Circulation

SYSTEMATIC REVIEW

Circadian-Regulated Cell Death in Cardiovascular Diseases

BACKGROUND: Over the past several years, a variety of human and animal studies have shown that circadian clocks regulate biological cardiovascular rhythms in both health and disease. For example, heart rate and blood pressure fluctuate over 24-hour daily periods, such that levels are higher in the morning and progressively decline in the evening.

METHODS AND RESULTS: It is interesting to note that the timing of the administration of various cardiac treatments can also benefit some cardiovascular outcomes. Circadian rhythms have been implicated in the pathogenesis of a number of cardiovascular diseases, including myocardial infarction, ischemia-reperfusion injury after myocardial infarction, and heart failure. Cell death is a major component of ischemia-reperfusion injury and posited as the central underlying cause of ventricular remodeling and cardiac dysfunction following myocardial infarction. It is notable that the time of day profoundly influences cardiac tolerance and sensitivity to cardiac injury.

CONCLUSIONS: Herein, we highlight the novel relationship between circadian rhythms and homeostatic processes that governs cell fate by apoptosis, necrosis, and autophagy. Understanding how these intricate processes interconnect at the cellular level is of paramount clinical importance for optimizing treatment strategies to achieve maximum cardiovascular outcome.

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Clinical Perspective

What Is New?

- Circadian clocks regulate biological cardiovascular rhythms in health and disease.
- Lifestyle disturbances to the circadian rhythms such as shift working, jet lag, or daylight saving time shifts can increase the incidence of fatal and non-fatal cardiovascular diseases.
- The mechanism of action of many cardiovascular drugs is subjected to circadian control. Circadian-dependent regulation of drug administration or chronotherapy can increase drug efficacy and reduce off-target effects.
- A major component of myocardial infarction is cell death, which is subjected to circadian control.

What Are the Clinical Implications?

- The time of day of therapy should be considered by physicians and healthcare providers to maximize benefits to patients with cardiovascular disease.
- Alterations in normal environmental conditions within intensive care units disrupt circadian rhythms that negatively influence cardiovascular outcomes.
- Implementing protocols for the time of day of therapy will have a major positive impact on heart disease outcomes and susceptibility to myocardial infarction.

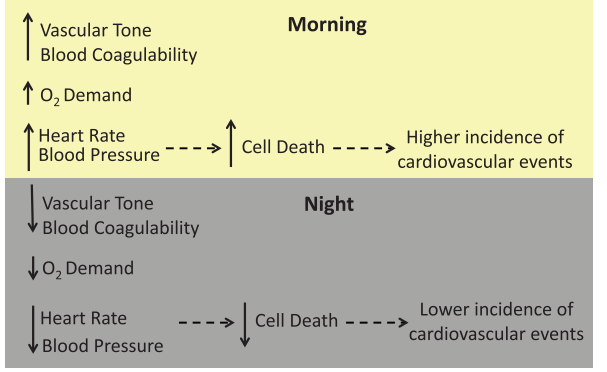

Figure 1. Circadian rhythm regulates the physiological and pathological function of the heart.
During the night period, vascular tone and blood coagulability are lower. This is accompanied by reduced heart rate and blood pressure and reduced cell death. Altogether, it leads to a lower incidence of having cardiovascular events. In the morning hours, the vascular tone and blood coagulability are increased, together with increased oxygen demand, increased heart rate and blood pressure, and activation of the cell death process. The combination of all these processes leads to increased incidence of cardiovascular events.

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide.[1] A growing body of experimental and clinical evidence points toward a significant link between cardiac dysfunction and intrinsic circadian biology. There is now substantial and compelling evidence that diurnal changes in the normal daily 12-hour light and 12-hour dark cycle profoundly influence the physiology and pathophysiology of the cardiovascular system (Figure 1). It is notable that one of the most profound effects of the circadian rhythm on cardiovascular function is its influence on blood pressure (BP) and heart rate (HR). These parameters exhibit diurnal rhythmicity with BP higher in the morning and lower in the evening. Endothelial cell function, platelet aggregation, and thrombus formation are also known to exhibit normal circadian rhythms that follow a cycle of 24 hours. Of note, the onset of many CVDs or cardiac events, such as myocardial infarction (MI), arrhythmias, stroke, heart failure, and sudden cardiac death, also exhibits diurnal variation with higher incidence of events occurring in the early morning.[2]

Perhaps one of the most highly studied forms of cardiac disease is ischemia-reperfusion (I-R) injury. During ischemia, impaired coronary blood flow results in tissue hypoxia, which can provoke severe cellular tissue injury, resulting in adverse cardiac remodeling and dysfunction.[3] Timely and effective reinstitution of blood flow (reperfusion) is the most effective modality for limiting myocardial cell death and ventricular dysfunction. However, it is well known that reoxygenation of ischemic cardiac tissue and reenergization of mitochondria may concurrently exacerbate cardiac cell damage through the generation of reactive oxygen species and the activation of cell death programs on reperfusion.[4] Although several studies have evaluated different clinical pharmacological therapies for mitigating I-R injury following MI,[5,6] early reperfusion is still the primary determinant of functional recovery following I-R injury. Indeed, the critical concept that time is muscle is reflected by the refinement in emergency department protocols for rapid reperfusion with thrombolytic therapy, cardiac catheterization, and bypass surgery.[5,7] Despite these advanced treatments, a significant number of individuals with coronary artery disease do not respond well to revascularization therapy following MI. Indeed, many individuals experience diminished cardiac performance and adverse pathological remodeling that ultimately lead to heart failure.[6] The mechanisms that underlie contractile dysfunction post-MI reperfusion remain poorly understood. At the cellular level, defects in the expression of calcium-handling proteins, metabolic regulators, and the components of mitochondrial respiratory chain, as well, have all been purported as underlying causes of I-R injury.[8] However, one unifying theme posited to explain underlying cardiac dysfunction and ventricular remodeling associated with I-R injury is the loss of cardiomyocytes through the activation of cell death programs (Figure 1).[9] Cell death during I-R injury is viewed

as a major underlying cause of ventricular remodeling and cardiac dysfunction following MI. In this context, cardiomyocyte death programs activated during I-R include but are not limited to apoptosis, necrosis, ferroptosis, parthanatos, pyroptosis, autophagic-dependent cell death, mitochondrial-mediated cell death, and others.[9–11] In particular, many of these forms of cell death involve mitochondria, identifying them as a major signaling platform for cardiomyocyte death. At present, the mechanisms by which circadian rhythms impinge on mitochondrial function and cell death signaling are not well defined. However, there is emerging evidence that expression of several genes associated with longevity and cell survival are transcriptionally regulated in a circadian-dependent manner,[12,13] highlighting the importance of the circadian mechanism as a determinant of cellular homeostasis and survival.

Here we provide a general overview of circadian rhythms and discuss their impact on cellular processes that regulate cell death in CVDs. Furthermore, we highlight the clinical applications of circadian rhythm biology and discuss the concept of chronotherapy as a strategy for achieving better outcomes in patients with CVDs.

