced macrophage activation (16,51). Furthermore, Rev-ERB α regulate enhancer-derived RNAs, suppressing the expression of nearby genes, including Mmp9 and CX3C chemokine ligand receptor 1 (Cx3cr1; one of the most expressed genes in microglia in mice and humans, is implicated in numerous microglial functions) in macrophages (78), and Rev-ERB α modulate the inflammatory infiltration of macrophages by inhibiting the expression of Ccl2 (79). It remains elusive whether clock genes control local macrophage proliferation.

6. Circadian rhythm and inflammatory immunoreactions

The levels of immune inflammatory immunoreactive cells and pro-inflammatory cytokines have an obvious daily rhythm (80) and functionality of the immune system has been linked to the circadian clock (81). A recent study reported that circadian disruption by sleep fragmentation accelerates atherosclerosis development by increasing the number of circulating monocytes (82). Consequently, disturbed circadian clock function may contribute to the risk of atherosclerosis through the pro-inflammatory state. Based on *in vivo* and *in vitro* experiments, the circadian rhythm is closely linked to inflammatory immunoreactions (52,83). In cells lacking Bmal1, lower expression of BMAL1 may affect mediators of inflammation and oxidative stress (52). Keller *et al* (74) indicated that Bmal1 mRNA expression in macrophages was lowest at the active phase (zeitgeber time 12). Furthermore, in animal models of inflammatory bowel disease (51), the expression of inflammatory factors in Clock gene-mutant mice was decreased compared with that in wild-type mice. In addition, the circadian rhythm expression of IFN- γ disappeared in Per2-mutant mice (84). In an arthritis model (85), simultaneous knockout of Cry-1 and Cry-2 led to an increase in TNF- α and aggravated the inflammatory response. In addition, the clock genes Rev-ERB α and ROR α have important roles in inflammatory immunoreactions (86,87).

7. Conclusions

Numerous studies have suggested that the circadian rhythm is significant regarding several aspects of atherosclerosis,

including glycometabolism, lipid metabolism, endothelial cell dysfunction, VSMC phenotype and inflammatory immune-reactions. However, the mechanism of specific clock genes involved in the atherosclerotic process remains elusive. Thus, further research on the relationship between the circadian rhythm and atherosclerosis is required. There is much to learn about the circadian rhythm, promoting a healthy lifestyle that includes sufficient, regular sleep, this may provide novel therapeutic targets and preventative measures to simultaneously slow the development of atherosclerosis and reduce cardiovascular mortality.

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Availability of data and materials

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Authors' contributions

ZZ, JD and XW conceived and designed the article. ZZ, BY, CL, XZ and TZ collected related articles and analyzed the relevant literature. ZZ and BY wrote the manuscript and drew the figures. JD, CL, XZ, TZ and XW revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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