Review

Circadian regulation of cancer cell and tumor microenvironment crosstalk

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Circadian rhythms regulate a remarkable variety of physiologic functions in living organisms. Circadian disruption is associated with tumorigenesis and tumor progression through effects on cancer cell biological properties, including proliferation, DNA repair, apoptosis, metabolism, and stemness. Emerging evidence indicates that circadian clocks also play an influential role in the tumor microenvironment (TME). This review outlines recent discoveries on how cancer cell clock components (including circadian clock and clock genes/proteins) regulate TME biology and, reciprocally, how TME clock components affect tumor growth, metastasis, and therapeutic response. An improved understanding of how clock components regulate the symbiosis between cancer cells and the TME will inform the development of novel clock-oriented therapeutic strategies, including immunotherapy.

Circadian rhythm and cancer: an old story but a new direction

The circadian rhythm (see Glossary) is an evolutionarily conserved phenomenon that regulates the rhythmicity of physiologic, behavioral, and biochemical functions in living organisms [1,2]. One of the best-known circadian rhythms is the sleep–wake cycle, in which the term circadian means ‘around a day’ [3]. Circadian rhythms are regulated by circadian clocks in mammals. Over the past few decades the connection between circadian clocks and tumorigenesis has been well studied. Mechanistically, circadian clock disruption can promote tumor growth and progression by affecting key cancer cell biological properties [1]. However, cancer cells do not live and flourish in isolation, are surrounded by various stromal cells and factors in the TME. Typically, the TME comprises extracellular matrix (ECM) and a variety of cells including innate myeloid cells [e.g., tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs)], neutrophils, and dendritic cells, lymphocytes [e.g., T cells and natural killer (NK) cells], cancer-associated fibroblasts (CAFs), and endothelial cells [4]. In recent years the importance of TME in affecting tumor progression and therapeutic efficacy has become widely recognized and multiple TME-targeted therapies have been developed [5]. Moreover, context-dependent cancer cell–TME symbiotic interactions have been demonstrated to be critical for tumor progression and therapy resistance [6–8]. Improving the molecular understanding of the circadian clock in tumorigenesis and cancer cell–TME symbiotic interactions is currently a major focus of cancer research. Therefore, we need to understand whether and how circadian clocks are essential for regulating TME biology and cancer cell–TME symbiosis. In this review we first discuss the influence of circadian clocks on cancer hallmarks and TME biology. Second, we highlight and discuss how cancer cell clock components (including circadian clock and clock genes/proteins) regulate TME biology and, reciprocally, how TME clock components affect tumor growth, metastasis, and therapy resistance. Finally, we discuss the immunotherapeutic potential of targeting clock component-regulated cancer cell–TME symbiosis. We believe that this emerging area of interest in cancer biology has provided and will continue to provide insights leading to novel and effective treatments for cancer patients.

Highlights

Circadian clocks contribute to tumor growth and metastasis by regulating the biology of cancer cells and the tumor microenvironment (TME), as well as their symbiotic interactions.

Cancer cell clock components affect TME biology via regulation of angiogenesis, tumor-promoting inflammation, and immune escape.

TME clock components affect tumor progression via regulating cancer cell biological properties directly and by affecting the protumor TME indirectly.

Characterizing clock component-regulated cancer cell–TME symbiosis might reveal unique therapeutic strategies, and targeting this symbiosis could increase the efficiency of immunotherapy.

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The molecular circadian clock and its effect on the biology of cancer cells and the TME