OVERVIEW OF THE CIRCADIAN RHYTHM

Virtually all tissues of the body have a circadian mechanism that controls the timing of many key biological reactions in response to a variety of environmental cues.[14] The molecular machinery for regulation of circadian rhythms is highly conserved and found in almost every cell type. The circadian mechanism is regulated centrally by input from the hypothalamic master pacemaker, which uniformly synchronizes cells of the body to their intrinsic body time. In mammals, the hypothalamic master clock resides in the suprachiasmatic nucleus (SCN), and it coordinates the daily rhythms of peripheral oscillators or clocks in the different cells of the body.[15] As shown in Figure 2, the mammalian circadian machinery within the SCN receives signals initiated by changes in light that are transmitted by dedicated photoreceptors within the retinal cells of the eyes.[15] The SCN then transmits information to the rest of the body via a combination of neural and humoral signals.

The molecular composition of the mammalian circadian clock involves a well-defined set of genes and proteins that form a hierarchical network of positive and

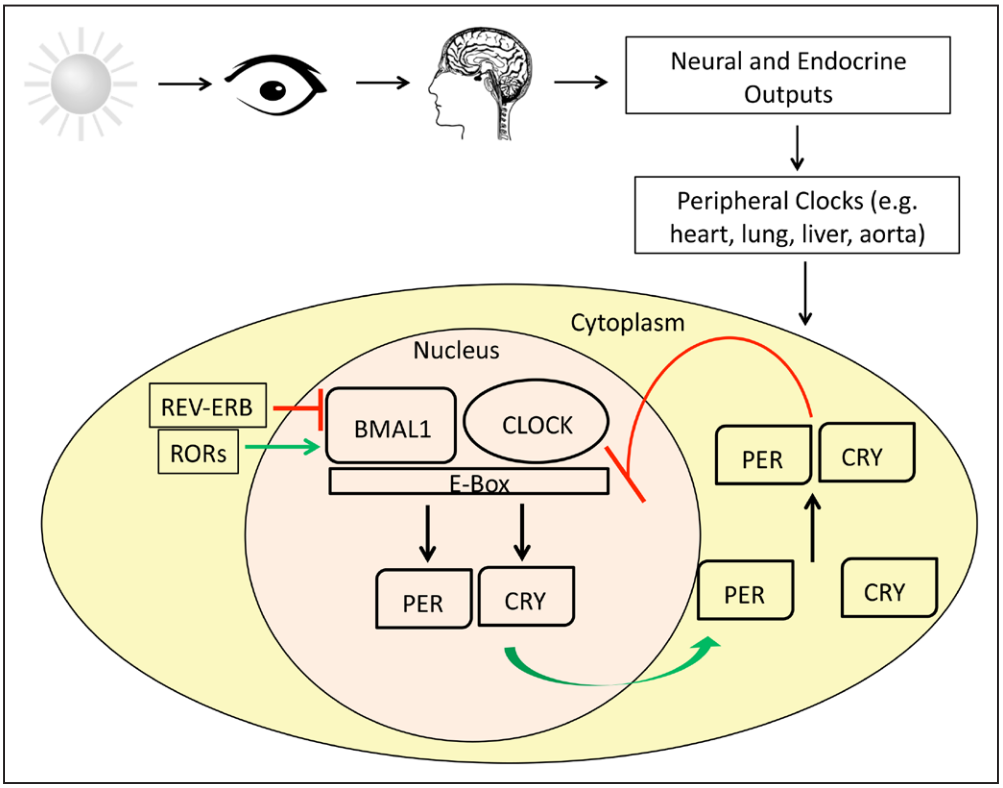

Figure 2. Molecular components of the circadian clock.
The mammalian circadian machinery starts with light signals that are absorbed by photoreceptors located in the eyes and then transmitted to the suprachiasmatic nucleus. The molecular composition of the circadian system involves transcriptional feedback loops that start with the circadian genes *CLOCK* and *BMAL1* that, on activation, heterodimerize in the nucleus and bind E-box DNA elements on target gene promoters. This step is followed by activation of *PERIOD* (Per1 and Per2) and *CRYPTOCHROME* (Cry1 and Cry2) genes. *PER* and *CRY* heterodimerize in the cytoplasm and inhibit the transcriptional activity of *CLOCK:BMAL1* dimers. Heterodimerization of *CLOCK:BMAL1* also initiates transcription of the orphan nuclear-receptor genes *Rev-Erbα/β* and *RORα/β*. ROR proteins can initiate *BMAL1* transcription and REV-Erb can inhibit it. BMAL1 indicates brain and muscle Arnt-like protein-1; CLOCK, circadian locomotor output cycles kaput; CRY, CRYPTOCHROME; PER, period; and RORα/β, retinoic acid receptor–related orphan receptors α/β.

negative feedback loops that regulate mRNA and protein expression of key components of the clock machinery, and many biological processes, as well.[15] In brief, the positive arm of the circadian mechanism involves transcriptional activation of the genes *Clock* (circadian locomotor output cycles kaput) and *Bmal1* (brain and muscle Arnt-like protein-1), which heterodimerize within the nucleus and bind to E-box DNA elements on target gene promoters.[15] This leads to the transcription of genes in the negative arm of the circadian mechanism, namely *Periods* (*Per1* and *Per2*) and *Cryptochromes* (*Cry1* and *Cry2*). The subsequent dimerization of *PER* and *CRY* within the cytoplasm inhibits the transcriptional activity of *CLOCK:BMAL1* dimers. Degradation of *PER* and *CRY* is required to abrogate the inhibitory effects on *Clock* and *Bmal1* transcription.[15] Hence, *PER* and *CRY* provide an important feedback mechanism to limit *CLOCK:BMAL1* activation. *CLOCK:BMAL1* heterodimers also regulate transcription of the orphan nuclear-receptor genes *Rev-Erbα/β* and *RORα/β,* which also provide an additional layer of transcriptional control over the circadian mechanism machinery. It is notable that, whereas ROR proteins can activate transcription of *Bmal1,* REV-ERB conversely inhibits transcription of *Bmal1*. These positive and negative feedback loops provide a mechanism for circadian rhythms to fine-tune target gene expression.[15]

In addition to the SCN, each organ system is regulated by intrinsic circadian rhythms that are controlled by intracellular circadian genes and proteins referred to as peripheral clocks. The mechanisms that govern peripheral clock activity are believed to be the same as that of the central clock, but lead to the output of organ-specific time-of-day genes, proteins, and physiological processes.[14]

Both the central clock and the peripheral clocks are endogenously generated and self-sustained by an intrinsic period of ≈24 hours.[14] Although the circadian clock mechanism outputs govern many biochemical processes as a function of the time of the day or night, the mechanism can also operate in the absence of light/dark cycles. In this case, the endogenous clocks are dynamically regulated and can be modified by other external cues, such as food intake, temperature, or environment. The environmental stimuli that synchronize the 24-hour circadian rhythm are known as zeitgebers, from the German word meaning time giver.[15] In this regard, zeitgebers reportedly influence circadian rhythms in a cell- and tissue-specific manner. For example, it was previously shown that glucose could serve as a zeitgeber for fibroblast circadian control in culture. In this study, the authors showed that readdition of glucose to the culture media following glucose deprivation was sufficient to resynchronize circadian gene oscillations following nutrient deprivation that was attributed to downregulation of *Per1* and *Per2* mRNA levels. However, whereas glucose had little to no effect on circadian gene expression in cardiomyocytes,[16] norepinephrine resynchronized rhythmic expression of circadian clock genes.[17] Furthermore, angiotensin II has been shown to be a zeitgeber in the vascular smooth muscle cells. Treatment of vascular smooth muscle cells with angiotensin II induced synchronous cyclic expression of *Per2* mRNAs. This effect was abolished by CV11947, a specific angiotensin II type 1 receptor antagonist.[18]

Given that both central and peripheral clocks are driven by distinct zeitgebers raises these questions: Why are both types of clocks needed? What synchronizes and regulates each clock? Under which conditions do the peripheral and central clocks communicate? Which clock is more dominant under a given physiological or pathological condition? These intriguing questions and others will need to be answered in future studies.

