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REVIEW ARTICLE

The role of circadian clocks in cancer: Mechanisms and clinical implications

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Abstract Circadian rhythm refers to the inherent 24-h cycle oscillation of biochemical, physiological and behavioral functions, which is almost universal in eukaryotes. At least 14 core clock genes have been reported to form multiple chain feedback loops that confer intrinsic circadian rhythmicity onto the molecular clock. Accumulating evidence has shown that the circadian gene dysfunction resulted from single nucleotide polymorphisms (SNPs), deletions, epigenetic modification, and deregulation is strongly associated with cancer risk. In the present review, we describe the composition of circadian rhythm system. We highlight the function and mechanism of clock genes in cancer pathogenesis and progression. Moreover, their potential clinical implications as prognostic biomarkers and therapeutic targets have been addressed.

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Introduction

Biological rhythms regulate many metabolic and physiological processes rhythmically, with a natural cycle of about 24 h. If the peak of rhythm occurs in the daytime and the trough at night, it can be further described as circadian rhythm.[1] Almost all the behaviors and physiological activities of lives, including bacteria, fungi, plants, fruit flies, fish, mice, and human beings, obey a 24-h circadian rhythm,[2] such as mammalian feeding behavior, sleep/wake pattern, hormone and immune system.[3] In mammals, the suprachiasmatic nucleus (SCN) is the master clock located in the brain, that is, the main circadian pacemaker. It can sense light signals and then transmit them to peripheral clock system, such as liver, muscle, skin, and other tissues, initiating transcription factors to drive tissue-specific gene expression in a paracrine manner.[4,5] In the present review, we describe the composition of circadian rhythm system. We highlight the function and mechanism of clock genes in cancer pathogenesis and progression (Fig. 1 and Table 1). Moreover, their potential clinical implications as prognostic biomarkers and therapeutic targets have been addressed.

Clock genes and circadian rhythm

At least 14 core clock genes have been reported, including period 1, 2, 3 (*PER 1, 2, 3*), cryptochrome 1, 2 (*CRY1, 2*), timeless (*TIM*), casein kinase Iϵ (*CKIϵ*), circadian locomotor output cycles kaput (*CLOCK*), brain and muscle arnt-like protein-1 (*BMAL1*), retinoic acid related orphan receptor α, β, γ (*RORα*, *RORβ*, *RORγ*), retinoic acid receptor-related orphan receptor α, β (*REV-ERBα*, *REV-ERBβ*), neuronal PAS domain protein 2 (*NPAS2*), and deleted in esophageal cancer1, 2 (*DEC1, 2*).[6] The transcription-translation feedback loop (TTFL) exists in mammalian circadian rhythms. In the core loop, a CLOCK-BMAL1 heterodimer binds to the E-box in the promoter of clock-controlled genes (CCGs) such as *PER*s, *CRY*s, *ROR*s, and *REV-ERB*s, to initiate transcriptional programs.[7,8] In turn, the transcriptional products PER and CRY proteins can compose a transcriptional repressor complex to inhibit the CLOCK-BMAL1 heterodimer through protein—protein interactions or recruitment of the transcription termination complex. On the other hand, RORs act as a transcriptional activator while REV-ERBs as transcriptional repressors, which competitively bind to ROR response elements (ROREs) to dynamically regulate BMAL1 transcription.[9] Thus, cell-autonomous oscillation resulting from multiple chain feedback loops confers intrinsic circadian rhythmicity onto the molecular clock.[10]

Besides regulation of the circadian rhythm, clock molecules are also intimately involved in other physiological processes. Mammalian *Period* genes encode three homologous proteins: PER1, 2, and 3.[11] PER2 exerts an essential role in controlling cell proliferation through regulating the expression of several downstream genes, such as *CCNA*, *CCNB1*, *CCND1*, *CCNE*, *TP53*, and *MYC*.[12] Both PER1 and PER2 are involved in DNA damage response pathways, and their aberrant expressions might lead to malignant transformation of cells by altering cell cycle progression and checkpoint responses to DNA damage. BMAL1 is widely implicated in aging, cardiovascular disease, immune