The circadian system is composed of central and peripheral clocks [9]. The central clock is located in the anterior hypothalamic suprachiasmatic nucleus (SCN), which can function autonomously and coordinate peripheral clocks in the body by sending signals [10]. At the molecular level, the circadian rhythms emerging from central and peripheral clocks are relatively similar. The central molecular circadian clock machinery is regulated by transcription–translation feedback loops (TTFLs) [1,2,11,12]. The aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL, also named brain and muscle ARNT-like protein 1 (BMAL1)) and CLOCK (circadian locomotor output cycles kaput) constitute positive factors of the feedback loop, which can bind to the E-box motif and promote the expression of transcription repressors, including cryptochrome (e.g., CRY1 and CRY2) and period (e.g., PER1, PER2, and PER3) genes. As the repressor arm of the TTFL, CRY and PER form a complex that enters the nucleus and suppresses the CLOCK–BMAL1 complex [13–15]. Furthermore, the CLOCK–BMAL1 complex can regulate the expression of nuclear receptors REV-ERBα/β (also known as NR1D1/2, nuclear receptor subfamily 1, group D, members 1/2) and retinoic acid receptor-related orphan receptors (RORs), which in turn repress and activate BMAL1, respectively, thus constituting a second feedback loop [1,2].

Circadian clock disruption is involved in the pathogenesis of many types of diseases, including cancer [16,17]. Emerging evidence suggests that circadian clock disruption (e.g., night-shift work and ‘late-eaters’ who eat after 9:30 PM) increases cancer risk [18–20] and is associated with increased tumor metastasis in a variety of cancer types, including breast cancer [21,22], non-small cell lung cancer (NSCLC) [23], and colorectal cancer (CRC) [24] in humans. The importance of this line of research is also supported by mouse model studies showing that circadian clocks play a prominent role in tumorigenesis and in regulating the antitumor efficiency of radiotherapy [25,26] and chemotherapy [27,28]. Mechanistically, circadian clocks can affect tumor growth and progression via the regulation of multiple cancer hallmarks in cancer cells [29,30], which include the DNA damage response, apoptosis, cell cycle, and senescence [1,18,31,32], proliferation [1,2], metabolism [30,33,34], replicative immortality [35], and genome instability and mutation [34]. Cancer stem cells (CSCs) are a subpopulation of cancer cells that have self-renewal ability and markedly contribute to tumor initiation, metastasis, and therapy resistance [6]. Increasing evidence argues that circadian clocks are essential for maintaining CSC stemness in different cancer types, including acute myeloid leukemia (AML) and glioblastoma multiforme (GBM) [36–38]. Specifically, disruption of the circadian clock machinery pharmacologically (e.g., using the REV-ERB agonists SR9009 and SR9011) and genetically (e.g., using CLOCK and BMAL1 short hairpin (sh)RNAs) in AML leads to CSC differentiation [38], and impairs glioma stem cell (GSC) stemness in GBM and causes GSC cell-cycle arrest and apoptosis [36,37]. These results suggest that the core components of circadian clocks regulate key cancer cell biological properties across cancer types.

Beyond its impact on cancer cells described earlier, circadian clock disruption also influences the TME and the interactions between cancer cells and the TME. For example, in a CRC–CAF coculture model, the biological clocks of CRC cells are modulated to enhance the CRC malignant phenotype via regulating cell metabolism, viability, and apoptosis, and induce chemotherapy resistance [39]. Moreover, in vivo findings from breast cancer and melanoma mouse models demonstrate that circadian clock disruption not only significantly enhances cancer cell proliferation, dissemination, stemness, and metastasis but also induces an immunosuppressive TME by increasing the proportion of TAMs and regulatory T cells (Tregs), thus inducing macrophage polarization towards an anti-inflammatory phenotype, and decreasing the infiltration and activity of lymphocytes.
of CD8$^+$ T cells [40,41]. Similarly, computational analysis of gene expression data from cancer patient samples has revealed that clock genes are associated with immune cell infiltration and cancer cell proliferation [42]. Together, these findings suggest that circadian clocks play a crucial role in regulating cancer cell biological properties, TME biology, and their potential symbiotic interactions. In the following, we highlight specific circadian clock components in cancer cells or in cells of the TME that affect cancer cell–TME crosstalk, and discuss their therapeutic potential.

The effect of cancer cell clock components on TME biology

TME is educated by cancer cells and plays an important role in tumor progression [4,8,43]. This section summarizes the role and underlying mechanisms of cancer cell clock components in regulating TME biology, including angiogenesis, tumor-promoting inflammation, and immune evasion (Figure 1).

