

# Circadian clock as a possible control point in colorectal cancer progression (Review)

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**Abstract.** The circadian rhythm is generated at the cellular level by a molecular clock system that involves specific genes. Studies have revealed that circadian clock disruption is a control point in cancer progression. Colorectal cancer (CRC) is one of the cancers closely associated with circadian disruption. In the present review, the involvement of the circadian clock in CRC development was summarized. Abnormal expression of certain clock genes has been found in patients with CRC and their correlation with clinicopathological features has also been explored. The period and cryptochrome 2 (Cry2), Sirtuin1 (SIRT1) and neuronal PAS domain protein 2 (NPAS2) genes were reported to have tumour suppressor properties. Conversely, Cry1, brain and muscle ARNT-like-1, circadian locomotor output cycles kaput (CLOCK) and timeless may aggravate CRC progression, but these findings are not consistent and require to be confirmed by further research. Circadian scheduling also indicated advantages in chemotherapy treatments for patients with CRC by

increasing the maximum tolerated doses and decreasing toxicities. Dysfunction of the molecular CLOCK system disrupted cellular processes to accelerate colon tumorigenesis, such as metabolism, cell cycle, DNA damage repair, proliferation and apoptosis, epithelial-mesenchymal transition and stemness. The clock gene network and how the dynamics of the system influence CRC were discussed.

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## 1. Background

Biological rhythms exist in our internal bodies and their functions vary according to oscillations with a 24-h light/dark cycle (1). The circadian time-keeping system is controlled by both central and peripheral oscillators. The suprachiasmatic nuclei of the hypothalamus are the central pacemaker (2,3) and certain interconnected specific clock genes control the peripheral circadian rhythm. The discovery of the circadian locomotor output cycles kaput (CLOCK) and its heterodimeric partner brain and muscle ARNT-like-1 (BMAL1) led to the first site of circadian oscillations (4,5). The transactivation of E-box-containing genes by the CLOCK:BMAL1 complex was able to activate Period (Per) genes, i.e. Per1-3 and Cryptochrome (Cry) genes, i.e. Cry1 and Cry2, which then negatively repressed CLOCK:BMAL1 transcription. As auxiliary feedback loops, REV-ERB and retinoic acid receptor-related orphan receptors (RORs) bind to a common response element known as the REV-ERB/ROR response element (RRE) and their intrinsic repressive and inductive activities have been proposed to contribute to CLOCK function and BMAL1 transcription (6,7) (Fig. 1). Histone acetylation induces an open chromatin conformation that is thought to activate gene expression (8). Deacetylation would shift back to condensing chromatin and silencing gene expression. The enzymes that

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**Abbreviations:** CRC, colorectal cancer; mCRC, metastatic CRC; CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like-1; Per1, period1; Cry1, cryptochrome 1; TIM, timeless; SIRT1, sirtuin1; RORs, retinoic acid receptor-related orphan receptors; RRE, REV-ERB/ROR response element; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MMR, mismatch repair; HAT, histone acetyltransferases; HDAC, histone deacetylases; DDR, DNA damage response; ATM, ataxia telangiectasia mutated; pCR, pathological complete regression; Beva, bevacizumab; CSCs, cancer stem-like cells; EMT, epithelial-mesenchymal transition; DPD, dihydropyrimidine dehydrogenase; NPAS2, neuronal PAS domain protein 2; OS, overall survival; miR, microRNA; MSH2, MutS homolog 2

**Key words:** circadian clock, colorectal cancer, period, cryptochrome, CLOCK, BMAL1, TIM

participate in these transitions are histone acetyltransferases (HATs) and histone deacetylases (HDACs) (9). CLOCK is an enzyme with HAT activity (10) and CLOCK specifically acetylates nonhistone targets, such as BMAL1 (11). Sirtuin1 (SIRT1) is an NAD<sup>+</sup>-dependent HDAC, and SIRT1 and CLOCK converge in a coordinated manner. SIRT1 activity depends on nicotinamide phosphoribosyl transferase, which is the rate-limiting enzyme involved in NAD<sup>+</sup> synthesis, which is regulated by CLOCK:BMAL1 (12,13). The functions of other clock genes, such as timeless (TIM) and timeless-interacting protein, in the circadian system remain to be fully elucidated.