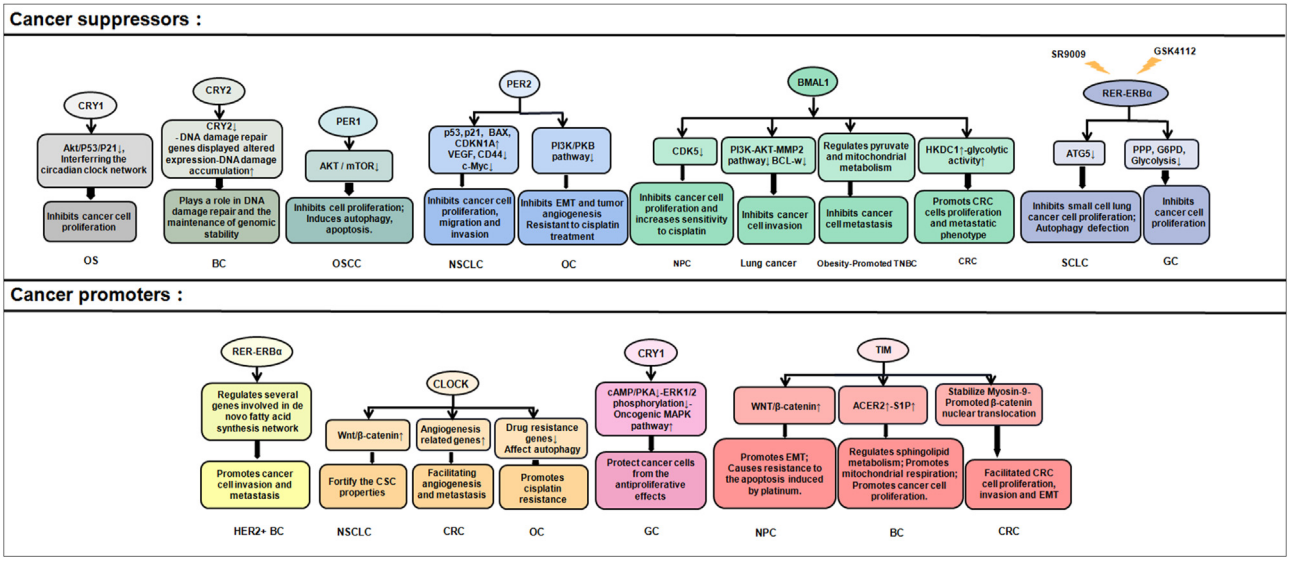

Figure 1 Proposed regulatory mechanism of clock genes in cancer. AC, adenyl cyclase; ACER2, alkaline ceramidase 2; ATG5, autophagy regulate gene 5; BC, breast cancer; CDK5, cyclin-dependent kinase 2; CSC, cancer stem cell; CRC, colorectal cancer; DOX, doxorubicin; EMT, epithelial—mesenchymal transition; GC, gastric cancer; G6PD, glucose-6-phosphate dehydrogenase; IL8, interleukin 8; ISO, isoproterenol; MDM2, mouse double minute 2; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9; NPC, nasopharyngeal carcinoma; NSCLC, nonsmall cell lung cancer; MRP2, ATP binding cassette subfamily C member 2; OV, ovarian cancer; OSCC, oral squamous cell carcinoma; PFKFB3, 6-phosphofructokinase-2/fructose-2,6-bisphosphatase; P-gp, P-glycoprotein; PPP, pentose phosphate pathway; SP1, specific protein 1; S1P, sphingosine 1-phosphate; TNBC, triple-negative breast cancer; TNFα, tumor necrosis factorα; u-PA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

Table 1 The function and mechanism of clock genes in cancer.

Clock genes	Expression in tumor tissue	Regulatory mechanism	Effect of clock genes on cancer progression	Reference
Cancer type: Lung cancer				
PER1	Downregulated	Unclear	Inhibits cancer cells invasion; Inducing the cell cycle arrest and apoptosis.	Lin, Y. S., et al. (2020) Gery, S., et al. (2007)
PER2	Downregulated	PER2-P53, P21, BAX, CDKN1A↑, VEGF, CD44, c-MYC↓	Inhibits cancer cells proliferation, migration and invasion	Xiang, R., et al. (2018) Lin, Y. S., (2020)
PER3	Downregulated	Unclear	Induces cancer cells apoptosis and inhibits migration	Tang, W., et al. (2018)
BMAL1	Downregulated	BMAL1-PI3K/AKT/MMP2 pathway↓ BCL-w↓	Suppresses cancer cells invasion	Jung, C.H., et al. (2013)
REV-ERBα	Unclear	REV-ERBα-ATG5↓	Inhibits small cell lung cancer cells proliferation; Autophagy defection.	Verlande, A., et al. (2021) Shen, W., et al. (2020)
TIM	Upregulated	Unclear	Loss of TIM inhibits cancer cells proliferation, induces cancer cells apoptosis, sensitized cancer cells to doxorubicin and cisplatin.	Zhang, Y. et al. (2020) Yoshida, K., et al. (2013)
CLOCK	Upregulated in the CSC-like cells	CLOCK-WNT/β-catenin↑	Fortify the CSC properties of cancer cells	Jiang, P., et al. (2020)
Cancer type: Colorectal cancer				
BMAL1	Unclear	HKDC1↑-Glycolytic activity↑	Promots CRC cells proliferation and metastatic phenotype	Fuhr, L. et al. (2018) Zeng, Z. L. et al. (2014)
TIM	Upregulated	TIM↑-Stabilize Myosin-9-Promoted β-catenin nuclear translocation; TIM↓-γH2AX↑-CHK1 and CDK1 phosphorylation↑-G2/M phase arrest.	Facilitated CRC cell proliferation, invasion and EMT	Cao, M. et al. (2021) Neilsen, B. K., et al. (2019)
CRY1	Upregulated	Unclear	Promoted the proliferation and migration of CRC cells	Yu, H. et al. (2013)
CLOCK	Upregulated	Angiogenesis related genes↑(Including HIF-1α and VEGF)	Facilitated EMT in CRC cells	Wang, Y. et al. (2017)
Cancer type: Breast cancer				
BMAL1	Downregulated	Regulates pyruvate and mitochondrial metabolism, Relates to the recruitment and infiltration of immune cells.	Downregulation of BMAL1 is associated with higher risk of metastasis in obesity-promoted TNBC	Ramos, C. A., et al. (2020)
TIM	Upregulated	TIM ↔SP1-ACER2↑-S1P↑	Regulates cancer cells sphingolipid metabolism; Promotes mitochondrial respiration; Promotes cancer cells proliferation.	Zhang, S., et al. (2020)
CRY2	Downregulated	CRY2↓-DNA damage accumulation↑(Exist in ER-/PR- BC)	Loss of CRY2 confers cancer cells aggressive phenotypes	Hoffman, A. E., et al. (2010) Liu, L., et al. (2017)

