The tumour suppressing role of the circadian clock

Critical Review

The Tumour Suppressing Role of the Circadian Clock

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Abstract

The circadian clock and the ~24 h rhythms it generates are essential in maintaining regular tissue functioning. At the molecular level, the circadian clock comprises a core set of rhythmically expressed genes and gene products that are able to drive rhythmic expression of other genes to generate overt circadian rhythms. It has recently come to light that perturbations of circadian rhythms contribute to the development of pathological states such as cancer, and altered expression and/or regulation of circadian clock genes has been identified in multiple tumour types. This review summarises the important role the circadian system plays in regulating cellular processes, including the cell cycle, apoptosis, DNA repair, the epithelial-to-mesenchymal transition, metabolism and immunity and how its dysregulation has widespread implications and could be a critical player in the development of cancer. Understanding its role in cancer development is important for the field chronotherapy, where the timing of chemotherapy administration is optimised based on differences in circadian clock functioning in normal and cancer cells. This has been found to influence the patient response, minimising the side effects commonly associated with chemotherapy. © 2019 IUBMB Life, 9999 (9999):1–10, 2019

Keywords: circadian clock; cancer; tumour suppressor

INTRODUCTION

The 2017 Nobel Prize in Physiology or Medicine was awarded to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their discoveries of the molecular mechanisms that control circadian rhythms. These 24 h clocks align metabolic and physiological processes, at cellular and systemic levels, to the external light and dark cycle. The circadian clock plays a critical role in tissue functioning and its dysregulation is implicated in several disease states (1–4). The suprachiasmatic nuclei (SCN), in the hypothalamus, receive light signals from the retina, via the retinohypothalamic tract, and act as a central clock to synchronise or "entrain" peripheral clocks, present in nearly all nucleated cells in the body, to the environment (5). While light is the most important entrainment signal (or Zeitgeber), temperature changes, activity, meal times and social cues have also been shown to play a role in synchronising our peripheral clocks (6).

Circadian clocks consist of a network of interlocking transcription-translation feedback loops. These feedback loops result in fluctuating levels of circadian clock gene and protein expression throughout the day. The first transcription-translation feedback loop contributing to the molecular clock involves the transcription factors circadian locomotor output cycles kaput (CLOCK) and ARNTL (aryl hydrocarbon receptor nuclear translocator-like protein 1) (BMAL1), which activate the

Abbreviations: *CLOCK, Circadian Locomotor Output Cycles Kaput; BMAL1, ARNTL (Aryl hydrocarbon receptor nuclear translocator-like protein 1); PER, Period; CRY, Cryptochrome; RORα, RAR-related orphan receptor alpha (NR1F1); REV-ERBα, NR1D1; REV-ERBβ, NR1D2; PASD1, PAS domain containing 1; PIWIL2, piwi like RNA-mediated gene silencing 2; NPAS2, Neuronal PAS domain protein 2; MEF, mouse embryonic fibroblasts; DEC1, Deleted in esophageal cancer 1; SR9009, Stenabolic*

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transcription of *PERIOD 1*, *2* and *3* (period [*PER*]) and *CRYPTOCHROME 1* and *2* (cryptochrome [*CRY*]) genes (7). The PER and CRY proteins, upon forming a complex, then repress CLOCK and BMAL1 activity to complete the loop (7). The *PER* and *CRY* genes are highly transcribed at the beginning of the day resulting in the formation of a repressive complex by these proteins, which as the end of the day nears, decreases their transcription. BMAL1 levels are further controlled by an additional regulatory loop involving the activating RAR-related orphan receptor alpha (NR1F1) (RORα) and repressing REV-ERBα/β (REV-ERBα: NR1D1; REV-ERBβ: NR1D2) nuclear receptor transcription factors (7). The overall pace of the molecular clock is governed to a great extent by post-translational modifications, including phosphorylation, ubiquitination and sumoylation, which determine the stability and nuclear translocation of the circadian clock proteins (7).