Shifts in wake and sleep schedules or sleep deprivation desynchronize the circadian rhythm. Emerging evidence suggests that impaired circadian rhythms may be linked to causal webs of cancer initiation (14,15). Colorectal cancer (CRC) is one of the cancers that is closely associated with circadian disruption (16-18). CRC is the world's fourth most deadly cancer and approximately one in four CRC cases will be diagnosed with metastatic CRC (mCRC) (19). Multiple genetic factors contribute to the development of carcinogenesis of the large bowel, such as amplification of human epidermal growth factor receptor 2 (HER2), as well as KRAS, NRAS and BRAF mutations (20,21), chromosomal instability such as microsatellite instability (MSI) or mismatch repair (MMR) (22), which all provide a basis for optimal therapeutic intervention for individuals. Surgical excision is the first choice of treatment for CRC and chemotherapy that is given in combination with different molecular therapies (such as bevacizumab, panitumumab, cetuximab, regorafenib and aflibercept) significantly extends overall survival (OS) (23). Experimental data further indicated how the clock gene network exerts an influence on CRC progression, offers potential biomarkers for prognosis, serves as a guide for treatment decisions and provides molecular targets for treating CRC.

## 2. The intestine harbours a circadian clock

Rhythmic processes were already characterized within the intestine prior to the identification of the molecular clock. Bowel movements usually occur during the day and certain studies have suggested that a potential biological clock exists in the bowel. Research has indicated that colonic motor activity is minimal during sleep but increases significantly at morning awakening (24). Rao *et al* (25) determined a significant increase in colonic pressure activity after awakening. Other physiologically relevant factors, such as absorption rhythms (26) and mucosal enzymes (27), also exhibited their lowest activities at night (Fig. 2). Furthermore, the rhythmicity of circular muscle contractility and intracolonic pressure were absent in Per1/2 double-knockout mice (28). In addition, the gut microbiota exhibits circadian oscillations in both mice and humans; Per1/2 double-knockout mice lose rhythmic fluctuations in microbiota compositions and time shift-induced jet lag also disrupts diurnal oscillations in commensal bacterial abundances (29).

Studies have determined that clock genes have an important role in intestinal physiological activities. Per1-2, Cry1, CLOCK, BMAL1 and REV-ERB $\alpha$  are expressed in the epithelial cells of the colon and exhibit circadian rhythmicity (30). Per2 and BMAL1 in the myenteric plexus and epithelial

cells have an important role in coordinating gastrointestinal functions, such as cell proliferation and migration (31), and rectal cell proliferation fluctuates during the day in colon cancer (32). BMAL1 was also reported to regulate intestinal drug disposition function (33).

These results clearly support that the circadian system is strongly connected with the gastrointestinal tract.

## 3. Associations between CRC and circadian clock genes

Clinical research indicated that disruption of the circadian rhythm accelerated CRC progression (34,35). Loss of circadian rhythms was also observed to potentiate CRC initiation in an Apc<sup>min</sup> model (36). Abnormal expression levels of circadian clock genes were observed in CRC tissues and current studies suggested that clock genes, mainly including Per, Cry, ARNT1 (BMAL1) and CLOCK, may influence colon cancer progression.

**Period genes.** Period genes are identified most frequently in CRC. Per1-3 were reported to have tumour suppressor properties, but certain studies reached different conclusions (Table I). A total of seven studies determined that the expression of Per1 was significantly decreased in tumour tissue compared to healthy mucosa (37-44). Furthermore, one study indicated a decrease in Per1 in undifferentiated tumours, while no differences were obtained in differentiated colon carcinomas (45), but two studies found no significant differences in Per1 between colon tumours and healthy mucosa (46,47). Mostafaie *et al* (40) demonstrated a correlated decrease in Per1 and estrogen receptor- $\beta$  in colorectal tumours. Furthermore, six studies reported that Per2 expression was significantly decreased in tumour tissues (39,48-52). Hasakova *et al* (50,53) found that Per2 was downregulated in tumour tissues of male patients with CRC, but Krugluger *et al* (45) determined that Per2 expression exhibited no differences in colon tumour tissues; the following six studies also confirmed this (38,40-42,47). Another six studies examined the gene expression of Per3 and all found significant decreases (37-39,41,42,54). In addition, Alexander *et al* (55) found a Per3 gene length polymorphism, and the 5-repeat Per3 VNTR sequence may increase the chance of colorectal adenoma formation.