(*continued on next page*)

Table 1 (*continued*)

Clock genes	Expression in tumor tissue	Regulatory mechanism	Effect of clock genes on cancer progression	Reference
REV-ERBα	Unclear	Regulates several genes involved in de novo fatty acid synthesis network	Be required specifically for the survival of HER2+ BC cells	Kourtidis, A., et al. (2010)
Cancer type: Ovarian Cancer				
PER2	Downregulated	PER2↓-PI3K/PKB↑, EMT↑, cell apoptosis↓, Inflammatory response and drug efflux↑	Inhibits tumor growth and metastasis; Inhibits tumor angiogenesis; Associated with cisplatin-resistance of OC cells.	Wang, Z., et al. (2016)
CLOCK	Upregulated	Upregulation of drug resistance genes (P-gp, MRP2) Affect autophagy	Promotes cancer cells cisplatin resistance	Xu, H., et al. (2018) Sun, Y., et al. (2017)
Cancer type: Gastric cancer				
REV-ERBα	Downregulated	REV-ERBα- G6PD, PFKFB3↓-PPP, Glycolysis↓ REV-ERBα↓-Cleaved caspase3↓BCL-2/BAX ratio↑	Inhibits cancer cells proliferation	Wang, X., et al. (2018) Tao, L., et al. (2019)
CRY1	Upregulated	CRY1-cAMP/PKA↓-ERK1/2 phosphorylation↓-Oncogenic MAPK pathway↑	Protect cancer cells from the antiproliferative effects of ISO.	Huang, Q., et al. (2020) Jang, J. et al., et al. (2018)
Cancer type: Nasopharyngeal carcinoma				
BMAL1(ARNTL)	Downregulated	BMAL1-CDK5↓	Inhibits cancer cells proliferation and increases sensitivity to cisplatin	Peng, H., et al. (2019) Q.Y He et al. (2018)
TIM	Upregulated	TIM-WNT/β-catenin↑	Promotes the EMT; Causes cancer cells platinum resistance.	Liu, S. L., et al. (2017)
Cancer type: Osteosarcoma				
CRY1	Downregulated	CRY1↓-Akt/P53/P21↑ Interfering the circadian clock network	Inhibits cancer cells proliferation	Zhou, L., et al. (2018)
Cancer type: Oral squamous cell carcinoma				
PER1	Downregulated	PER1- AKT/mTOR↓	Regulates cancer cells proliferation, autophagy, apoptosis.	Yang, G., et al. (2020)

ACER2, Alkaline ceramidase 2; ATG5, Autophagy regulate gene 5; BC, Breast cancer; CDK5, Cyclin-dependent kinase 2; CDKN1A, Cyclin Dependent Kinase Inhibitor 1A; CSC, Cancer stem cell; CRC, Colorectal cancer; DOX, doxorubicin; EMT, Epithelial—Mesenchymal Transition; ER, estrogen receptor; G6PD, Glucose-6-phosphate Dehydrogenase; HER, human epidermal growth factor receptor 2; IL8, Interleukin8; ISO, Isoproterenol; MAPK, mitogen-activated protein kinase; MDM2, Mouse double minute 2; MMP2, Matrix metalloproteinase-2; MMP9, Matrix metalloproteinase-9; MRP2, multidrug resistance protein 2; OC, Ovarian Cancer; PFKFB3, 6-phosphofructokinase-2/fructose-2, 6-bisphosphatase; PPP, Pentose Phosphate Pathway; PR, progesterone receptor; SP1, Specific protein 1; S1P, Sphingosine 1-phosphate; TNFα, Tumor necrosis factor α; uPA, urokinase-type plasminogen activator; VEGF, Vascular endothelial growth factor, γH2AX, Phosphorylation of H2AX.

diseases and cancer. Several studies have shown that BMAL1 controls cell cycle and proliferation.[13] CRYs are a class of blue light-sensitive flavoproteins found both in plants and animals.[14] High CRY1 expression level has been observed of SCN, and mice with excised SCN show increased tumor growth.[15] CRY2 plays a unique role in regulating DNA damage repair and maintaining genomic stability.[16] Nuclear receptors REV-ERBs and retinoic acid receptor-related orphan receptor RORs participate in the regulation of multiple physiological processes, including glucose and lipid metabolism, adipocyte differentiation, and immunity.[17] They also play key roles in glial activation and neuroinflammation.[18] TIM is originally recognized to be a molecular cog in the Drosophila biological clock. Mammalian TIM (mTIM) is identified as a potential circadian clock component due to its sequence similarity with insect TIM (dTIM), but its role in clock regulation remains debatable.[19] In mammalian brain, *TIM* mRNA expression

displays rhythmic,[20] and the interaction between TIM and PERs or CRYs has been identified.[21,22] However, these interactions are not necessary to support a direct circadian role of mTIM. In addition, mTIM also functions in maintaining genome stability and replication checkpoint control.[23]