The expression patterns of ~40% of mammalian genes oscillate with circadian rhythm (in at least one organ in the body), suggesting they are regulated by the circadian clock, as well as numerous non-coding RNAs (8). Clock-controlled genes include genes involved in fundamental cellular processes, including the cell cycle, DNA damage response and metabolism. CLOCK itself is a transcription factor with histone acetyltransferase activity, and can therefore exert epigenetic control, reportedly acetylating lysine resides in histone H3 and H4, as well as non-histone proteins, such as BMAL1 and the glucocorticoid receptor (9, 10). The circadian clock clearly has a major influence on cellular functioning, exemplified further by the fact that not only does the circadian clock regulate the transcriptome and epigenome, but recently, extensive circadian regulation of the phosphoproteome was revealed, with more than 25% of phosphorylation sites being found to oscillate daily (11). Furthermore, widespread circadian regulation of the nuclear proteome has also been recently described, with over 10% of nuclear proteins exhibiting a circadian nuclear accumulation (12).

Perturbations in circadian clock function have a profound impact on the cell, disrupting numerous cellular pathways, and are thereby likely to contribute to many of the hallmarks of cancer. This review acts to summarise the molecular mechanisms by which altered circadian clock function can influence tumourigenesis, and highlight the tumour suppressive role of the circadian clock. It focuses on the circadian regulation of cellular processes such as the cell cycle, apoptosis, DNA repair, epithelial-to-mesenchymal transition, metabolism and immune function and how the aberrant regulation of these processes by circadian clock dysfunction can contribute towards cancer development. Importantly, circadian output processes can typically feed back into the clock, thus augmenting the loss of control exhibited by cancer cells, and reinforcing the central role of the circadian clock in cancer biology.

THE CIRCADIAN CLOCK-CANCER CONNECTION

Conflicting entrainment signals can cause the endogenous circadian clocks to become desynchronized from external time and/or one another. This circadian disruption has been correlated with increased cancer risk and progression. This correlation has been evident in shift workers and the International Agency for Research on Cancer classified shiftwork that results in circadian disruption as a probable carcinogen (group 2A carcinogen), in the same category as ultraviolet radiation, benzo(a) pyrene and acrylamide (compared to tobacco smoking, e.g., which is a group 1 carcinogen) (13). A positive correlation has also been observed between the level of "light at night" in a country and the prevalence of breast cancer, where countries with the highest levels of night-time illumination were those with the highest incidence of breast cancer (14).

Mouse studies have supported this connection, for example, Papagiannakopoulos et al. showed that physiologic disruption of circadian rhythms by altering the light cycle accelerates lung tumourigenesis (15). Interestingly, meal timing, which also acts to entrain peripheral circadian clocks, had a considerable impact on cancer growth, where mice with access to food for restricted times (zeitgeber times 1–6, i.e., the first 6 h after light onset) had reduced cancer growth compared to those fed ad libitum (16). This coincided with the induction of rhythmic expression of critical genes in the tumours, in response to meal timing, including cellular stress response and cell cycle genes, which did not occur in mice fed ad libitum.

It is disruption of the circadian clock itself which associates with cancer, as loss of circadian oscillations after SCN ablation (including loss of the 24 h rest-activity cycle, the daily rhythms of serum corticosterone level, lymphocyte count and body temperature rhythm) leads to significantly increased tumour growth (17). While it is still unclear how circadian disruption contributes toward tumourigenesis, it has been suggested that circadian disruptions may interfere with natural fluctuations in hormone levels, for example, melatonin. Beckett and Roden highlight the cancer-suppressive actions of melatonin, including its ability to scavenge free radicals (18), enhance DNA repair (19) and regulate multiple signal transduction cascades (20), as well as synchronise peripheral clocks (21). While a direct link has not yet been proven, disruption in natural melatonin levels is likely to affect many cellular processes that could promote cancer, and the resultant desynchronisation of circadian machinery have profound consequences (22, 23).

CIRCADIAN RHYTHM DISRUPTION IN CANCER

Peripheral clocks are largely synchronised in a healthy organism, where the core clock genes oscillate in phase across organs (8). This maintenance of "circadian homeostasis" is critical for the clock to perform its protective and tumour suppressing role. It is becoming increasingly apparent that cancer cell clocks commonly lose this equilibrium, and become disrupted. In fact, it has been suggested that perturbations induced by a single oncogene, for example, Ras, are sufficient to deregulate the circadian clock. Ras overexpression, in human keratinocytes, rat