Figure 1. Clock components in cancer cells affect tumor microenvironment (TME) biology. Clock components, including circadian clock and clock genes/proteins (e.g., CLOCK, ARNTL/BMAL1, PER, CRY, RORs, and REV-ERBα) in cancer cells or cancer stem cells regulate the expression and secretion of soluble factors (e.g., HIF1α, ARNT, VEGF, OLFML3, and other unidentified factors). These secreted factors modulate TME biology, including endothelial cell (EC) biology (e.g., promoting angiogenesis and antiangiogenic therapy resistance), the infiltration of myeloid cells (e.g., macrophages, neutrophils, monocytes, dendritic cells, MDSCs, and mast cells). Myeloid-derived suppressor cells (MDSCs): a subset of myeloid cells in tumor tissues and in periphery of tumor-bearing hosts that have an immunosuppressive function.

Regulatory T cells (Tregs): a population of specialized T cells that suppress antitumor immune responses.

Single-cell RNA sequencing (scRNA-seq): a technology that can determine the gene expression profiles of individual cells, thus offering a better understanding of the phenotype, state, and function of an individual cell in the context of its microenvironment.

Stemness: a molecular process underlying the core stem cell property of self-renewal.

Symbiosis: a type of relationship between two species or cell types in which at least one component benefits.

The Cancer Genome Atlas (TCGA): a landmark cancer genomics program that characterizes >20 000 primary cancer and matched normal samples across 33 cancer types.

Type 17 T helper (Th17) cells: a subpopulation of CD4$^+$ T cells which are characterized by the production of high levels of IL-17.

Tumor-associated macrophages (TAMs): macrophages present in tumor tissues that display a protumor and immunosuppressive function.
Angiogenesis is a TME-associated cancer hallmark in which new blood vessels form from existing vasculatures [44–46]. Soluble factors secreted by cancer cells in the TME favor tumor angiogenesis, thus promoting tumor growth and metastasis [44,45]. We highlight recent findings that underscore the crucial role of cancer cell clock components in regulating the expression of angiogenic factors such as hypoxia-inducible factor 1α (HIF-1α), aryl hydrocarbon receptor nuclear translocator (ARNT), and vascular endothelial growth factor (VEGF). Genetic studies have demonstrated that the expression of these proangiogenic factors is decreased upon CLOCK shRNA knockdown and increased upon CLOCK overexpression in human CRC cells [24]. Furthermore, VEGF expression in xenograft tumors (including sarcoma, lung carcinoma, and melanoma) is increased under hypoxic conditions, fluctuates rhythmically in a circadian fashion (peaking during the light phase and decreasing around the early dark phase), and is transcriptionally inhibited by PER2 and CRY1 [47]. Accordingly, the antitumor effects of antiangiogenic therapies are more potent when drugs are administered at ZT 2 than that at ZT 14 (ZT 0 is designated as lights on and ZT 12 as lights off) [47]. Furthermore, antiangiogenic therapy can upregulate BMAL1 expression in CRC cells, which in turn induces therapy resistance via upregulation of VEGF [48]. These results highlight a crucial role of cancer cell clock components in influencing tumor angiogenesis and antiangiogenic therapy efficiency.

Inflammation is a key hallmark of cancer [46], supported mainly by the infiltration of innate myeloid cells [49]. Unbiased profiling of The Cancer Genome Atlas (TCGA) datasets (https://www.cancer.gov/tcga) followed by functional studies with mouse models have highlighted a strong connection between CSCs and myeloid cells because cancer cell stemness inversely correlates with antitumor immunity signatures across cancer types [6,50]. Specifically in GBM, high CLOCK levels in GSCs correlate with increased microglia in the TME via transcriptional regulation of the chemokine olfactomedin-like 3 [36]. In addition to microglia, cancer cell clock components also contribute to the infiltration of other types of myeloid cells in mouse models and cancer patients. For example, in kidney renal clear cell carcinoma (KIRC) and breast cancer, the expression of clock genes (e.g., CLOCK, ARNTL, and PER3) in cancer cells fluctuates rhythmically and is associated with the infiltration of macrophages, neutrophils, and dendritic cells [31,51]. Together, these findings support the concept that cancer cell clock components may influence tumor-promoting inflammation via effects on myeloid cell-mediated inflammation.