Clock genes and cancers

Changes in modern human lifestyles are prone to circadian rhythm disruption, which causes pathological conditions, such as sleep disturbances, depression, endocrine system dysregulation, obesity, metabolic diseases, particularly cancer.[5,24–28] International Agency for Research on Cancer (IARC) conclude that night work is probably carcinogenic to humans.[29] Recent studies have shown that the circadian gene dysfunction resulted from single nucleotide polymorphisms (SNPs), deletions, epigenetic modification, and deregulation is strongly associated with cancer risk.[30,31]

Lung cancer

Lung cancer has the highest mortality rate worldwide,[32] which is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) based on pathological characteristics. NSCLC is mainly divided into adenocarcinoma (ADC) and squamous cell carcinoma (SCC) by histological phenotype.[33] The integration and analysis of The Cancer Genome Atlas (TCGA) data show that high CRY2, BMAL1, and RORA expressions are associated with a favorable outcome in ADC patients. Whereas high expression of DEC1 correlates to poor overall survival (OS) in patients with SCC.[34]

"Tumor suppressors" in lung cancer: PERs, BMAL1, and REV-ERBα

PER1, 2, 3 expressions are downregulated in NSCLC tissues compared with the matched non-tumor tissues, and DNA hypermethylation and histone H3 acetylation are potential mechanisms for PER1 silencing. Forced expression of Per1 resulted in the cell cycle arrest and apoptosis of NSCLC cells.[35] PER2 significantly inhibited NSCLC growth and metastasis *in vivo* might through up-regulating the expression of the tumor suppressor genes *BAX*, *TP*53, and *CDKN1A*, while suppressing the expression of the proto-oncogenes vascular endothelial growth factor (*VEGF*), *CD44*, and *MYC*.[36,37] PER3 was also observed inactivated by promoter hypermethylation in NSCLC tissues and can be restored by demethylation drug 5-Aza treatment.[38] Thus, loss of PERs might promote NSCLC progression and serve as a novel biomarker for NSCLC diagnosis and prognosis.[39]

BMAL1 might act as a tumor suppressor in lung cancer. Forced expression of BMAL1 hampered cancer cell growth and invasion by inhibiting the PI3k/AKT/MMP-2 pathway and antagonizing BCL-w activity.[40] REV-ERBα functions as a transcriptional repressor and can bind to the promoter of autophagy regulate gene 5 (*ATG*5) to repress its transcription, leading to defective autophagy. SR9009, a special synthetic agonist of REV-ERBs, pharmacologically activates REV-ERBα in a post-translational manner to impair autophagy activity, enhancing cytotoxicity both in chemosensitive and chemoresistant SCLC cells.[41]

"Tumor promoters" in lung cancer: CLOCK, and TIM

CLOCK expression has been found enriched in lung cancer stem-like cells (CSCs), and CLOCK depletion suppressed cell stemness, exemplified by the reduced expression of CD133, CD44, Sox2, Nanog and Oct 4. A chemopreventive agent, epigallocatechin gallate (EGCG), can reduce the CSC-like properties of NSCLC cells by targeting CLOCK to inactivate the Wnt/β-catenin pathway.[42]

High TIM expression has been observed in NSCLC tissues, and positively correlates to tumor size, tumor-node-metastasis (TNM) stage, lymph node metastasis, and clinical prognosis.[43,44] *TIM* silencing inhibited cell proliferation and clonogenic growth, induced apoptosis in lung cancer cells, and sensitized cancer cells to doxorubicin and cisplatin treatment.[44,45] In that, CLOCK and TIM overexpression might contribute to the pathogenesis, progression, and poor prognosis of NSCLC.[43]

Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death globally.[46,47] Recent evidence supports that BMAL1 behaves as a tumor suppressor, whereas TIM, CRY1, and CLOCK, might function as tumor promoters in CRC.

"Tumor suppressor" in CRC: BMAL1

BMAL1 can act as a metabolic regulator. A disruption of BMAL1 induced a time-dependent metabolic reprogramming, namely an increased glycolytic activity, through up-regulating hexokinase HKDC1. The metabolic alteration led to a faster proliferation, and a more metastatic phenotype in CRC cells.[48] In addition, forced BMAL1 expression impeded CRC cell proliferation and sensitized cells to oxaliplatin by activating ATM signaling, resulting in G2/M phase arrest of cancer cells. Moreover, BMAL1 expression was associated with the prognosis of oxaliplatin-based chemotherapy in advanced CRC. CRC patients with high BMAL1 expressions showed significantly longer progression-free survival (PFS) and OS than those with low BMAL1 levels.[49]