fibroblasts and colorectal cancer cells, leads to an increase in the circadian period of *BMAL1* oscillations, whereas inhibition shortens the period length (24). Furthermore, several studies have shown that tumour cells are arrhythmic or circadian clock gene oscillations are greatly suppressed. This has been shown in vitro, where Kiessling et al. showed that B16 melanoma cells are arrhythmic, yet rhythmicity of circadian clock genes is restored using Dexamethasone, resulting in reduced proliferation and tumour growth (25). Breast cancer cells have similarly been shown to display arrhythmic patterns of circadian clock gene expression, and while serum shock can induce oscillation of some circadian clock genes, the amplitude is greatly reduced compared to normal breast epithelial cells, suggesting a defective circadian clock mechanism (26–29). Interestingly, despite this, distinct circadian expression profiles of numerous clock-controlled genes can be detected in breast cancer cells after synchronisation (29), as well as rhythmic fluctuations of microRNAs (27). Fuhr et al. recently found that 16.0% (3,998) of all genes were oscillating in SW480 colon cancer cells and 14.8% (3,693) were oscillating in its metastatic counterpart, SW620. However, only 5.5% (1,385) of all genes oscillate in both cell lines, which point to a switch in the 24 h oscillating gene expression profile as cells progress from primary tumour to metastatic (30).

In vivo, absent, or very weak, rhythmic profiles of circadian clock genes have been described in Glasgow osteosarcoma and pancreatic adenocarcinoma tumour tissue (16, 31). Interestingly, Huisman et al. found that colorectal tumour metastases in the mouse liver displayed absent circadian clock gene oscillations, and that the presence of these metastases resulted in a shift in circadian clock periodicity in surrounding liver and kidney tissue, where liver tissue displayed a phase advance, and kidney tissue a phase delay in circadian clock gene oscillations. This suggests that the circadian rhythm is not only disrupted in tumour tissue but can also result in a disruption in the surrounding peripheral clocks (32).

Circadian rhythms can be dampened by oncogenic induction of repressor proteins, whose expression is usually restricted to germline tissues. For example, upon oncogenic transformation, the cancer/testis antigen PAS domain containing 1 (PASD1), which is a paralog of CLOCK, can inhibit the CLOCK/BMAL1 complex and lead to the suppression of the clock's imperative oscillations, as well as lengthening the period of *PER2* oscillations in U2OS osteosarcoma cells (33). Similarly, the cancer/testis antigen piwi like RNA-mediated gene silencing 2 (PIWIL2) can repress circadian rhythms in HeLa cervical cancer cells by suppressing GSKβ-induced degradation of BMAL1 and CLOCK, as well as inhibiting the transcriptional activation of *PER2* and *REV-ERBα* promoters (34).

Not all studies, however, have shown suppressed circadian clock gene oscillations in cancer cells/tumour tissue. Comas et al. found that fibrosarcoma cells and mouse sarcomas in vivo display significant rhythmic profiles of *PER2*- and *BMAL1*-driven luciferase expression, similar to that found in normal tissues, although with slightly reduced amplitude (35). Moreover, U2OS osteosarcoma cells are often used as a cell line in which to study circadian rhythms due to the robust circadian oscillations observed in these cells (36). It is unclear at present what distinguishes cancer cells displaying functional circadian oscillations from arrhythmic cancer cells.

CORE CIRCADIAN CLOCK GENES ARE DYSREGULATED IN CANCER CELLS

In addition to suppression of circadian clock oscillatory activity, it has been demonstrated that circadian clock genes are downregulated in many cancer cells and this has been suggested to be a contributor to many cancer phenotypes (37–39). In a microarray study comparing the gene expression patterns of cervical cancer tissue to normal cervical epithelium, we previously showed that *PER2* is amongst the 10 most significantly downregulated genes in cervical cancer patient tissue compared to normal (40, 41). Overexpression of specific circadian genes has been observed to inhibit cancer features, including proliferation, migration and invasion (38, 42, 43) and dose-dependently suppress tumour growth (44), highlighting the importance of circadian clock downregulation in cancer biology. It has been reported by Cadenas et al. that the expression of circadian clock genes (most significantly *CRY2*, *PER2* and *PER3*) is inversely correlated to the expression of proliferation-associated genes (the proliferation metagene) in breast cancer patient tissue (45). Furthermore, these circadian clock genes expression levels were lower in aggressive cancers compared to low grade and non-metastasising tumours, and consequently, loss of *CRY2*, *PER2* and *PER3* circadian clock gene expression associated with poorer prognosis (45). This further supports the notion that circadian clock genes might play a role in tumour suppression. It must be noted that cancer cells not only feature repression of individual circadian clock genes, but that the co-ordinate co-expression of circadian clock genes is also reported to be compromised. Under normal circumstances, the *CRY* and *PER* genes display highly co-ordinated temporal expression patterns, as their protein products homo- and heterodimerise and function together in the negative regulatory feedback loop of the circadian clockwork (46, 47). Cadenas et al. found that, the more aggressive and metastatic the tumour presents, the lower the correlation of expression between *CRY* and *PER* gene expression, in particular, between *CRY2* and *PER3*, or *PER2* and *PER3* (45). Surprisingly, timeless gene expression, unlike other circadian clock genes, is observed to be higher in cancer cells and tissue compared to its expression in healthy tissue (48). Its elevated expression has in fact been proposed to be a poor prognostic marker in certain cancer types (49).