Immune escape is another critical hallmark of cancer [46], a process that involves in modulating lymphocyte biology and the expression of immune checkpoint molecules. Multi-omic analyses of KIRC and lung cancer have shown that clock genes (e.g., CLOCK, ARNTL, CRY1, CRY2, PER1, PER2, PER3, RORA, and NR1D1) not only regulate the circadian rhythm and transcription factor activity of cancer cells but also correlate with the infiltration of lymphocytes, including B cells, CD8+ T cells, and CD4+ T cells [31,32]. Similarly, patient-derived GSCs exhibit circadian oscillation independently of tumor genetics [37], and CLOCK expression in GBM patient tumors correlates with a decreased level of activated CD8+ T cells [36]. Moreover, genome mutation analysis has revealed that clock genes are frequently mutated in cancer patients, which in turn can induce genome instability, are associated with T cell (e.g., CD8+ and CD4+ T cell) exhaustion, and correlate with upregulation of immune inhibitory molecules, including programmed death-ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) [52,53]. These results suggest an association between clock components in cancer cells and the infiltration of lymphocytes and the expression of immune checkpoint molecules, thus potentially contributing to immune escape.

Collectively, these findings highlight that cancer cell clock components affect TME-related cancer hallmarks (including angiogenesis, inflammation, and immune escape), and suggest that...
blockade of circadian clock-associated cancer cell–TME crosstalk may inhibit tumor progression. Nonetheless, the available data provide a roadmap for further investigations of molecular mechanisms underlying the uncharacterized cancer cell clock component-associated TME characteristics, such as the presence and function of CAFs, Tregs, MDSCs, and the ECM.

Impact of TME clock components on tumor progression and therapy resistance

In addition to cancer cells and CSCs, the circadian clock can intrinsically regulate the behavior and function of the TME including immune cells [54–56], fibroblasts [57], and endothelial cells [58]. Given the importance of symbiotic cancer cell–TME interactions in tumor biology [36,59–61], we highlight the role of clock components in the TME of mouse models that affect cancer cell biological properties (Figure 2). For example, in a 4T1 breast cancer mouse model, clock genes (e.g., Clock, Arntl, Per2, Cry1, and Nr1d1) are rhythmically expressed in cells of the TME to induce the expression and secretion of Wnt family member 10A (WNT10A). Following secretion, WNT10A can increase cancer cell stemness through upregulation of aldehyde dehydrogenase 3 family, member A1 (ALDH3A1) [62], an enzyme whose activity is a characteristic of CSCs and correlates with tumor malignancy [63]. As a result, time-of-day effects for the efficacy of administering an ALDH inhibitor have been observed in this mouse model [62]. In MC38 colon and EO771 breast cancer mouse models, the expression of clock gene Per2 in the TME is crucial for metastatic colonization via inducing a pre-metastatic niche [64]. Furthermore, in a B16F10 melanoma mouse model, cisplatin treatment in the evening induces less toxicity than the same treatment administered in the morning. Notably, this difference is not apparent in Per1/Per2 knockout (KO) mice [65]. As a result, tumor-bearing Per1/Per2 KO mice showed extended survival and a more robust immune response (e.g., increased CD4+ and CD8+ T cells) relative to tumor-bearing wild-type (WT) mice following cisplatin treatment [65]. Taken together, these findings from distinct mouse models highlight an essential role of TME clock components in regulating cancer cell stemness, metastasis, and therapy resistance, and in so doing support