"Tumor promoters" in CRC: TIM, CRY1, and CLOCK

TIM expression was up-regulated in CRC tissues relative to the normal counterparts, and was closely associated with the TNM stage and OS of patients with CRC.[50] CREB-binding protein (CBP)/p300-mediated H3K27 acetylation contributed to the enhanced expression of TIM.[50] In addition, ERK activation has been shown to promote TIM expression in cancer.[51] TIM bound to Myosin-9 to enhance its stability, thus promoted the nuclear translocation of β-catenin. The activation of β-catenin pathway induced by TIM facilitated CRC cell proliferation, invasion, and EMT *in vitro* and *in vivo*.[50] Depletion of TIM increased γH2AX (a marker of DNA damage) and caused G2/M phase arrest by enhancing the phosphorylation levels of checkpoint kinase 1 (CHK1) and cyclin-dependent kinase 1 (CDK1), thus limiting cell proliferation.[51]

Both CRY1 and CLOCK expressions were found significantly up-regulated in CRC tissues compared to the non-cancerous tissues, and were associated with the clinicopathological characteristics of CRC, including lymph node metastasis and TNM staging.[52–54] The PFS and OS of CRC patients with low CRY1 levels were significantly longer than those with high CRY1 expressions.[52] CRY1 was highly expressed in the majority of CRC cell lines, and CRY1 overexpression promoted the proliferation and migration of CRC cells.[52]

A study reported that genetic variants (SNPs, rs3749474 and rs1801260) in the CLOCK gene significantly correlated with the OS of CRC patients.[55] Thus, CLOCK gene polymorphisms might serve as an independent prognostic biomarker for CRC. Higher CLOCK level was observed in CRC cell lines with high metastatic potential. Forced expression of CLOCK up-regulated angiogenesis-related genes including HIF-1α and VEGF, and facilitated EMT in CRC cells.[53]

Breast cancer

Breast cancer (BC) is featured with the highest mortality rate among gynecological cancers. Circadian clock molecules CRY2 and BMAL1 function in tumor suppression, whereas REV-ERBα and TIM exert a tumor-promoting effect in BC.

"Tumor suppressors" in BC: CRY2, and BMAL1

Higher promoter hypermethylation of *CRY2* gene and significantly reduced CRY2 expression have been found in BC tissues relative to normal tissues.[56] *CRY2* knockdown promoted the accumulation of mutagen-induced DNA damage in BC cells.[16] Three SNPs (rs11038689, rs7123390 and rs1401417) in CRY2 are closely correlated with postmenopausal BC risk. Intriguing, this association only exists in women with estrogen receptor (ER) and progesterone receptor (PR) negative tumors, but not with ER/PR positive BC.[56] Overall, methylation status, as well as genetic variants of *CRY2* gene, might be useful BC biomarkers.

BMAL1 might serve as a tumor suppressor in BC. Down-regulation of BMAL1 correlates to higher risk of metastasis in BC patients. A study in an obesity/hyperinsulinemia mice model showed that BMAL1 inhibited triple-negative BC (TNBC) tumor growth and lung metastasis in obese mice rather than lean ones. Loss of BMAL1 and hyperinsulinemia obesity orchestrated to accelerate mitochondrial metabolism and reshape inflammatory tumor microenvironment by enhanced recruitment of macrophages and decreased infiltration of $CD8^+$ T cells. These actions conferred survival and metastatic advantages to BC cells.[57]

"Tumor promoters" in BC: REV-ERBα, and TIM

NR1D1 gene that encodes REV-ERBα is located on ERBB2 (HER2)-containing 17q12-21 amplicon, suggesting a role of REV-ERBα in $HER2^+$ breast cancer.[58] REV-ERBα was demonstrated to be required specifically for the survival of $HER2^+$ BC cells. As a transcription factor, REV-ERBα regulates several genes involved in de novo fatty acid synthesis network, exemplified by malate dehydrogenase 1 (MDH1) and malic enzyme 1 (ME1), which link glycolysis and fatty acid synthesis. The high-level fat synthesis and storage induced by REV-ERBα promoted the energy production necessary for the survival of $HER2^+$ BC cells.[58] Considering that $HER2^+$ associates with the aggressive form of BC, targeting REV-ERBs might be a promising approach for this malignancy.

High expression of TIM correlates with a poor clinical outcome, especially in patients with ER-positive BC. The oncogenic role of TIM might be associated with its regulation on sphingosine metabolism. As a coactivator, TIM can interact with specific protein 1 (SP1) to transcriptionally regulate alkaline ceramidase 2 (ACER2), thus promoting the synthesis of sphingosine 1-phosphate (S1P), one of the most important bioactive products of sphingosine metabolism.[59] ACER2 mediated the enhanced mitochondrial respiration and proliferation of BC cells induced by TIM.

Ovarian cancer

Ovarian cancer (OC) is one of the most common cancers worldwide in women with high incidence and mortality, and the clinical outcome of patients with OC directly correlates with the stage of disease at diagnosis.[60]

"Tumor suppressors" in OC: PER2

In OC tissue samples, PER2 expressions were found inversely correlated with the pathological stage.[61] The regulation on inflammatory factors as well as oncogenic signaling pathway might mediate the tumor-suppressive role of PER2 in OC.