The downregulation of circadian clock genes in cancer cells is frequently associated with hypermethylation of the promoter regions of these genes. For example, *BMAL1* is reported to be epigenetically silenced in ovarian cancer (50), and Kuo et al. showed that 37 of 53 breast cancer cell lines had hypermethylation of *PER1 PER2*, *CRY1* or *BMAL1* circadian clock gene promoters (51). However, chromatin modifications and DNA

methylation programmes are under the influence of the circadian clock, in order to maintain systemic and tissue-specific rhythms (52, 53). Joska et al. argue that because perturbation of the circadian clock will alter the methylation status, hypermethylation of clock genes in cancer cells may simply be a result of defective clock gene expression and its impact on DNA methylation patterns (53). Interestingly, however, reversing DNA methylation, via treatment with a methylation inhibitor, such as 5-aza-2′-deoxycytidine, regenerates endogenous circadian rhythms in RPMI8402 lymphoblastic leukaemia cells, via restoration of *BMAL1* expression (54), suggesting that hypermethylation of circadian clock genes might be a mechanism by which circadian rhythms are suppressed within tumour cells.

Single nucleotide polymorphisms (SNPs) in circadian clock genes also associate with cancer. For example, significant correlations exist between SNPs in the *CLOCK* (55, 56) and *CRY2* (57) genes and breast and colorectal cancer risk. Moreover, SNPs in *RORα* were associated with breast cancer risk (58). In fact, Reszka et al., reveal that *BMAL1, BMAL2, CLOCK,* neuronal PAS domain protein 2 (*NPAS2), CRY1, CRY2, PER1, PER3* and *TIMELESS* are all candidate breast cancer risk variants amongst night shift workers (59). To highlight the prevalence of circadian clock gene mutations, *CLOCK* mutations occurred in 53% of microsatellite unstable colorectal cancers (which account for ~15% of colorectal cancers) (56).

These studies highlight the altered regulation of the circadian clock in cancer cells. This, in turn, impacts many important cellular processes, including cell cycle regulation, apoptosis and DNA repair mechanisms, amongst others.

THE CIRCADIAN CLOCK GENES REGULATE PROGRESSION THROUGH THE CELL CYCLE

There is recent evidence suggesting a robust coupling between the circadian clock and cell cycle (60). The circadian clock impacts on the cell cycle by ensuring temporally controlled activation of genes necessary for transition into the next phases of the cycle. The cell cycle, on the other hand, influences the circadian clock by ensuring transcription silencing occurs during mitosis. Additionally, p53 has been found to impact on the clock and cell cycle in a bidirectional way, further ensuring coupling between the two oscillators (60, 61). The TIMELESS protein also exhibits a dual function being essential in both circadian timekeeping and DNA replication (62). It is thus apparent that the altered regulation of the circadian clock in cancer can affect cancer cell progression through the cell cycle. This is evidenced by studies showing that enhancing circadian clock function, in cancer cells, by restoring circadian rhythmicity, results in fewer cells entering the S phase, thereby inhibiting cancer cell proliferation and tumour growth (25).