CIRCADIAN RHYTHM AND CVD

As shown previously, zeitgebers regulate circadian rhythms in cardiomyocytes, fibroblasts, and vascular smooth muscle cells differentially in a cell- and context-specific manner to maintain a healthy functioning cardiovascular system. It is interesting to note that one of the most profound effects that circadian biology has on the cardiovascular system is reflected by the diurnal variation in BP and HR. The typical circadian rhythmicity of daily BP and HR is defined by a peak in the early morning, followed by gradual decline later in the day and early evening, with maximal decline during sleep. This variation in BP and HR in a 24-hour cycle can be influenced and modulated by intrinsic and environmental factors such as mental stress, physical activity, food consumption, temperature, noise, etc. A growing body of evidence suggests that atypical circadian variations in BP lead to an increased incidence of fatal and nonfatal CVDs[19] (Figure 1). For example, studies that monitored daily variations in BP determined that stable nighttime BP has a better predictive index of future cardiovascular events than the daytime BP or daily mean values of BP.[20] It is notable that individuals with a nondipper BP profile, ie, defined as individuals with impaired normal sleep-associated decline in BP, have an increased risk of developing left ventricular hypertrophy, heart failure, MI, and stroke in comparison with individuals who exhibit normal nighttime decline in BP.[21] Moreover, inverse dippers, or individuals whose BP rises during sleep, also have significantly higher occurrence of strokes than those with normal circadian patterns of BP variation,[22] suggesting that individuals with a BP profile that does not follow the normal circadian rhythm may be at higher risk for adverse cardiovascular events. Several clock genes were recently reported to play a key role in the daily regulation of BP.[22] Of note, *BMAL1*

was shown to be responsible for the daily variations in BP. In this study, global genetic deletion of *BMAL1* in mice disrupted circadian rhythm and the daily variation in BP that resulted in the loss of BP control and hypotension.[23] Other core circadian genes involved in BP regulation involve the negative circadian feedback regulators *Cry1* and *Cry2*. In contrast to genetic deletion of *BMAL1*, compound deletion of both *Cry1* and *Cry2* genes resulted in increased BP and hypertensive mice.[24] It is notable that *Per1* was also found to regulate the expression of several genes related to sodium transport in the kidney. In this study, urinary sodium excretion demonstrates a circadian pattern with a peak during active periods. This expression was diminished in *Per1* knockout mice, promoting *Per1* as a mediator of circadian BP rhythms via the regulation of distal nephron sodium transport genes.[25]

In addition to BP regulation, circadian rhythms and time of day also have a major impact on the onset and severity of cardiac injury following I-R and MI. At the cellular level, ischemia or hypoxia is sensed in cardiomyocytes by hypoxia-inducible factor 1 α (*HIF-1α*). *HIF-1α* is a basic helix-loop-helix PAS (Per-Arnt-Sim) domain transcription factor that localizes to the nucleus of hypoxic cells from inactivation of the pyrrole hydroxylase-VHL (Von Hippel–Lindau) degradation pathway. In the nucleus, *HIF-1α* binds to hypoxia response elements in the promoters of a wide range of genes involved in cell metabolism, cell survival, and cell death.[26] Although *HIF-1α* has been typically associated with transcriptional control of hypoxia-dependent gene transcription, *HIF-1α* has also been implicated in the circadian gene regulation through its association with *Clock*. In particular, it is widely believed that homology in the binding characteristics of *PER2* and *HIF-1α* permit cross talk between the circadian gene *Clock* and *Hif-1α* in regulation of vasopressin gene expression in the SCN. However, this apparent cross talk does not involve heterodimerization of *HIF-1α/CLOCK* at respective E-box elements,[27] suggesting that cross talk between mediators of hypoxia-induced cardiac cell injury and circadian pathways shares overlapping properties for target gene expression. Along these lines, cardiomyocytes expressing a stable form of *HIF-1α* were resistant to I-R injury, and expressed several genes including *HSP-70* and *Glut-4* associated with ischemic preconditioning.[28] This study raises the interesting possibility that *HIF-1α*– and *Clock*-regulated gene expression may be involved in ischemic preconditioning; however, further investigations are needed to support this notion.

In this regard, the incidence of MI is greater in the early morning,[2] a finding consistent with the poor tolerance of the myocardium to I-R injury during the sleep-to-wake transition.[29] Disruption of circadian rhythms in cardiomyocytes impairs the time-of-day oscillations in metabolism, and other physiological processes, as well, thereby increasing the susceptibility of cardiomyocytes to cell death activation and cardiac dysfunction.[29] One paradigm purported to explain the higher risk of MI in the morning hours has been linked to increased BP (discussed above) and coronary vascular resistance. It is believed that these otherwise normal diurnal oscillations in BP and vascular resistance may further compromise oxygen delivery in individuals with existing ischemic heart disease,[30] resulting in greater risk for MI. It remains to be established whether light/dark cycles also influence cardiomyocyte HIF1-α levels.

Concordant with these findings, the extent of myocardial injury following MI correlates directly with the time of day in which the ischemic insult occurred.[29] Indeed, a recent experimental study in rodents reported that circadian-dependent effects on MI mortality were greater in males versus females.[31] It is remarkable that 6 independent meta-analysis studies[32] that examined the link between the effects of daylight saving time shifts and the incidence of acute MI revealed that shifting the normal circadian rhythm by as little as 1 hour significantly influenced the incidence and risk for cardiovascular events. In particular, switching to daylight saving time in the spring significantly increased the incidence and risk of MI during the first 3 weeks after the transition; however, this effect was surprisingly greater in women than in men.[33] Conversely, switching back to daylight saving time in the fall had the opposite effect with a greater risk for MI in men than in women within the first week after the time change.[33] Moreover, people who self-identify as late chronotype (preverbal night owl) were found to have more problems adjusting to the spring saving time change[33]; it is tempting to speculate that these people have an increased risk for MI. The underlying triggers that account for the adverse cardiac events associated with disrupted circadian rhythms remain cryptic, but are postulated to result from a number of interceding factors including sleep deprivation, altered diurnal patterns of sympathetic activity, hormonal changes, and the production of inflammatory cytokines.[33] It is intriguing to note that none of the 6 meta-analyses provided information on duration and quality of sleep, circadian habits of the individual, and personal chronotype, which may have shed more insight into the subgroups that are at greater risk for MI following daytime changes. Another interesting link between disrupted circadian rhythms and poor prognosis following MI is illustrated in intensive care units (ICUs). Individuals within the ICU environment are subjected to noise and artificial light at night, and frequent disruption of normal sleep patterns because of diagnostic and therapeutic interventions, and these conspire to disturb circadian rhythms and sleep.[34] A 2-month study in the ICU setting demonstrated that one-fifth of critically ill patients admitted to the ICU had sustained some cardiac event including acute MI. Moreover, patients who had an acute MI were more likely to die by an incidence