A case-control study of OC patients showed that PERs expression significantly decreased in the circadian rhythm-disorder group (patients who work night-shifts) relative to the normal group (those who work day shift); whereas the expression of tumor-promoting inflammatory factors, such as programmed cell death receptor 1 (PD-1), programmed death-ligand 1 (PD-L1), and interleukin-6 (IL-6), were augmented.[62] The loss of *PER2* led to the activation of PI3K/AKT pathway and epithelial–mesenchymal transition (EMT), impaired cell apoptosis, and aggravated inflammatory response as well as drug efflux. These actions contributed to the cisplatin-resistance of OC cells.[63] The tumor-suppressive effect of PER2 was further validated in a xenograft mouse model of OC.[61] Thus, the rhythm-related factor PER2 should be a promising biomarker for diagnosis and therapeutic response of OC.

"Tumor promoters" in OC: CLOCK

The expression of circadian gene *CLOCK* is strongly associated with chemo-resistance of OC cells. The up-regulation of CLOCK expression endowed OC cells resistance to cisplatin treatment.[64] Up-regulation of drug resistance genes, such as P-glycoprotein (*P-gp*) and ATP binding cassette subfamily C member 2 (*MRP2*), and autophagy induced by CLOCK might mediate this process.[65]

Gastric cancer

Gastric cancer (GC) is the third leading cause of cancer-related deaths globally with high incidence, metastasis, and mortality rates. In contrast, the early diagnosis, radical

resection, and 5-year survival rates are relatively low.[66,67] Up-regulated *PER2* expression was observed in GC relative to the adjacent nontumor tissues. *CRY1* expression is significantly associated with advanced GC (stage III and IV).[68] Whereas decreased REV-ERBα expression relates to poor differentiation, TNM stage, and poor clinical outcome in GC patients.[69]

"Tumor suppressors" in GC: REV-ERBα

Compared with normal tissues, REV-ERBα expression was significantly decreased accompanied by downregulated cleaved caspase-3 and up-regulated BCL-2/BAX ratio in GC. Treatment with GSK4112, a REV-ERBα activator, induced apoptosis in GC cells. Considering its association with clinicopathological characteristics, REV-ERBα might be a potential biomarker for tumor development and prognosis, and therapeutic target for GC.[69]

"Tumor promoter" candidate in GC: CRY1

CRY1 acts as a potential oncogenic factor in GC. Although *CRY1* overexpression only slightly affected the proliferation and migration of GC cells, it protected cells from the antiproliferative effect of isoproterenol (ISO), a β-adrenergic receptor agonist. Through blocking the signal transduction between G protein-coupled receptor and adenyl cyclase (AC), CRY1 overexpression downregulated cAMP/PKA pathway, resulting in retained ERK1/2 phosphorylation level and the activation of oncogenic MAPK pathway. These actions thus facilitated the survival of GC cells.[70] Additionally, KS15, a chemical scaffold containing 2-ethoxypropionic acid, has been identified as a CRY inhibitor. It can block the interaction between CRYs and BMAL1 by directly binding to the C-terminal region of CRYs. This action impair the inhibitory effect of CRYs on CLOCK-BMAL1 feedback loop, thus activating E-box-mediated transcription.[71] Thereby, KS15 and other CRYs inhibitors might be applied to adjuvant chemotherapy by pharmacologically interrupting the transcriptional activity of the CLOCK-BMAL1 heterodimer.

Nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) represents one of the major subtypes of head and neck cancers, and mostly prevails in southern China and southeastern Asia.[72,73] The majority of patients are diagnosed with advanced stage (III and IV), which leads to poor prognosis.[74]

"Tumor suppressors" in NPC: BMAL1

Cisplatin-based concurrent chemotherapy is the standard regimen for metastatic nasopharyngeal carcinoma.[75] Cyclin-dependent kinase 5 (CDK5), a cell cycle G2/M phase checkpoint gene, might be a functional target in BMAL1-mediated proliferation suppression of NPC cells. Moreover, BMAL1 improved the sensitivity of NPC cells to cisplatin treatment *in vivo* by targeting CDK5.[76] In that, BMAL1/CDK5 axis might be a promising target for NPC therapy.

"Tumor promoters" in NPC: TIM

TIM plays an oncogenic role in NPC progression. TIM expression is significantly associated with clinical stage, T and N category, distant metastasis, and serum Epstein—Barr virus (EBV) DNA. High TIM expression correlates to poorer OS and progression-free survival (PFS) of NPC patients. Overexpression of TIM enhanced EMT, and activated the WNT/β-catenin pathway, which conferred NPC cells resistance to cisplatin-induced apoptosis.[77] Thereby, TIM should represent a valuable prognostic index and potential target for therapeutic gain of NPC.