Figure 2. Impact of tumor microenvironment (TME) clock components on tumor growth, metastasis, and stemness. CLOCK and BMAL1 in the TME can induce the secretion of WNT10A, which in turn upregulates ALDH3A1 in cancer stem cells (CSCs) to promote stemness, tumor growth, and metastasis. In addition, PER1 and PER2 in the TME can induce a pre-metastatic niche to promote metastasis and induce chemotherapy resistance and immunosuppression, thus promoting tumor growth. Abbreviations: ALDH3A1, aldehyde dehydrogenase 3 family, member A1; BMAL1, brain and muscle ARNT-like protein-1; CLOCK, circadian locomotor output cycles kaput; PER, period; WNT10A, Wnt family member 10A.
the potential of targeting TME clock components. As discussed earlier, TME comprises diverse cell types, and it will therefore be important to identify the specific cell types of the TME that contribute to clock component-regulated cancer cell biological properties and therapy resistance.

TAMs are the most prominent subpopulation of cells in the TME and play a critical role in influencing tumor growth and metastasis [66]. TAMs can be classified as polarized toward proinflammatory and ‘alternatively activated’ phenotypes which exhibit immune-stimulatory (antitumor) and immunosuppressive (protumor) effects, respectively [8,67]. It should be noted that some TAMs may not be polarized to either state [68]. When polarized to the immune-stimulatory or immunosuppressive phenotype, BMAL1 expression in macrophages is upregulated. Depletion of BMAL1 in macrophages downregulates mitochondrial metabolism via upregulation of HIF1α and reactive oxygen species (ROS) accumulation, and downregulation of nuclear factor erythroid 2-related factor 2 (NRF2), thus regulating the production of proinflammatory cytokines [69,70]. As a result, tumor growth and TAM alternative polarization are increased in myeloid-specific Bmal1 KO mice compared with Bmal1 WT mice [69]. Similarly, coinjection of cancer cells with Bmal1 KO macrophages promotes tumor growth and reduces the infiltration of CD8+ T cells compared with Bmal1 WT macrophages [69]. Thus, these emerging lines of evidence highlight that macrophage BMAL1 is critical for suppressing tumor growth and promoting an antitumor immune response (Figure 3).

Lymphocytes play a crucial role in tumor immunity, and their antitumor activities can be regulated by cell-intrinsic clock components (Figure 3). RORγt is a transcription factor that can control interleukin (IL)-17-producing CD4+ type 17 T helper (Th17) cell differentiation in a circadian clock-dependent manner [71]. Activation of RORγ by using its synthetic agonists enhances the differentiation and effector function of Th17 cells and reduces the level of Tregs by regulating the expression of cytokines/chemokines, co-stimulatory receptors, and immunosuppressive molecules [72]. Consequently, coculture and coinjection of RORγ agonist-treated CD8+ Tc17 cells and EG7 lymphoma cells increases apoptosis in vitro and inhibits tumor growth in vivo [72]. Moreover, translational studies in breast cancer and CRC mouse models have shown that activation of RORγ inhibits tumor growth and extends animal subject survival through an influence on the antitumor immune response [72]. It should be noted that the effect of RORγ activation on Tregs may not be due to its clock function because Tregs do not have intrinsic circadian oscillators [73]. In addition to RORγ, activation of RORα can maintain the balance of cholesterol metabolism in CD8+ T cells by attenuating the nuclear factor κB (NF-κB) pathway, which in turn enhances CD8+ T cell activity and function. Eventually, these activated CD8+ T cells induce CRC cancer cell apoptosis [74]. In summary, these findings suggest that the specific targeting of RORs in T cells is a potential approach for immunotherapies.