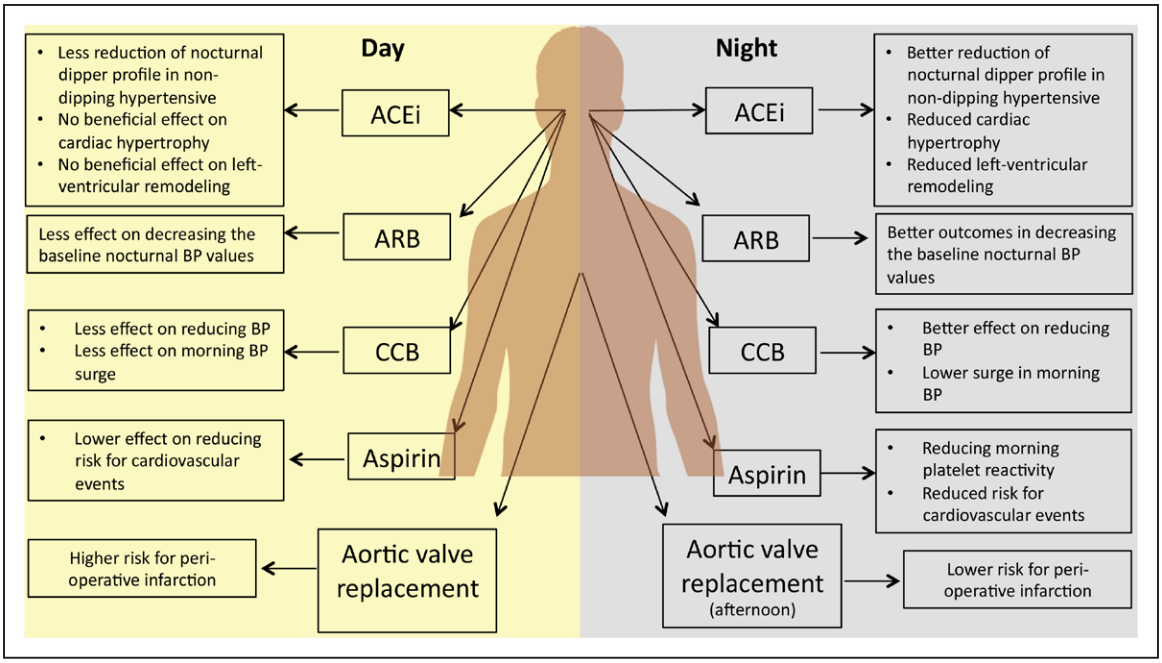

Figure 3. Chronotherapy in cardiovascular diseases.
Established therapies that are recommended to be taken in the dark phase to increase efficacy and improve outcome. Hypertensive medications, including ACEi, ARB, and CCB, administered at bedtime can increase the efficacy on blood pressure. Aspirin administered at bedtime can decrease the risk for cardiovascular events. Cardiac surgery performed in the afternoon can lower the risk of adverse cardiac events following surgery with improved patient outcomes. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BP, blood pressure; and CCB, calcium channel blocker.

of 50% attributable to ICU conditions.[35] Furthermore, disruptions in circadian rhythms in the ICU were also shown to be accompanied by abnormalities in the daily variations of BP and HR.[34]

The impact of circadian rhythm disturbances on increased risk of CVD is best illustrated in individuals who do shift work and travelers who experience jet lag.[36] The first study to document the impact of circadian rhythm on cardiovascular health and disease was published >30 years ago; it followed the cardiac status of paper mill workers for >15 years. The study showed that the incidence of ischemic heart disease was higher in shift workers than in normal daytime workers. These findings were independent of age or smoking history, emphasizing the importance of maintaining a normal circadian rhythm or sleep pattern for cardiovascular health.[37]

It is remarkable that the adverse effects of circadian rhythm disruptions have been shown to influence other CVD outcomes. For example, desynchrony between the external environment and endogenous circadian rhythms is exemplified in a hamster model of dilated cardiomyopathy. Here, tau+ hamsters develop dilated cardiomyopathy from a mutation in casein kinase 1, which is known to phosphorylate the circadian regulator *Per2*.[38] Moreover, a mutation in *CLOCK* leads to development of an age-dependent cardiomyopathy in male mice. It is intriguing that female mice overexpressing *CLOCK* are protected from developing age-dependent heart disease. Ovarian hormones play an important role in protecting female mice from heart disease even in the presence of circadian disruption.[39] Other studies have shown that *BMAL1* knockout mice have dampened cardiovascular circadian rhythms and develop an age-dependent dilated cardiomyopathy.[40] Knockout of *BMAL1* or deletion of all 3 isoforms of *Cry* in mice contributed to arterial stiffness and the impairment of extracellular matrix composition.[41] Furthermore, mutation in the *Per2* gene was associated with aortic endothelial dysfunction, decreased production of nitric oxide and other vasodilatory prostaglandins,[42] and Akt-dependent senescence and impaired ischemia-induced revascularization, as well.[43]

As summarized in Figure 3, a better understanding of the mechanisms that govern normal circadian rhythms on cardiovascular health may prove beneficial in reducing cardiovascular risk and improving patient outcomes.

CHRONOTHERAPY: RESETTING THERAPY IN ACCORDANCE WITH THE CARDIAC CIRCADIAN RHYTHM

The concept of chronotherapy aims to maximize the beneficial effects of drug therapy by coordinating treatment with the rhythmicity of the biological clock. The toxicity of a given drug is related to its rate of absorption, metabolism, and clearance from the body. Many

organ systems such as the liver and kidney that are involved in drug disposition are also dependent on the diurnal variation. Thus, the same medication administered under identical conditions may not exhibit the same pharmacokinetics and pharmacodynamics profile when given at different times of the day, thereby reducing its efficacy or even causing unwanted adverse effects of the drug.[44]

Perhaps one of the most clinically established examples of the impact of chronotherapy is with antihypertensive agents. Increases in visit-to-visit BP variability and dipping BP profiles are associated with an increased risk for MI, stroke, and heart failure. Recent studies found that individuals with increased BP have significantly lower survival rates and worse prognosis following MI and stroke than individuals with lower BP rates.[45] Chronotherapy studies with antihypertensive agents showed better regulation of BP rates in accordance with the time of the day of administration, which might translate into reduced future risk for CVDs and better prognosis. For example, angiotensin-converting enzyme inhibitors that lower BP when administered at the time of sleep may help to recapitulate dipper status in nondipping hypertensives.[46] Another clinical study demonstrated the benefit of timing angiotensin II receptor blocker medications.[47] In this study, the administration of the angiotensin II receptor blocker telmisartan to hypertensive individuals at bedtime caused significantly decreased baseline BP at night. According to this study, administration of telmisartan at bedtime reduced the prevalence of nondipping BP from baseline by 76% and increased the nighttime drop in BP toward a more normal dipper profile[48] (Figure 3). Calcium channel blockers such as nifedipine may also benefit from timed therapy in lowering BP. Indeed, nifedipine has been shown to efficiently reduce BP when administered after bedtime, and the morning surge in BP at the time of highest likelihood of adverse cardiovascular events, as well. Collectively, these findings suggest that nifedipine[49] and other antihypertensive therapies[44] should be administered preferentially at bedtime to maximize the therapeutic benefit on lowering BP (Figure 3).