Studies have also explored the relationships among period genes and clinical-pathological features of patients with CRC. Compared to tumours that did not express period genes, Momma *et al* (56) indicated that positive Per1 and Per2 staining was associated with larger tumour sizes. Tumours with positivity for Per2 expression tended to have a greater depth of invasion and were generally more advanced, and patients tended to exhibit poorer OS. He *et al* (37) reported that Per2 was correlated with immune cell infiltration and was associated with unfavorable prognosis in colon adenocarcinoma. Hasakova *et al* (50,53) determined that Per2 was significantly downregulated in tumour tissues of male patients with CRC, and a negative correlation between Per2 and microRNA (miR)-34a was found. Low expression of Per2 and high expression of miR-34a were associated with significantly better outcomes in male patients. However, certain studies obtained the opposite result, claiming that high Per2 expression was associated with better survival and that low expression of the Per1 gene was related to liver metastasis (42). Low expression of Per2 was significantly associated with CRC metastasis (49).

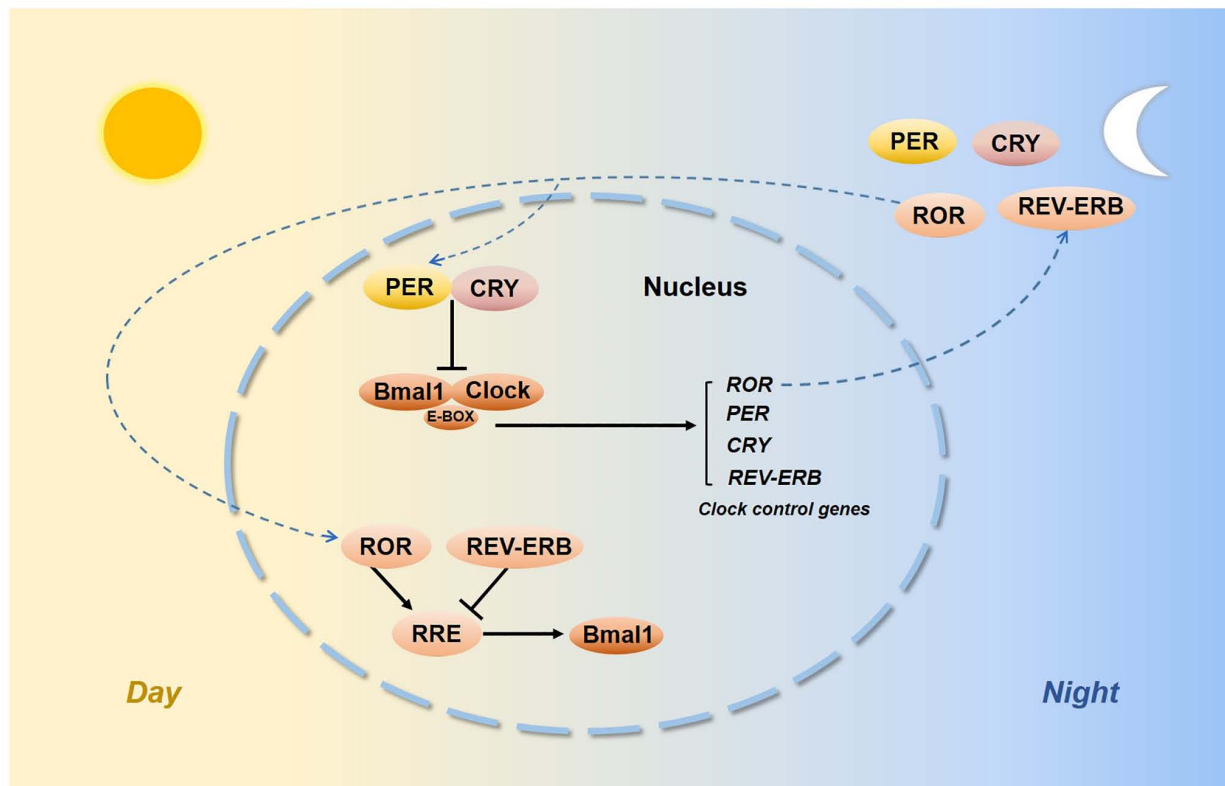


Figure 1. Molecular components of the circadian clock. CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like-1; PER, period; CRY, cryptochrome; TIM, timeless; SIRT1, sirtuin1; RORs, retinoic acid receptor-related orphan receptors; RRE, REV-ERB/ROR response element.