Another potential TME clock mechanism involves the infiltration of leukocytes, a population of immune cells that are essential for tumor development [75]. A growing body of evidence suggests that circadian clock-regulated promigratory molecules can trigger leukocyte migration. We highlight recent findings that reveal the role and molecular basis of circadian clock components in specific cell types of the TME (e.g., B cells, neutrophils, and endothelial cells) in regulating the expression of promigratory molecules and leukocyte migration (Figure 3). For instance, specific deletion of Bmal1 in B cells, neutrophils, or endothelial cells in mice abolishes time-of-day differences in the expression of promigratory molecules [e.g., integrin αL (CD11A), P-selectin glycoprotein ligand 1 (PSGL-1), intercellular adhesion molecule 1 (ICAM-1), or vascular cell adhesion protein 1 (VCAM-1)], which in turn ablate the arrhythmic homing of B cells, neutrophils, and leukocytes [58]. By contrast, CLOCK expression in endothelial cells can transcriptionally upregulate the genes encoding ICAM-1, VCAM-1, and C–C motif chemokine ligand 2 (CCL2),
thus increasing the adhesion of leukocytes to endothelium [76]. In addition to the CLOCK–BMAL1 complex, other clock components also regulate the infiltration and adhesion of macrophages and monocytes. For example, activation of REV-ERBα can suppress CCL2 and its downstream signals (e.g., ERK and p38), which in turn inhibits macrophage adhesion and migration [77]. Moreover, overexpression of CRY1 in endothelial cells can inhibit the expression
of inflammatory cytokines [e.g., IL-1β, IL-6, and tumor necrosis factor (TNF)-α], adhesion molecules (e.g., VCAM-1, ICAM-1, and E-selectin), and NF-κB pathway activation, all of which impair monocyte adhesion [78]. Finally, in vivo findings from syngeneic and xenogeneic leukemia cancer models show that clock-regulated recruitment and engraftment of leukemic cells/leukocytes increases tumor burden [58].

Beyond the cell types of the TME discussed earlier, we may also need to consider the complement system which has a close connection with circadian clocks [79] and plays a pivotal role in promoting tumor growth via triggering myeloid cell infiltration and suppressing the CD8+ T cell-mediated immune response [80]. Based upon the results of studies described earlier, we propose that TME clock components play a significant role in modulating cancer cell stemness, tumor growth, metastasis, and therapeutic efficacy. Multiple examples have highlighted a critical role of clock components in myeloid cells, lymphocytes, and endothelial cells that regulate tumor growth via modulation of cancer cell apoptosis, macrophage polarization, CD8+ T cell activity, and leukocyte tumor infiltration. These studies targeting TME clock components and their regulated factors may have therapeutic potential in disrupting TME–cancer cell symbiotic interactions.

**Therapeutic potential of clock-oriented immunotherapy**

TME-targeted therapeutic strategies have emerged as a promising approach for cancer treatment because of TME–cancer cell symbiotic interactions, and the critical role of the TME in regulating tumor progression and modulating the antitumor efficiency of standard-of-care therapies [5,6,8,81]. Among the diverse cell types in the TME, myeloid cells (e.g., TAMs and MDSCs) can suppress T cell (including CD4+ and CD8+ T cells)–mediated immune responses and immunotherapy efficiency [81–84], and induce antiangiogenic therapy resistance [85–88]. Similarly, CAFs can suppress immunotherapy efficiency via interactions with myeloid cells and T cells [89]. Based on these mechanistic studies, attempts have been made to develop TME-targeted therapies to overcome immunotherapy resistance [90]. For example, TAM-targeted therapies (e.g., targeting TAM phagocytosis [91,92] or reprogramming [93–96]), MDSC-targeted therapies (e.g., targeting MDSC infiltration [61,97–99] or activation [100–102]), CAF-targeting therapies (e.g., blocking CAF function or activation [103]), and antiangiogenic therapies [104] show robust synergistic effects with **immune checkpoint inhibitors (ICIs)** in mouse tumor models. More importantly, some of these combination therapies are currently in clinical trials for treating cancer patients [86,103–105].