It is important to note that chronotherapy has significant clinical benefits for the heart. Indeed, it has been shown experimentally that administration of angiotensin-converting enzyme inhibitors to mice with cardiac hypertrophy has greater benefit in reducing cardiac remodeling if the drugs are given at sleep time, in comparison with treatment in the morning. Mechanistically, many drugs target the products of rhythmic genes, and thus the administration of angiotensin-converting enzyme inhibitors at sleep time may target the nighttime rhythms in renin-angiotensin system targets[50] (Figure 3). Another example is chronotherapy for patients with obstructive sleep apnea. Treating with continuous positive airway pressure therapy at night suppresses adverse nocturnal sympathetic activity, lowers BP, and improves myocardial systolic function in patients with heart failure.[51]

An additional case that highlights the benefit of chronotherapy on CVD outcome is best illustrated by the time of day in which aspirin (acetylsalicylic acid) is administered. Chronic administration of aspirin has been proven to reduce the risk of recurrence of adverse cardiovascular events such as MI, ischemic stroke, and blood clots in high-risk individuals. Aspirin is a nonsteroidal anti-inflammatory drug that suppresses platelet activity. It is notable that platelet activity peaks during the morning hours, concordant with other risks of cardiovascular events such BP and vascular resistance. A recent study showed that aspirin taken at night had a greater effect on reducing morning peak platelet reactivity (stickiness) and thus could contribute to reduced risk of morning cardiovascular events such as MI in hypertensive individuals[52] (Figure 3). Another reason for taking aspirin at night is supported by microarray analysis that showed that Ptgs1, the target of aspirin, oscillates throughout the day. Because aspirin is known to have a short half-life, it is highly important to consider administering aspirin at the optimal time of day for improved efficacy.[13]

Moreover, a recent publication by Montaigne and colleagues[53] showed that time of day affects not only the efficacy of medications, but also cardiovascular surgical outcomes. Individuals who underwent morning surgery for aortic valve replacement were more likely to experience a major cardiovascular event (ie, a perioperative infarction) or other cardiac complications after surgery than individuals undergoing the same surgery in the afternoon. Moreover, these cardiac events were also associated with diminished left ventricular ejection fraction and higher incidence of acute heart failure within the first 500 days postsurgery. In contrast, individuals who underwent surgery in the afternoon did not display the same level of adverse risk for cardiac events as the early-morning cohorts.[53] The observations on the benefit of time-of-day treatment by Montaigne et al[53] were further substantiated by an experimental rodent study in which the manipulation of specific clock genes influenced circadian rhythm and cardiac resistance to I-R injury. In this study, pharmacological inhibition or genetic deletion of *Rev-Erbα* reduced cardiac damage at the time of sleep-to-wake transition, which was related to increased expression of the cell cycle regulator *CDKN1a/p21*[53] (Figure 3).

It is interesting to note that RNA-seq and DNA arrays that quantified transcriptomes of 12 mouse organs, including the heart, found that best-selling drugs and World Health Organization essential medicines directly target proteins that are encoded from genes that were found to oscillate rhythmically throughout the day.

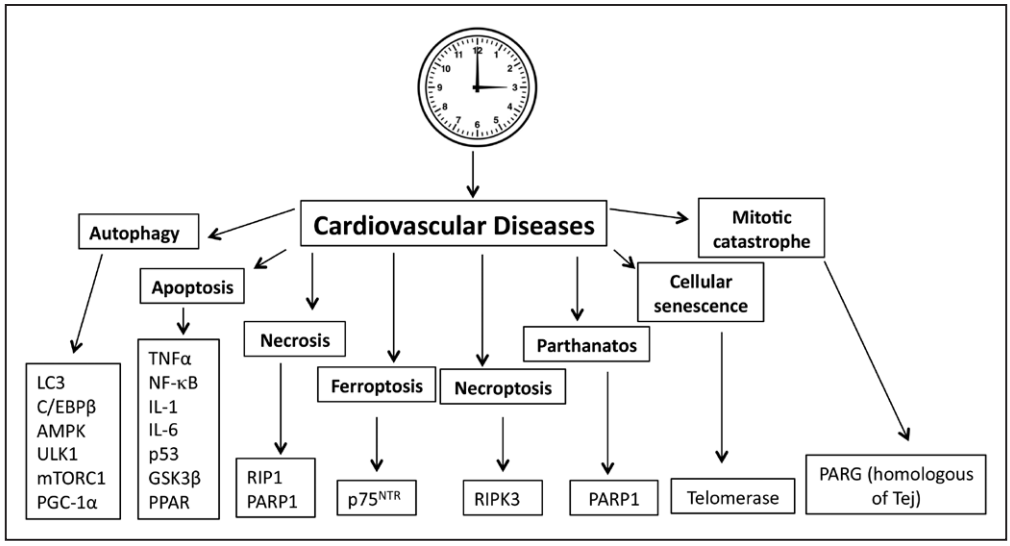

Figure 4. Circadian rhythm regulates cell death in cardiovascular diseases.
Scheme depicts established links and putative regulators involved in circadian control. AMPK indicates adenosine monophosphate–activated protein kinase; GSK3β, glycogen synthase kinase 3 β; IL, interleukin; mTORC1, mechanistic target of rapamycin complex 1; NF-κB, nuclear factor κ-B; PARG, poly(ADP-ribose) glycohydrolase; PGC-1α, peroxisome proliferator–activated receptor gamma coactivator 1-alpha; PPAR, peroxisome proliferator–activated receptor; TNFα, tumor necrosis factor α; and ULK1, unc-51-like kinase 1.

Moreover, many of these drugs have short half-lives of their active component, meaning that timing the administration of these drugs may significantly benefit their effects.[13]

To summarize, special attention should be given to the fact that circadian dynamics can increase or decrease a treatment above or below its threshold, respectively. Furthermore, with the increasing understanding of the significance of circadian rhythm on cardiovascular health, it is just a matter of time until physicians will start to include recommendations for drug timing to their patients' prescriptions. Indeed, as the world moves toward personalized medicine, optimization of cardiac drug administrations and procedures is just another step in tailoring treatment in ways that may lead to improved outcomes. To this end, researchers have been developing state-of-the-art approaches to determine our body's optimal time, by taking clinical samples a few hours apart for tests to assess a person's biological clock timing.[54] Although the underlying mechanisms that contribute to adverse cardiac remodeling following MI remain poorly understood, one unifying theme purported to contribute to impaired cardiac function and diminished ventricular performance following MI is the increased loss of functional cardiomyocytes through activation of various cell signaling pathways and cell death programs. Autophagy, apoptosis, and necrosis are the major signaling processes that get activated under stress conditions. However, the link between circadian rhythms and these processes in cardiomyocytes remains to be established. Herein, we highlight the existing literature demonstrating a link between circadian rhythm and cell death signaling processes (Figure 4).