Several important regulators of the cell cycle have been identified as clock-controlled genes and these genes likely contribute to the altered cell cycle upon circadian clock disruption. CYCLIN D1 and CYCLIN E, essential in cell cycle progression, show expression patterns which display circadian rhythms (63, 64). The expression of these cyclins increases upon the suppression of circadian clock gene expression facilitating increased cell proliferation (65). Negative regulators of the cell cycle similarly display circadian rhythms, including WEE1 and p21, where the CLOCK/BMAL1 dimer induces transcription of *WEE1* (66), which acts to inhibit CDK1 and CDK2, and BMAL1 indirectly regulates the transcription of p21, via its regulation of REV-ERBα/β and RORα/γ (67). The expression of p16 has also been shown to display circadian variation (64).

c-MYC is a key clock-controlled gene which influences cell cycle progression and plays an important role in sustaining the changes which occur with cellular transformation, via its control of both cellular growth and metabolism (68). It has been shown that lung tumours with low *PER2* have enriched *c-MYC* signatures, suggesting increased c-MYC activity (15). *PER2* and *BMAL1* mutant tumours similarly show a significant increase in the protein levels of c-MYC compared to control tumours (15). The activation of c-MYC is a key contributor to the exhibited increased movement through the cell cycle via its control of ~12–15% of the genes within the genome (69). The c-MYC protein also has the ability to alter metabolic pathways so that they confer a growth advantage to the cells, promoting tumourigenesis (36). Interestingly, deregulated expression of *MYC* disrupts the molecular clock by dampening *BMAL1* gene oscillations, revealing reciprocal regulation of *c-MYC* and the circadian clock (36). Bu et al. recently described how in c-MYC-driven tumours, the unfolded protein response acts to suppress BMAL1, thereby impacting circadian rhythms, but also inhibiting protein synthesis, and as such promoting cancer cell survival (70). Because at least 50% of cancers have aberrant MYC expression, MYC dysregulation could help to explain the disrupted circadian clocks commonly observed in cancers (36).

THE CIRCADIAN CLOCK GENES REGULATE APOPTOSIS

Not only does circadian clock gene overexpression have antiproliferative effects and affect the cell cycle, but it has also been shown to promote apoptosis. *PER2* overexpression induces apoptosis in breast cancer cells (71), lung cancer cells (72) and pancreatic cancer cells (42), where it was shown to result in the downregulation of anti-apoptosis genes, *c-MYC*, *BCL-XL* and *BCL-2*, and upregulation of pro-apoptotic genes, *p53* and *BAX* (72). Conversely, *mPer2* mutant cells (thymocytes from homozygous *Per2* mutant mice deficient in *Per2*-mediated transcription regulation) show reduced apoptosis in response to γ radiation, likely due to attenuated p53 induction (63). Similarly, *PER2* mutant mouse embryonic fibroblasts (MEF) cells show increased resistance to apoptosis under conditions of oxidative stress (73). *PER1* overexpression, on the other hand, sensitises cells to ionising radiation-induced apoptosis in colon cancer cells, while its downregulation reduces apoptosis (39). Apoptosis itself can act on circadian clock

genes, where for example, it has been shown that *PER2* expression is enhanced during flutamide-induced apoptosis (74).

PER2 mutant cells harbour a functional circadian oscillator, whereas *BMAL1* is the only gene whose deletion alone leads to complete loss of rhythmicity. In contrast to *PER2* mutant cells, apoptosis is enhanced in *BMAL1* mutant breast epithelial cells, in response to chemotherapeutic agents, Cisplatin and Doxorubicin (75). This suggests that interfering with *BMAL1* expression and abolishing circadian rhythms might be protective against tumour initiation following DNA damage insults. On the contrary, *BMAL1* overexpression in tongue squamous cell carcinoma cells and colorectal cancer cells sensitised these cells to paclitaxel and oxaliplatin, respectively (38, 76), suggesting that *BMAL1* modulation might have different effects on apoptosis induction depending on the type of chemotherapeutic drug used.

The link between the circadian clock and apoptosis is further highlighted in a study by Granda et al., showing that in bone marrow tissue expression of *BCL-2* and *BAX* vary in a circadian manner throughout the day, with their relative abundances occurring in antiphase to each other (77). Interestingly, in tumour-bearing mice, *BCL-2* rhythmicity is lost; suggesting altered circadian regulation of *BCL-2* expression in response to tumour formation (77).

The ability of cells to avoid apoptosis is a hallmark of cancer, and the link between circadian clock genes and apoptosis reinforces the circadian clock's tumour suppressor role.