Osteosarcoma and CRY1

Osteosarcoma (OS) is a primary malignant bone tumor that mainly affects children and adolescent.[78] CRY1 is characterized as a tumor suppressor in OS. Reduced CRY1 expression was found in human osteosarcoma relative to the normal tissues. *CRY1* silencing promoted the proliferation of osteosarcoma cells both *in vitro* and *in vivo*. The anti-proliferative effect of CRY1 not only relies on controlling the downstream signaling, exemplified by AKT/p53/p21 axis, but on interfering the circadian clock network. *Cry1* knockdown enhanced the expression of *Cry2*, *Per1*, *Per2*, *Per3*, *BMAL1*, and *Clock*, whereas reduced *Dec1*, *Dec2*, *CK1ϵ*, and *Npas2* expression in OC cells.[79]

Oral squamous cell carcinoma and PER1

Oral squamous cell carcinoma (OSCC) is the most common oral cancer with poor prognosis and high mortality.[80] PER1 was significantly downregulated in OSCC, and PER1 expression correlated with TNM clinical stage and prognosis of OSCC patients. PER1 exerts tumor-suppressive effect in OSCC cells by regulating autophagy, proliferation and apoptosis in an AKT/mTOR pathway-dependent manner.[81]

Clock genes and tumor microenvironment

The tumor microenvironment (TME) has been intimately implicated in tumorigenesis because within it, tumor cells communicate and interact with the surrounding noncancerous components, such as fibroblasts, macrophages, immune cells, capillaries, and extracellular matrix, contributing to the pathogenesis and progression of cancer.[82] Circadian genes mediate the interplay between tumor cells and the TME through regulation of downstream CCGs, which are involved in cell cycle, apoptosis, DNA-damage response, and chromatin modifications.[83]

On the one hand, the TME affects the circadian rhythmicity of tumor cells by interacting with or altering the expression of clock molecules as well as the downstream CCGs, accelerating cancer progression. Due to the compactness of solid tumor tissue, the lack of tumor microvessels, and the limitation of oxygen delivery, tumor cells are often confronted with a hypoxic TME. Hypoxia-inducible factor-1 (HIF-1) plays a pivotal role in adapting to hypoxia.[84,85] In CRC tissues, *CLOCK* showed a strong positive correlation with *HIF-1* and *VEGF* expressions.[54] CLOCK interacted with HIF-1 to transcriptionally boost VEGF expression, facilitating angiogenesis and metastasis in CRC.[54] Glioma-associated microglial cells, a key component harboring in the TME, play an essential role in the incidence and development of glioma. Co-culture with M2 phenotype

microglia resulted in up-regulated CLOCK, and decreased BMAL1 expression and apoptosis in glioma cells. Exosomal miR-7239-3p derived from M2 microglia mediated this process through inhibiting *BMAL1* expression and inducing EMT-associated protein expression.[86]

On the other hand, disruptions of circadian rhythm also contribute to the reshape of tissue microenvironment, possibly by induction of systemic inflammation and alteration of clock genes expression. A study in mice exposed to dark—light cycles simulating night shift work schedule (IN group) showed that gut microbiota and plasma exosomes were altered, and inflammatory macrophages in visceral white adipose tissue were increased by periodic shifts. Besides, plasma exosomes derived from IN group reduced pAKT/AKT responses to exogenous insulin, and altered clock genes expression in differentiated adipocytes.[87]

Clock genes and cancer metabolism

Cancer cells frequently undertake deregulated metabolism.[88—94] Circadian clocks are broadly implicated in metabolic process through interacting with key metabolic regulators. HIF-1α, a core glycolytic regulator, has been identified a direct transcriptional target of NPAS2. NPAS2 promoted glucose metabolic reprogramming through up-regulating glycolytic genes and downregulating mitochondrial biogenesis in hepatocarcinoma (HCC) cells, and HIF-1α mediated this process.[95] PER2 is also involved in glucose metabolism. It has been demonstrated to enhance glucose storage to liver glycogen in response to feeding and acute fasting.[96] Fructose-2,6-bisphosphatase (PFKFB3) and glucose-6-phosphate dehydrogenase (G6PD) involved in glycolysis and pentose phosphate pathway (PPP) are both verified direct targets of REV-ERBα. The repression of PFKFB3 and G6PD gene transcriptions induced by REV-ERBα hampered glycolytic flux and PPP pathway in GC cells.[97] Besides, REV-ERBs are well recognized as lipid metabolism regulators. REV-ERBs agonists strongly inhibited the expressions of several lipogenic enzymes, including fatty acid synthase (FASN) and stearoyl-CoA desaturase 1 (SCD1).[98]

Importantly, oncogenic factors are involved in the interplay between circadian clock system and metabolism, which results in circadian rhythm disorder and metabolic dysrhythmia in cancer cells. Oncogenic MYC functions as a core metabolic regulator to drive malignancy. As a transcription factor, it binds to E-boxes sites of the genome, which are identical to the binding sites of CLOCK-BMAL1 heterodimeric transcription factor. This action disrupted the clock system in cancer cells. *CRY*, *PER* and *REV-ERBs* have been identified as direct MYC target genes, with the exception of *ARNTL* (BMAL1). MYC induced REV-ERBα to inhibit BMAL1 expression and oscillation in neuroblastoma cells. Moreover, the circadian oscillation of critical metabolites in glycolysis and glutamine metabolism was disturbed by the activation of MYC, thus promoting biosynthesis for the growth of cancer cells.[99,100]

Chronotherapy in cancer

Chronotherapy emerges as a strategy that employs the circadian variation in cell division, tissue turnover, and drug metabolism to optimize therapeutic efficacy and minimize adverse effects.[101] NCBI database has shown 4626 cases of clinical trials related to circadian rhythms from 1965 to March 2022, and among them, 163 clinical trials are conducted in cancer.