CIRCADIAN RHYTHM AND AUTOPHAGY

Autophagy is a highly conserved evolutionary process that regulates protein degradation, organelle turnover, and recycling of cellular components during cellular stress. The autophagic process starts with the formation of autophagosome, in which its contents are subsequently degraded by fusion with the acid-rich lysosomes, which allows for the release of amino acids and other cellular nutrients that can then be used for energy production.[55]

Autophagy in the heart is essential for the maintenance of cardiovascular homeostasis and function. However, excessive or insufficient autophagy beyond a given threshold could be detrimental and contribute to cardiac dysfunction and disease pathogenesis.[55] In the context of I-R injury, initiation of autophagy during the early ischemic phase before reperfusion is protective, whereas late or delayed activation of autophagy during reperfusion is detrimental.[56] A better understanding of the link between circadian regulation, autophagy, and cardiovascular function may lead to improved optimization of treatments and cardioprotection following MI. As will be described below, the circadian-dependent control of autophagy has been well established.

The first evidence for a link between autophagy and circadian regulation was established in the early 1970s. Using electron microscopy, Pfeifer demonstrated that the presence of autophagic vacuoles and atrophy of the liver followed a diurnal pattern in meal-fed rats in comparison with nutrient-deprived controls.[57] In subsequent studies, the same group showed that autophagy also followed a diurnal pattern in kidney tubules

of normal rats with a greater number of autophagic vacuoles present in the early morning and declined levels during the evening and at night.[58] Furthermore, and perhaps most compelling, was the finding that the volume and numeric density of autophagic vacuoles in the heart also followed a diurnal pattern, which peaked during the late-light phase and later declined toward the early-dark period.[59] Collectively, these studies demonstrate that peripheral regulated circadian-dependent autophagy is operational in many organ systems and may play a key role in tissue and organ repair. This may explain why maintaining sleep hours is critical for normal physiological processes and tissue maintenance. Other studies looked at more specific molecular markers for autophagy, such as microtubule-associated protein 1 light chain 3, and reached the same conclusion that autophagic flux follows a diurnal rhythm.[60]

The circadian regulation of autophagy was also found in eukaryotic cells and includes several oscillating genes in the autophagy pathway.[61] In yeast, microarray studies demonstrated that >50% of the yeast genome is cyclically controlled during metabolic restriction. Autophagic gene regulation appeared to follow a specific temporal expression pattern that was accompanied by reduced metabolic functions.[61] This observation supports the idea that cell homeostasis and cell death events occur in synchrony, which is highly conserved even in a simple eukaryotic cell. Although the circadian signals that govern rhythmic autophagic flux in mammalian cells and in eukaryotic cells are diverse, the autophagic rhythms themselves may reflect a conserved attribute of cellular homeostasis and cell death control, as will be presented below.

The transcriptional regulation of autophagic rhythm was shown to involve both circadian effectors and metabolic signals. For instance, *C/EBPβ* (the CCAAT-enhancer–binding protein β) is among the most prominent of these factors to be implicated in the cyclic regulation of autophagy.[62] *C/EBPβ* belongs to a larger family of transcription factors and has a basic leucine zipper structure that is required for the induction of autophagy in response to starvation.[62] *C/EBPβ* was found to be expressed in a rhythmic manner and is controlled by both circadian and nutritional signals. It is notable that knockdown of *C/EBPβ* in vivo eliminated circadian-regulated autophagy. Moreover, liver-specific *BMAL1* knockout mice showed disrupted rhythmic regulation of autophagy, altered *C/EBPβ* levels, and reduced autophagic gene expression.[60] Another interesting finding from this study was the link between the cyclic regulation of autophagy and the reliance of autophagic rhythm on nutritional signals. Low autophagic flux occurred in accordance with feeding that followed the onset of the dark phase.[60] However, 24-hour starvation did not affect the rhythmicity of expression of several autophagy genes in the liver,[60] supporting the conclusion reached by Pfeifer in the 1970s.[57] This observation suggests that the zeitgebers of the circadian system directly affect the temporal and spatial autophagy gene expression.

An additional stress response pathway that functionally links autophagy and rhythmicity of the circadian is through the adenosine monophosphate–activated protein kinase (AMPK). AMPK is a prominent energy-sensing kinase that triggers different catabolic pathways, including glucose uptake and oxidative metabolism. AMPK is also responsible for the delay of various anabolic processes including lipid, protein, and carbohydrate biosynthesis. In cardiomyocytes, unc-51–like kinase 1 activation by AMPK notably was found to be crucial for the initiation of autophagy and mitochondrial homeostasis.[63] Moreover, a parallel study revealed that the mechanistic target of rapamycin complex 1 phosphorylation site in unc-51–like kinase 1 was responsible for regulation by AMPK.[64] Hence, together, AMPK can activate autophagy by dual mechanisms, one involving the direct activation of unc-51–like kinase 1 and the other by abrogating the inhibitory effects of mechanistic target of rapamycin complex 1 on unc-51–like kinase 1.[63,64] It is interesting to note that both AMPK and mechanistic target of rapamycin exhibit rhythmic regulation.[65] In mouse hepatocytes, AMPK activity and nuclear localization follow a cyclic rhythm that was inversely associated with *Cry1* nuclear expression. It is notable that the activation of AMPK destabilized Cry proteins and normal rhythmic oscillations. Furthermore, mice with disrupted AMPK pathways demonstrated significant impairment in peripheral clock activity.[65] The ability of AMPK to control cyclic expression of genes associated with metabolism such as mechanistic target of rapamycin through regulation of *Cry1* suggests a close relationship between autophagic mechanisms and circadian rhythms.

It is notable that the peroxisome proliferator–activated receptor gamma coactivator 1-alpha (PGC-1α) has been shown to interact with components of the circadian system, indicating another relationship between the circadian rhythm, mitochondrial function, and regulation of cell death. The correlation between PGC-1α and circadian gene expression suggests that PGC-1α may regulate mitochondrial turnover in a circadian system–dependent manner. It is not surprising that *PGC-1α* knockout mice exhibited abnormalities in light-dark cycles, followed by metabolic changes in body temperature. Furthermore, close analyses of *PGC-1α*–deficient fibroblast cells and mice with liver-specific lack of *PGC-1α* proved that this transcription factor is essential for cell-autonomous clock function.[66]

CIRCADIAN RHYTHM AND APOPTOSIS

Apoptosis is a form of programmed cell death that involves nuclear DNA fragmentation without inflammation. The biochemical pathways for apoptotic cell death

have been well documented and are known to involve both an extrinsic death receptor pathway and an intrinsic mitochondrial-regulated pathway. These pathways are highly integrated with necrosis and autophagy and have been extensively reviewed.[67] It is well documented that apoptotic cell death is most noticeable in the myocardium during various CVDs, including heart failure, I-R, and MI.[68] Apoptosis activation typically involves a cellular trigger or signaling mechanism that initiates the process. In most but not all cases, reactive oxygen species are generated by mitochondrial and other cellular sources, including NADPH oxidases and monoamine oxidase during ischemic injury.[67] At the molecular level, apoptosis is characterized by activation of caspases, notably death effector caspases 3, 6, and 7.[67] Although apoptosis is activated during early ischemia, it is tremendously amplified on reperfusion, presumably from the reenergization of mitochondria.[68] Disruption of cellular homeostasis throughout ischemia leads to cellular damage. These disruptions are especially noticeable in both calcium homeostasis, which has a prominent role in the I-R-mediated caspase activation during apoptosis, and mitochondrial permeability transition pore opening during necrosis, as well. Hence, the release of internal Ca^{2+} from the endoplasmic reticulum and mitochondria sites is likely a key triggering event in both apoptotic and necrotic cell death pathways.[69] Cyclic variation of apoptotic death was described a few years after the discovery that autophagy was regulated in a circadian-dependent manner.[57] Studies in the small intestine of mice demonstrated the dependency of circadian rhythm on apoptotic cell death after the irradiation exposure. As expected, the peak time for the induction of apoptosis was higher in the morning than in the evening hours.[70]