DNA REPAIR MECHANISMS ARE CONTROLLED BY CIRCADIAN CLOCK GENES

Aside from their regulation of apoptotic proteins, the circadian clock genes are also known to control genes involved in DNA repair and the DNA damage response (39). For example, PER1 protein can directly interact with DNA damage proteins such as ATM and CHK2 in HEK293T cells, as well as HCT116 colon cancer cells, and the interaction is enhanced in cells treated with ionising irradiation (39). In fact, *PER1* plays a key role in activating the ATM checkpoint pathway. Similarly, the TIMELESS protein interacts with CHK1 and the kinase ATR in HEK293T cells, and this interaction is stimulated by treatment with UV or hydroxyurea, to induce DNA damage (62). TIMELESS is also recruited to sites of laser-induced DNA damage upon directly binding the DNA damage sensing protein PARP-1 in HEK293T and U20S osteosarcoma cells (78), and has been found to be essential in DNA repair by homologous recombination. Knock-out of TIMELESS protein in U20S cells results in a drastic impairment of this process (79). While the circadian clock acts to regulate DNA repair mechanisms, conversely, DNA damage, such as that due to exposure to ionising radiation, can act on the circadian clock, by phase advancing *PER2* promoter-driven oscillations in Rat1 fibroblasts (80). The kinase ATM conveys information about compromised genome integrity to the clock components to initiate this clock resetting (80).

Kang and Sancar revealed how DNA excision repair in mouse brain tissue exhibits a circadian rhythm, due to circadian control of the nucleotide excision repair factor, Xeroderma Pigmentosum, complementation group A (XPA) which shows oscillating patterns of expression (81). Altered regulation of the circadian clock in cancer cells thus likely impacts DNA repair pathways. This has implications for cancer treatment, where chemotherapeutic drugs induce a DNA damage repair response. Monitoring DNA repair in normal and cancer cells and timing the administration of chemotherapy accordingly, could result in improved drug efficacy and reduced toxicity (81).

THE CIRCADIAN CLOCK INFLUENCES EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT) AND METASTASIS

Downregulation of circadian clock genes in cancer cells has also been correlated with increased cell migration and invasion. Studies have shown that inhibition of *CRY1* (82), *PER2* (83) or *BMAL1* (84) results in enhanced cell migration or invasion via the Akt signalling pathway, in osteosarcoma (*CRY1* and *PER2*) and lung cancer and glioma cells (*BMAL1*). In addition, *BMAL1* knockout, resulting in loss of circadian rhythmicity, results in increased invasion of breast cancer cells (75). The EMT is a key step in cancer progression, and enables cancer cell invasion and metastasis. It has been found that in addition to influencing cell migration and invasion, the circadian clock regulates other EMT processes, for example, circadian disruption due to light-at-night has been found to result in disruption of melatonin's circadian rhythm, leading to inhibition of GSK3β activity and resultant β-catenin activation (using an in vivo tissue-isolated human breast tumour xenograft model system), and is thus likely to drive epithelial-to-mesenchymal transition in breast cancer patients (85).

PER2 recruits transcriptional co-repressors to OCT1-binding sites of the *TWIST1* and *SLUG* promoters (in breast epithelial cells), thereby repressing the expression of these EMT genes. The deregulation of *PER2* in tumour development thereby leads to the activation of EMT gene expression and associated EMT processes (86). Moreover, it has been found that the EMT regulators, *SMAD3* and *SNAIL*, show circadian expression in human fibroblasts and mesenchymal stem cells, as well as in mouse liver in vivo, and that *CLOCK/BMAL1* upregulates *SMAD3* promoter activity (87). On the contrary, TGF-β, via SMAD3 phosphorylation, can act to reset the clock, both in Rat1 fibroblasts and in the kidney and adrenal gland in vivo. It does this by upregulating expression of the circadian clock genes deleted in oesophageal cancer 1 (*DEC1*) and *CRY1* (88).