Current T cell-targeted immunotherapies include those that block inhibitory immune checkpoints, and approaches that boost adaptive immunity by genetically engineering T cells with chimeric antigen receptors (CARs) and T cell receptors [5]. Given the importance of circadian clock-regulated cancer cell–TME crosstalk in T cell biology discussed earlier, increased understanding of this relationship could lead to strategies to increase immunotherapy efficiency. Preclinical studies in mouse models have demonstrated that administration of a RORγ agonist can enhance the antitumor activity of Th17 cells modified for expression of a CAR, and this **chimeric antigen receptor (CAR) T cell therapy** provided long-term protection against tumors [106]. In addition, clock component-regulated cancer cell–TME crosstalk has been associated with enhanced tumor infiltration of immunosuppressive myeloid cells, which in turn impairs the infiltration and activation of CD4+ and CD8+ T cells, as well as upregulating immune checkpoint molecules [31,32,36,51–53]. These findings suggest that targeting clock component-regulated TME-cancer cell crosstalk might increase the antitumor efficiency of ICIs. Indeed, this concept is supported by evidence from clinical studies where the antitumor efficacy of nivolumab (anti-PD1 antibody) in advanced NSCLC patients is significantly higher in the morning treatment group than in the afternoon treatment group [107]. Moreover, a clinical trial testing an RORγ agonist in
combination with an anti-PD-1 antibody for metastatic NSCLC patients is underway (NCT03396497). These studies suggest that clock components may have a clinical impact on ICI therapy response. Further studies focusing on understanding clock component-regulated interactions between cancer cells and the immune system will help to design and develop novel and effective clock-oriented immunotherapies.

Concluding remarks
Although it is well established that circadian clock disruption is linked to an increased risk of cancer, details of the molecular mechanisms underlying this association are limited [29]. This review has highlighted some of the molecular circuitry that underlies clock component-regulated crosstalk between cancer cells and cells of the TME, including innate myeloid cells, lymphocytes, and endothelial cells. Currently, available results suggest a clock component-regulated cancer cell–TME symbiosis that is essential for sustained tumor growth, and that affects therapeutic response to distinct treatments including immunotherapies.

The regulation of clock component-oriented cancer cell–TME crosstalk is complex. As discussed earlier, BMAL1 depletion in GSCs inhibits tumor growth by reducing the infiltration of immunosuppressive microglia in GBM – a result that supports an oncogenic effect of BMAL1 expression [36]. However, BMAL1 expression in macrophages exhibits a tumor-suppressive effect, as indicated by the finding that BMAL1 deficiency promotes an immunosuppressive phenotype that stimulates tumor growth in melanoma [69]. Such results highlight that clock component-regulated cancer cell–TME interactions are context-dependent. Owing to this complexity, cancer cells and cells of the TME in different cancer types can express distinct immune checkpoint molecules, and these might provide various ICI treatment options. Further studies using mouse genetic models and advanced technologies, such as cytometry by time of flight (CyTOF) and single-cell RNA sequencing (scRNA-seq) [108,109], will increase our understanding of the context-dependent signaling axis (including immune checkpoint profile) underlying clock component-regulated cancer cell–TME interactions. Such knowledge will enable a more informed use of ICI therapies that, in turn, will lead to improved outcomes for cancer patients receiving ICI therapy.

In summary, recent studies have provided information highlighting the influence of clock component-regulated cancer cell–TME interactions on tumor growth, metastasis, and therapy resistance. However, multiple open questions remain regarding the molecular mechanisms underlying this symbiosis and potential approaches for disrupting these symbiotic interactions (see Outstanding questions). We anticipate that future studies will increase our understanding of these interactions, and in so doing will reveal novel approaches for cancer treatments.

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Declaration of interests
The authors declare no conflicts of interest.

References

Outstanding questions
Which clock components and related cancer cell–TME interactions are the drivers that regulate tumorigenesis, metastasis, and therapy resistance? What are the specific differences and similarities regarding cancer cell–TME symbiosis among different cancer components in specific cancer types?

What signaling pathways are crucial for the regulation of clock component-regulated cancer cell–TME interactions?

Can scRNA-seq and CyTOF identify new clock component-regulated cancer cell–TME interactions and new immune checkpoint molecules during this interaction?

What is the molecular mechanism underlying interactions between cancer cell clock components and the adaptive immune response?

Does clock component-regulated cancer cell–TME symbiosis affect the antitumor activity of immunotherapies and conventional therapies, and if so how? Will targeting this symbiosis have synergistic effects with immunotherapies or conventional therapies? If yes, what is the best combination strategy?
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