Apoptosis and the Extrinsic Death Pathway

Apoptosis can be initiated by 2 biochemical pathways: the extrinsic death receptor pathway and intrinsic mitochondrial pathways. The extrinsic pathway has been studied in the context of tumor necrosis factor α (TNFα), which has been shown to be synthesized and secreted by cells in a circadian-dependent manner.[67,71] In one study, deletion of *Cry1* and *Cry2* resulted in a higher production of TNFα, whereas ectopic expression of *Cry1* in the mouse embryonic fibroblast germ line in which *Cry1* and *Cry2* were deleted showed a significant reduction in TNFα.[72] Further studies revealed that nuclear factor κB, a major transcription factor activated downstream of TNFα receptor signaling, was impaired in cells deficient in *Cry*.[73] Given that TNFα is associated with inflammation and cardiac cell death during ischemic injury, the fact that TNFα production and subsequent nuclear factor κB signaling can be modulated in a circadian-dependent manner is profound and has important clinical implications for the design and timing of delivery of agents designed to inhibit cardiac cell death and ischemic injury. In addition, several other cytokines including interferon γ, interleukin (IL)–1, and IL-6 are also known to follow a diurnal variation of expression. For example, whereas interferon γ expression in wild-type mice varied in a circadian-dependent manner, interferon γ levels in mice defective for Per2 activity failed to follow circadian regulation.[74] It is notable that IL-1 levels were reduced in *Per2*-defective mice, suggesting that *Per2* may directly regulate *IL-1* gene promoter activity.[74] Moreover, IL-6 was found to be increased in the morning and reduced in the afternoon in individuals with rheumatoid arthritis, consistent with the daily clinical manifestations of disease.[75] Furthermore, individuals with obstructive sleep apnea and disrupted circadian rhythms exhibited increased IL-6 levels in comparison with non–sleep apneic controls.[76] Taken together, these findings demonstrate that cytokines that regulate vital cellular processes for normal cellular homeostatic function are regulated in a circadian-dependent manner, with the loss of circadian control contributing to the pathogenesis of many human pathologies.

Apoptosis and Intrinsic Mitochondrial Death Pathway

In the context of the intrinsic mitochondrial death pathway, morphological damage to the cell caused by caspase activation and mitochondrial outer membrane damage leads to the amplification of death signals through the activation of certain proapoptotic members of the Bcl-2 family members including, Bax, Bak, and Bnip3 (Bcl-2 Nineteen Kilodalton Interacting Protein 3).[67] Moreover, permeability change to the inner mitochondrial membrane resulting in permeability transition pore opening is a major event of necrotic cell death as discussed below. However, mitochondrial permeability transition pore opening can be suppressed by several mechanisms, including the phosphorylation of glycogen synthase kinase 3 β. The suppression of apoptosis by glycogen synthase kinase 3 β has been linked to inhibition of the tumor suppressor protein p53 and attenuation of ATP hydrolysis during ischemia.[77] Furthermore, p53 has also been shown to play a central role in apoptotic cell death following DNA damage and the intrinsic apoptosis pathway.[78] Although p53 protein does not appear to be regulated in a circadian-dependent manner, it was shown to directly interact with core components of the molecular clock such as BMAL1, CRY, and PER.[79] A large-scale RNA interference–based genetic screen identified *BMAL1* as a putative regulator of p53, suggesting that *p53* promoter activity may be regulated by *BMAL1*.[80] *CRY*, on the other hand, was found to downregulate p53 activity given that *Cry* mutations sensitized p53-deficient cells to TNFα-mediated apop-

tosis by interfering with nuclear factor κB and glycogen synthase kinase 3 β signaling.[73,81] Furthermore, p53 has also been shown to transcriptionally activate the Bcl-2 death protein Bnip3 and provoke autophagic cell death in cardiomyocytes.[82] It is notable that we identified a link between mitochondrial injury and cell death of cardiomyocytes during hypoxic injury that directly coupled to the transcriptional activation of *Bnip3*.[82] Bnip3 is uniquely distinguished from other members of the Bcl-2 gene family for several important and salient reasons. One unique feature of Bnip3 that sets it apart from other Bcl-2 family members is its inducible expression during ischemia or hypoxic stress. Bnip3 can trigger mitochondrial perturbations on the inner mitochondrial membrane, resulting in impaired respiration, loss of $\Delta\Psi m$, mitochondrial permeability transition pore opening, and cell death. Bnip3 can serve as a docking site for autophagy proteins such as light chain 3II or Beclin-1 for mitochondrial clearance by mitophagy or promote cell death via apoptosis or necrosis.[83] Indeed, previous work from our laboratory demonstrated that interventions such as genetic knockdown or mutations of Bnip3 defective for mitochondrial targeting were each sufficient for suppressing mitochondrial injury and cell death induced by Bnip3 during hypoxia. It is important to note that, in comparison with wild-type littermates, *Bnip3*$^{-/-}$ mice were resistant to mitochondrial defects and cell death induced by the chemotherapeutic drug doxorubicin.[83] Hence, these studies identify Bnip3 as a key signaling factor involved in the mitochondrial dysfunction, mitophagy, and cell death of cardiomyocytes.

Another significant player in the intrinsic apoptosis pathway is the peroxisome proliferator–activated receptors (PPARs) family. PPARs are expressed in the cardiovascular system including the endothelial cells, vascular smooth muscle cells, and monocytes/macrophages, where they exert their cardiovascular protective actions.[84] One member of this family, PPARγ, is involved in the diurnal rhythmicity of BP and HR. PPARγ was shown to exhibit circadian expression in the aorta, and also to activate the *BMAL1* promoter. Genetic knockout studies demonstrated that loss of function of PPARγ in vascular endothelial cells or smooth muscle cells attenuated diurnal oscillation in BP and HR.[85]

CIRCADIAN RHYTHM AND NECROSIS

Necrosis was initially thought to be an accidental or unregulated form of cell death during ischemic injury. However, there is growing evidence that, like apoptosis, necrosis is a regulated form of programmed cell death.[86] Necrosis is characterized by morphological changes to the cells that are distinguished from apoptosis. These differences, which include intracellular swelling, dissolution of nuclear envelope, mitochondrial dysfunction, and rupture of the cell membrane, collectively trigger inflammation at the site of injury.[86] There is a paucity of available information regarding the interplay between programmed necrosis and the circadian mechanism.

It is interesting to note that microarray analysis of different circadian genes in clock mutant *Drosophila* flies revealed that RIP1 (receptor-interacting protein 1) was markedly increased in comparison with wild-type flies.[87] Furthermore, the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP1), which has overlapping properties with necrotic cell death pathways, was also increased in *clock* mutant flies.[88] Both PARP1 and RIP1 are known to be markedly activated in the myocardium following I-R, in parallel with the reported decline in contractile function and myocardial NAD+ and ATP content in infarcted hearts.[89,90] It was also previously suggested that PARP1 and RIP1 exhibit some overlapping properties with PARP1-mediated cell death dependent on RIP1.[91] Again, given the close association between RIP1 and PARP1 for the regulation of cell death during ischemic injury, and based on the clinical outcomes data (ie, greater risk for MI and cardiac injury in the morning), it will be interesting to see the impact of circadian-regulated input on the expression of genes and signaling pathways that govern cardiac cell fate in the normal and diseased heart.