The link between the circadian clock and EMT is reinforced through studies showing an association between altered circadian clock gene expression and metastasis. For example, high expression of *BMAL1* and low expression of *PER1* have been associated with high rates of liver metastases in colorectal cancer patients (89). Additionally, high expression of various circadian clock

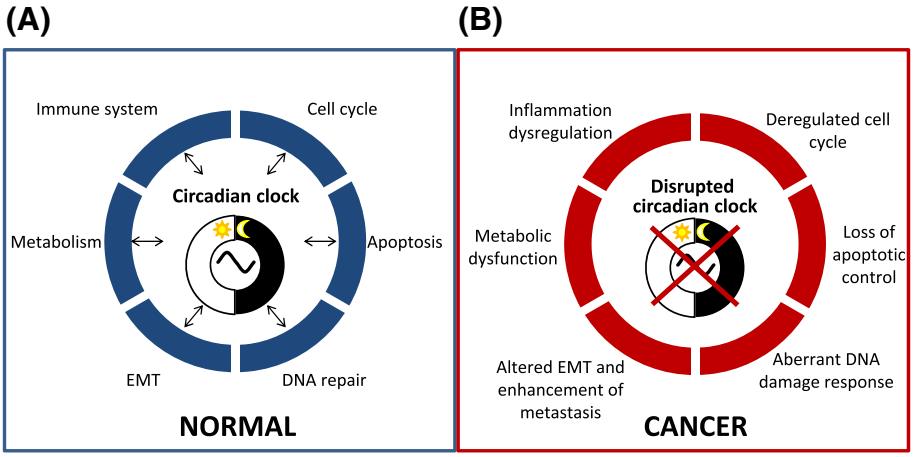

FIG 1 *The effect of a disrupted circadian clock on cellular processes. A, The circadian regulation of cellular processes confines activities to a particular time of day and ensures temporal homeostasis. B, A disrupted circadian clock accompanies cancer development, resulting in deregulated cellular processes, and facilitating cancer progression. Arrows indicate bidirectional interaction between the clock and cellular processes; EMT, epithelial-to-mesenchymal transition.*

genes has been associated with metastasis-free survival of breast cancer patients (45).

THE CIRCADIAN CLOCK REGULATES METABOLISM

It is well known that the circadian clock drives daily rhythms in metabolism. In fact, it has recently been reported that ~50% of mouse liver metabolites are circadian-regulated (90). Conversely, meal timing is known to entrain the circadian clock. It is apparent that under normal conditions the two processes are coupled (91), and unsurprisingly, dysregulation of the circadian clock can result in a disrupted circadian metabolome, which can play a significant role in the progression of cancer. Recently, Kettner et al. showed in mice that prolonged circadian disruption induced by simulated jet lag, induces liver cancer via global liver metabolic dysfunction (3). Similarly, Masri et al. showed that lung cancer rewires the circadian hepatic metabolome, and thereby alters cyclic energy expenditure, lipid metabolism and insulin and glucose signalling (92). At a more molecular level, Altman et al. showed that altered *PER2* and *BMAL1* circadian gene expression and oscillation induced by oncogenic *MYC* in osteosarcoma cells alters oscillation of glucose and glutamine metabolism, and this is speculated to provide an advantage to cancer cells (36). In support, Papagiannakopoulos et al. report that *mPer2*$^{tm1Brd/J}$ mutant lung cancer cells, although rhythmic, have increased rates of glucose consumption and increased levels of lactate excretion compared to wildtype cells, as well as increased glutamine usage, suggesting a role for PER2 specifically in regulating these metabolic processes (15). This increased metabolic activity can act to fuel tumour progression. Finally, a recent study by Fuhr et al. revealed that there is a shift in the expression of circadian-controlled genes in the progression from primary tumour to metastatic colon cancer cells, and this causes a shift in the metabolic profile (30). These authors also show that *BMAL1* knock-down in a primary tumour colon cancer cell line results in increased glycolysis and a more metastatic phenotype (30).

THE CIRCADIAN CLOCK INFLUENCES THE IMMUNE SYSTEM

Further to regulating metabolism, the circadian clock has also been shown to influence immune-regulated processes (93). The immune response is highly dependent on the time of day, possibly to allow for maximal immune reactions at times when encounters with pathogens are most likely to occur. On the contrary, the immune system is able to regulate the circadian clock. A comprehensive bidirectional communication between the two systems has recently been described (94). The link between the two processes is evidenced by the bidirectional regulation of clock proteins and cytokines, and TNFα has been found to be the major bridging element between the two systems in Hodgkin lymphoma cells (94). Clock proteins have been shown to temporally regulate cytokine expression (95), for example, secretion of proinflammatory cytokines, TNFα and IL6, displays circadian oscillation in macrophages (96). Furthermore, mice display temporal variations in serum IL-6 following endotoxin challenge, and these variations are absent in mice with *BMAL1*-deficient myeloid cells. This is due to suppression of *REV-ERBα* expression in *BMAL1*-deficent cells (97). In turn, cytokine expression influences clock protein expression, where, for example, TNF-α and IL-1β can inhibit the activity of the BMAL1/CLOCK dimer in fibroblasts and therefore inhibit expression of circadian clock genes, including the *PERIOD* genes (98).