Studies have shown that chronochemotherapy achieves reasonable responses in cancer therapy relative to traditional dosage regimens. 5-FU is a clinical first-line drug used for several common solid malignancies, and its metabolism displays circadian variation.[102] Capecitabine is an oral pre-prodrug of 5-FU. Continuous chronomodulated 5-FU or capecitabine treatment showed improved tolerability and efficacy of the drugs, and reduced mucosal toxicity in cancer patients compared to the regular regimen. These effects might be attributed to the circadian rhythmicity of 5-FU degrading enzyme, dihydropyrimidine dehydrogenase (DPD), and the target enzyme, thymidylate synthase (TS).[103,104] In addition, in a randomized phase II trial in patients with advanced NPC, compared to regular cisplatin administration with intensity-modulated radiation therapy (IMRT), cisplatin chronotherapy combined with IMRT achieved better outcomes for side effects and immune function, and enhanced the tolerance for treatment.[105] The diurnal changes of circulating hormones, such as melatonin, might mediate the systemic circadian activity. It has been reported that the presence of melatonin, which level peaks at late night, increased doxorubicin (DOX) toxicity. Hence, administrated with DOX in the mid-morning, when the circulating level of melatonin was low, reduced cardiotoxicity in cancer therapy.[106]

Recently, chronomodulated treatment has also been applied in immune checkpoint blockage (ICB) therapy. A study aimed at exploring the dependence of the adaptive immune system on circadian rhythms was carried out in 481 patients with melanoma treated with immune checkpoint inhibitors. Because adaptive immune responses occurred more robust when the initial stimulation was administered in the daytime than in the evening, scheduling infusions of immune checkpoint inhibitors before mid-afternoon facilitated a more effective immune-mediated antitumor response and prolonged OS of the patients.[107]

Summary

The emerging role of proper clock maintenance is highlighted by intimate association between circadian disorder and a variety of diseases, including cancer.[108] Aberrant expression of circadian clock genes has been observed in multiple types of cancer and been implicated in cancer incidence and development, therapeutic response, and prognosis. In NSCLC, TIM expression negatively correlates to the clinical prognosis of patients. In CRC, patients with higher BMAL1 expressions have longer PFS and OS, and the polymorphism of CLOCK (rs3749474 and rs1801260) is also associates with the OS of patients. CRY2 SNPs (rs11038689, rs7123390 and rs1401417) correlate with postmenopausal BC risk, and high expression of TIM predicts a poor outcome of patients with ER-positive BC. In addition, REV-ERBα in GC, TIM in NPC, and PER1 in OSCC, all display predictive value for the OS of patients. Thus, the genetic variants and altered expression of clock genes might become novel biomarkers for clinical diagnosis and prognosis prediction in cancer.

Among the clock gene family members, PERs and BMAL1 function as tumor suppressors, Whereas CLOCK and TIM as tumor promoters in diversified cancers. Others, such as REV-ERBα and CRYs, might play differential, even opposite roles in different types of cancer. For most of the family members act as transcription factors, in the scenario of specific cancer cells, they might be recruited to different transcriptional activator or repressor complex, thus boost different transcriptional programs of target genes and the downstream signaling pathways. These actions might partially account for the differential role of specific clock gene in various cancer types. It should be noted that so far, the documentaries on the role of circadian clocks in cancer are preliminary. With deeply exploration of the dysfunction of circadian system and clock genes in tumorigenesis and progression, the comprehensive facet of clock gene family should be further updated and outlined.

Circadian components interact with metabolic enzymes and intra- or extra-oncogenic factors derived from the TME to construct a complicate network, which might account for the circadian desynchrony and metabolic dysrhythmia in cancer cells.[27,86] Notably, nuclear receptors REV-ERBs and RORs are druggable targets and the synthetic ligands targeting these receptors might be useful in the treatment of circadian rhythm desynchrony and metabolic disturbance.[109,110] REV-ERBs agonists exert antitumor effects against a variety of oncogenic factors.[98,110] Among them, the REV-ERBs agonist SR9009 affects metabolism and exerts cytotoxic effects on glioblastoma and lung cancer cells.[41,109,111] Another REV-ERBs synthetic agonist, SR9011, shows inhibitory effect on the growth of glioblastoma without causing significant toxicity.[109] Currently, chronotherapy has attracted increasing attention as an adjuvant mode combined with radiochemotherapy and immune therapy. The therapeutic indexes of regular regimen are substantially improved by chronomodulated administration and are of particular significance in cancer therapies. Profound understanding the intricate network among circadian clock molecules, metabolism, and the TME, might immensely contribute to revealing the role of circadian clocks in tumorigenicity and malignancy, improving the chronotherapeutic efficacy, and establishing more novel temporal treatment strategies.

Author contributions

HL and XL conceived and designed the study. HL prepared the manuscript. YL, RH and WL assisted in manuscript editing. XL reviewed and edited the manuscript. All authors reviewed and approved the final version of manuscript.

Conflict of interests

The authors declare that they have no conflict of interest.

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