CIRCADIAN RHYTHM AND OTHER CELL DEATH TYPES

In addition to classical forms of cell death (apoptosis, necrosis), the Nomenclature Committee on Cell Death has defined forms of cell death that differ morphologically from traditional forms of cell death, described above. Because most of these new forms of cell death types were only recently described within the past few years, there is a scarcity of information regarding the role of these death programs in the cardiovascular system and regulation by circadian control. In this section, we provide an overview of the available data regarding new cell death types that are defined by the Nomenclature Committee on Cell Death. We have limited our discussion to forms of cell death known to be regulated by circadian rhythm in various tissues and models. For a comprehensive review on this topic, the reader is referred to Galluzzi et al.[92]

Ferroptosis

Ferroptosis is a form of iron-regulated cell death that is characterized by the accumulation of lipid hydroperoxides that is dependent on iron-mediated reactive oxygen species production.[92] Although there are limited reports on the role of ferroptosis in the heart, one study in the brain identified ferroptosis to be subject-

ed to circadian regulation and linked to the transcription factor nuclear factor-E2–related factor 2 (*Nrf2*). In this regard, Nrf2 was found to protect neurons from cytotoxicity induced by iron overload through the brain-derived neurotrophic factor that was shown to regulate Nrf2 in astrocytes in a circadian rhythm–dependent manner. Previous studies suggested brain-derived neurotrophic factor stimulates Nrf2 via receptor combination of the truncated form of TrkB.T1 and $p75^{NTR}$. $p75^{NTR}$ is known to be regulated by Clock and BMAL1, implying that the activation of Nrf2 via brain-derived neurotrophic factor may be regulated by the circadian system.[93] It is unclear, however, whether ferroptosis in the heart is subjected to the same level of circadian rhythm control.

Necroptosis

Necroptosis is another form of regulated cell death that starts with perturbations of the extracellular or intracellular microenvironment. At the molecular level, necroptosis is dependent on the activation of RIPK3 (receptor-interacting serine/threonine-protein kinase 3).[92] Disrupted circadian rhythm is a known risk factor for inflammatory bowel disease, which is characterized by mucosal and intestinal inflammation. Genetic ablations of circadian clock function or environmental disruptions in circadian rhythms were shown to increase susceptibility to severe intestinal inflammation and worsen the state of the disease. These changes in circadian regulation were found to be accompanied by necroptotic cell death, mediated by RIPK3. The intestinal necroptosis following disrupted circadian rhythm increased mitotic cell cycle arrest via Per1/2, which was controlled by the nuclear kinase Wee1.[94] Whether necroptosis itself or components of the pathway are subjected to circadian control in the heart is unknown and remains an active area of investigation in our laboratory.

Parthanatos

Parthanatos is driven by the hyperactivation of PARP1. In particular, parthanatos occurs in response to severe alkylating DNA damage, oxidative stress, hypoxia, hypoglycemia, and inflammatory cues.[92] Previous studies showed that PARP1 oscillates in a circadian manner, and PARP1 knockout mice have disrupted food entrainment through a mechanism that involves clock. PARP1 regulates the feeding signal in mice by modifying the expression of clock and its downstream transcriptional circuitry for rhythm generation.[95]

Cellular Senescence

Cellular senescence refers to the inability of cells to proliferate while remaining viable and metabolically active. The morphological changes that define senescence are flattening, intracellular vacuolization, cellular and nuclear enlargement, and altered chromatin structure.[92] Studies performed on senescent cells showed that their circadian rhythmicity was significantly weaker than in young cells. The introduction of senescent cells to telomerase reversed these changes. It is interesting to note that the implantation of young cells into old mice completely reentrained their circadian rhythm; however, the opposite implantation of senescent cells into young mice failed to effectively reentrain the circadian system.[12] Additional studies also showed that BMAL1 deficiencies may result in the development of premature senescence in different tissues, including lungs, liver, and spleen.[96] Altogether, these findings suggest that senescence disrupts the ability of cells to transmit circadian signals effectively and that defects in circadian components stimulate senescence. It remains to be elucidated whether senescence-mediated cell death is circadian-regulated in cardiomyocytes.

Mitotic Catastrophe

Mitotic catastrophe impedes the proliferation and survival of cells that are unable to complete mitosis because of wide DNA damage, defects of the mitotic machinery, or failure of mitotic checkpoints.[92] Poly(ADP-ribose) glycohydrolase has a central role in regulating cell fate following DNA damage. Irradiated poly(ADP-ribose) glycohydrolase–deficient cells displayed centrosome amplification that may result in the induction of mitotic catastrophe. Tej, the poly(ADP-ribose) glycohydrolase homologue in *Arabidopsis*, plays a crucial role in period length of the *Arabidopsis* circadian oscillator. This finding may give a clue for a possible regulation of mitotic catastrophe by circadian rhythms.[97] Again, whether the underlying events that lead to mitotic catastrophe in plants have analogous counterparts in the mammalian heart remains unknown.

CONCLUSIONS

It is notable that the Nobel Prize in Physiology or Medicine was awarded in 2017 to Jeffrey C. Hall, Michael Rosbash, and Michael W. Young for their exciting early discoveries relating to the molecular circadian clock. Now we are focused on how this applies to clinical medicine. A recent flurry of human and animal studies clearly demonstrates that circadian clocks play a crucial role in many aspects of healthy cardiovascular homeostasis and disease pathogenesis and pathophysiology. We are now starting to understand the molecular basis for this in the heart. It is remarkable that the quantification of transcriptomes of 12 mouse organs, including the heart, reveals that up to 43%

of all protein-coding genes have circadian rhythms in transcription in an organ-specific manner. It is important to note that several studies have used high-throughput technologies to directly characterize diurnal gene and protein rhythms in the heart[98] under circadian and diurnal conditions, providing a window into the importance of timing for cardiac pathways. However, although the first evidence of circadian gene signatures of autophagic, apoptotic, and necrotic cell death pathways is embedded in these and other studies, the specific pathways remain to be discovered. Elucidating these pathways is especially important for understanding how time of day directly and profoundly influences heart diseases, for example, the tolerance and susceptibility of the heart to I-R injury.[99]

In summary, the studies highlighted in this review lead to the novel suggestion that a window of optimal inhibition of cell death programs in a circadian-dependent manner is crucial for the maintenance of cardiovascular homeostasis and function (Figure 4). Excessive or insufficient levels of cell death may lead to the development of cardiac dysfunction and heart failure. Because the link between cell death activation and circadian rhythm appears highly complex, global targeting of putative regulators of cell death may not be universally beneficial in migrating I-R injury because certain death effectors, like caspases, can also mediate diverse cellular processes such as cardiac hypertrophy.[100] Therefore, careful consideration of targeting cell death pathways in relation to circadian control is warranted. Future studies will undoubtedly address the complex interplay between homeostatic processes such as autophagy, and programmed cell death, and circadian rhythms in the normal diseased heart. This will lead to innovative approaches to benefit treatment and outcomes for patients with CVD.

ARTICLE INFORMATION

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Disclosures

None.

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