These studies suggest that disturbing the circadian clock can impact inflammation and thereby cancer development. For example, *CRY* depletion leads to constitutive elevation of proinflammatory cytokines in fibroblasts and macrophages derived

from arrhythmic Cry1–/–, Cry2–/– double knockout mice, via increased NFκB signalling induced by increased cAMP production (99). This leads to a low-grade chronic inflammatory status. Low-grade chronic inflammation is known to be a contributor to cancer development.

THE ROLE OF THE CIRCADIAN CLOCK IN TUMOUR PROMOTION

It must be noted that although the circadian clock has many tumour suppressing functions, specific circadian clock genes can promote tumourigenesis in certain contexts (100). Colorectal cancers often display higher *CLOCK* or *BMAL1* expression compared to normal tissue (89, 101, 102), and overexpression of *CLOCK* in fact increases the proliferation of colorectal cancer cells, while downregulation decreases proliferation (103). *CLOCK* and *BMAL1* overexpression have also been reported in Erα-positive breast cancer (104), mesothelioma (105) and leukaemia (106), where their inhibition reduces cell growth. Importantly, overexpression of *BMAL1/CLOCK* in the above studies often coincides with downregulation of *PER* genes, suggesting that the circadian clock function itself is still compromised. These studies suggest that there is a complex interplay between individual circadian clock genes and cell proliferation, and further research is needed to better understand how the circadian clock genes act in concert to regulate tumourigenesis.

CONCLUSIONS

In summary, the circadian clock controls fundamental cellular processes, including, but not limited to, the cell cycle, apoptosis, DNA repair, EMT, metabolism and inflammation (Fig. 1A). As such, it acts as a barrier to transformation and tumour progression by ensuring temporal cellular homeostasis. In turn, many cellular processes regulate the clock itself, resulting in interdependencies between pathways. An increasing number of studies are revealing that circadian clock deregulation accompanies tumourigenesis. The associated impact on cellular processes reveals the circadian clock to be a central player in cancer biology (Fig. 1B). Knowledge of the link between the circadian clock and tumorigenesis is important in furthering our understanding of exactly how maintaining the circadian clock can suppress the cancer phenotype, but could also be exploited for therapeutic purposes. Sulli et al. recently showed that activation of REV-ERB proteins, using REV-ERB agonists SR9009 (stenabolic) and SR9011, resulted in cancer cell death, while normal cell viability was unaffected (107). In addition, SR9009 reduced tumour growth in vivo and no toxicity was observed (107). This suggests that pharmacological activation of circadian clock proteins could be an effective anticancer strategy. It is not only the targeting of the circadian clock machinery that could have therapeutic potential, but exploiting circadian clock deregulation in cancer could increase the efficacy of existing cancer treatments. It has been shown that higher treatment efficacy and lower levels of toxicity can be achieved if the timing of treatment is linked to the circadian clock. Hence, knowledge of clock-controlled processes and their circadian dysregulation in cancer cells is crucial to developing and optimising chronotherapy schedules that deliver maximum benefit to the patient.

Limitations of the current body of literature include a lack of understanding regarding the extent to which the deregulated circadian clock acts as a cause or consequence of cancer development. Additionally, care must be taken in extrapolating the effect of the circadian clock on cellular pathways from in vitro experiments in tissue culture, to whole animals, where the biology of circadian rhythms is profoundly complex. Finally, broad terms like "clock dysfunction" make it difficult to elucidate the role of the circadian clock in mediating cancer biology; as this term covers a wide range of disruptions to the circadian clock, including disturbances to the body-wide clock, tissue-specific rhythms and cell-autonomous clocks. Ongoing efforts at investigating the circadian clock in cancer development are needed, taking cognisance of these limitations.

CONFLICT OF INTEREST

No potential conflicts of interest are disclosed.

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