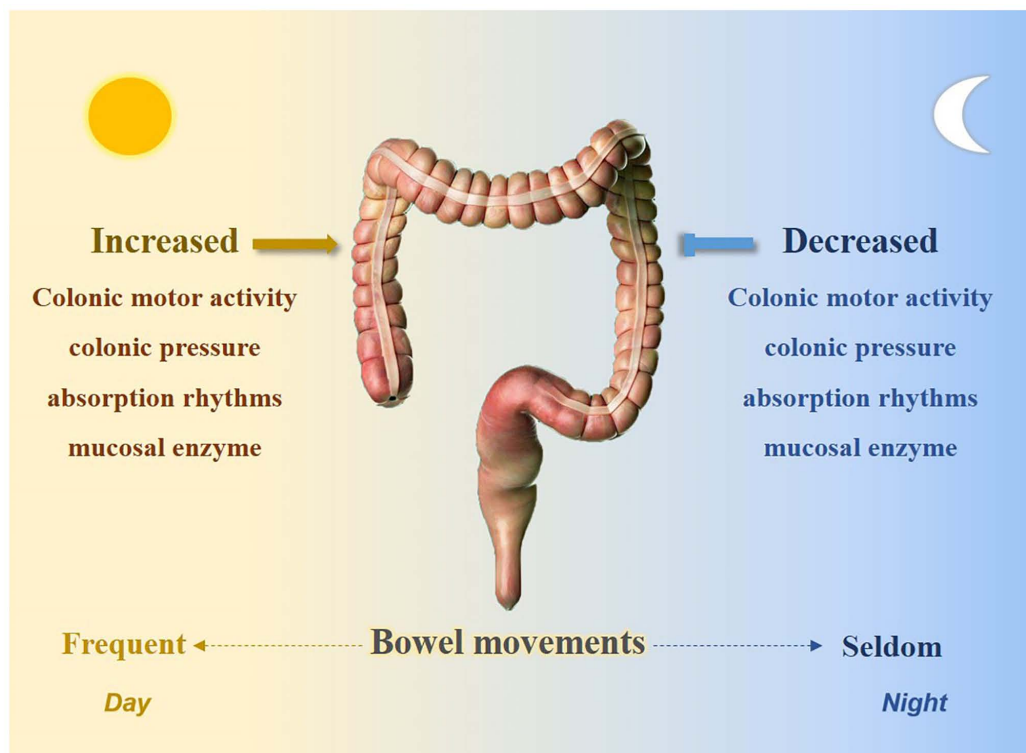


Figure 2. The intestine harbors a circadian clock.

Mazzocchi *et al* (39) indicated that lower expression of *Per1* and *Per3* in tumour tissues may be suggestive of a poorer survival prognosis. Wu *et al* (43) next determined that low

expression levels of *Per1* and *miR-192* were correlated with unfavourable survival in patients with stage III/IV CRC but with better OS rates in patients with stage II CRC. In addition,

Table I. Studies exploring the expression and clinical significance of period genes in patients with CRC.

Author, year	Gene	Expression in CRC	Observations	(Refs.)
Mazzocchi, 2011	Per1	Decreased	Low expression of Per1 may indicate a poor survival prognosis	(39)
Oshima, 2011	Per1	Decreased	Low expression of Per1 was associated with liver metastasis	(42)
Wu, 2016	Per1	Decreased	Low expression of Per1 was associated with unfavorable survival of patients with stage III/IV CRC, but with favorable OS of patients with stage II CRC	(43)
He, 2022	Per1	Decreased	Not specified	(37)
Karantanos, 2013	Per1	Decreased	Not specified	(38)
Orhan, 2019	Per1	Decreased	Not specified	(41)
Lu, 2015	Per1	No differences	Not specified	(46)
Nemeth, 2011	Per1	No differences	Not specified	(47)
Momma, 2017	Per1	Not mentioned	Positive Per1 staining was associated with larger tumor size.	(56)
Mostafaie, 2009	Per1	Not mentioned	Correlated decrease of Per1 and estrogen receptor- $\beta$ in CRC	(40)
Krugluger, 2007	Per1	Not mentioned	Per1 decreased in undifferentiated tumors, while no difference was found in differentiated CRC	(45)
He, 2022	Per2	Not mentioned	High expression of Per2 was associated with immune cell infiltration and associated with unfavorable prognosis in colon adenocarcinoma	(37)
Karantanos, 2013	Per2	No differences	Not specified	(38)
Mostafaie, 2009	Per2	No differences	Not specified	(40)
Nemeth, 2011	Per2	No differences	Not specified	(47)
Mazzocchi, 2011	Per2	Decreased	Not specified	(39)
Orhan, 2019	Per2	No differences	Not specified	(41)
Oshima, 2011	Per2	Not mentioned	High expression of Per2 associated with better outcomes	(42)
Krugluger, 2007	Per2	No differences	Not specified	(45)
Lu, 2015	Per2	Not mentioned	Per2 expression was higher in the pCR group than in the non-pCR group	(46)
Aroca-Siendones, 2021	Per2	Decreased	Low expression of Per2 was significantly associated with CRC metastasis	(49)
Hasakova, 2018/2019	Per2	Decreased	Low expression of Per2 was associated with better outcomes in male patients with CRC	(50,53)
Wang, 2011	Per2	Decreased	Not specified	(48)
Wang, 2016	Per2	Decreased	Not specified	(51)
Xiong, 2022	Per2	Decreased	Not specified	(52)
Momma, 2017	Per2	Not mentioned	Tumors with positive Per2 expression tended to exhibit a greater depth of invasion and were generally more advanced, and patients may have poorer OS	(56)
He, 2022	Per3	Decreased	Not specified	(37)
Karantanos, 2013	Per3	Decreased	Not specified	(38)
Mazzocchi, 2011	Per3	Decreased	Lower expression of Per3 tended to be associated with poorer survival rates	(39)
Orhan, 2019	Per3	Decreased	Not specified	(41)
Oshima, 2011	Per3	Decreased	Not specified	(42)
Wang, 2012	Per3	Decreased	Incidence of death was higher in patients with Per3-negative CRC	(54)
Alexander, 2015	Per3	Not mentioned	Regarding Per3 gene length polymorphism, the 5-repeat Per3 VNTR sequence may increase the chance of colorectal adenoma formation	(55)

CRC, colorectal cancer; Per1, period 1; OS, overall survival; pCR, pathological complete regression.

Štorcelová *et al* (57) identified certain cell cycle regulatory genes related to the Per2 expression in human CRC tissues.

Substantial evidence indicates that the cell cycle occurs with a daily rhythm. Healthy mice exhibited rhythmicity of Per1, Per2, Wee1 and p21 in the intestine, but the circadian rhythmicity was significantly reduced in tumours (58). Knocking down Per2 subsequently inhibited p53 and caused CRC cells to acquire malignant biological features (52). Studies have explained how periodic genes regulate CRC progression by interfering with the cell cycle. DNA double-strand breaks activate the DNA damage response (DDR). The DDR is initiated by kinases such as ataxia telangiectasia mutated (ATM) (59) and the G1-S checkpoint is regulated by serine/threonine-protein kinase checkpoint kinase 2 (CHK2), whereas the G2/M checkpoint is regulated by CHK1 (60). Gery *et al* (61) reported that Per1 promoted DNA damage-induced apoptosis by interacting with ATM and CHK2. In addition, overexpression of c-Myc is associated with CRC (62); in Per1-knockout mice and Per2 mutant mice, c-Myc expression levels are elevated without restriction, inducing excess cyclin D1 expression that increases proliferation (63). Of note, Per2 has been indicated to be suppressed in colon cancer cell lines, which increased cyclin D and cell proliferation (64). Another study also supported that Per2 functions in tumour suppression by regulating the cell cycle (65).

Crosstalk between the circadian clock and Wnt/ $\beta$ -catenin signalling has been reported to be involved in CRC.  $\beta$ -catenin is a main signal transducer of the Wnt pathway (66) and activation of the Wnt/ $\beta$ -catenin pathway promotes cell proliferation and invasion (67). Wood *et al* (64) demonstrated that downregulation of Per2 in colon cells increased  $\beta$ -catenin expression. In *in vivo* experiments, when compared to Apc(Min/+) mice, Per2-mutant Apc(Min/+) mice developed smaller colonic polyps. A study from the same group, by Yang *et al* (68), discovered that increased levels of  $\beta$ -catenin in Apc(Min/+) mice could destabilize the Per2 protein. In addition, period genes were reported to inhibit drug resistance in CRC cells. Per2 was significantly upregulated when glucose metabolism was restricted and thus weakened the ability of cancer cells to survive chemotherapy (69). Per3 was reported to be downregulated in drug-resistant CRC cells and cancer stem-like cells (CSCs). Overexpression of Per3 strengthened the 5-fluorouracil-induced inhibitory effects on colorectal CSCs, while knockdown of Per3 decreased its inhibitory effects. In addition, Per3 overexpression decreased stemness markers, such as CD44, CD133 and SOX2. It was confirmed that Per3 reduces the chemoresistance and self-renewal capability of CRC by inhibiting both Notch and Wnt/ $\beta$ -catenin signalling (70). Calcium significantly prevents tumorigenesis of CRC and Per3 significantly upregulates calcium to prevent CRC tumorigenesis (71).

**Cry genes.** Various studies have examined the expression of Cry1 and Cry2 in CRC samples (Table II). Further evidence has indicated that Cry2 suppresses colon tumour growth, but the role of Cry1 in cancer progression remains controversial. Compared to matched normal mucosa, gene expression of Cry1 was reported to be upregulated in colon carcinoma tissues by three studies (37,72,73), but two other studies claimed that the expression of Cry1 did not differ significantly between

groups (39,46). Cry2 was identified to be downregulated in CRC tissues in three studies (37,39,50,72). Another study retrospectively analysed CRC tissue samples from patients with pathological complete regression (pCR) and non-pCR and suggested that the expression of Cry2 was higher in the pCR group (46).

The Cry1 and Cry2 genes were also associated with adverse clinical-pathological features in patients with CRC. It was determined that high Cry1 expression was correlated with poor OS rates in patients with CRC (73). Another study also indicated that high expression of Cry1 was associated with lower OS and disease-free survival at five years in patients with CRC (49). Hasakova *et al* (50,72) indicated that the expression of Cry1 and Cry2 was correlated differently with sex or tumour location in colon carcinoma tissues. Cry1 expression increased significantly in females with distant metastases, while females without distant metastases frequently exhibited downregulation of Cry2 expression. Better survival rates were associated with low expression of Cry2, high expression of Cry1 in tumour tissue was associated with an unfavorable survival prognosis, but this was not observed in males. In addition, higher Cry1 expression in right-sided colon tumour tissues was associated with worse survival rates in females, and the expression of Cry1 in the left-sided colon tumour tissues was higher than in the adjacent tissue in males. Furthermore, Cry2 expression was associated with tumour location in males with grade 2 cancer. However, Mazzocchi *et al* (74) indicated that patients with CRC with elevated Cry1 or Cry2 expression levels had poorer survival rates. The Cry1 gene was particularly decreased in elderly female patients with CRC and tumours located in the transverse colon tended to exhibit lower Cry expression levels.

WEE1 was reported to affect the timing of the M phase (75) and downregulation of WEE1 was identified in CRC (76). Studies have indicated that both Cry1 and Cry2 deletions lead to elevated WEE1 levels (63,77) and thus affected the cell cycle and proliferation of CRC. However, this requires to be confirmed by further research.

**BMAL1 and CLOCK genes.** CLOCK and its heterodimeric partner, ARNTL1 (BMAL1), are the core components of the circadian clock system, and modified expression of these two genes in CRC tumour tissues is related to clinico-pathological features (Table III).

BMAL1 may be a biomarker for CRC treatment prognoses. Studies indicated that the expression levels of BMAL1 decreased by half in mCRC tumour tissues, high expression levels of BMAL1 were associated with reduced efficacy of the anti-angiogenic drug bevacizumab (Beva) and patients who had low expression levels of BMAL1 tended to have good responses to Beva therapy (78); furthermore, upregulation of the BMAL1 gene was correlated with liver metastasis in patients with CRC (42). Patients with CRC with high BMAL1 expression exhibited a significant enrichment of epithelial-mesenchymal transition (EMT) and invasion-associated gene signatures (79). Another study suggested that BMAL1 expression decreased in CRC (37), and low BMAL1 expression was significantly associated with metastasis (49). However, Karantanos *et al* (38) determined that the BMAL1 gene expression levels were higher in cancerous tissues, but no correlation was found with the clinical significance.

Table II. Studies exploring the expression and clinical significance of CRY genes in patients with CRC.

Author, year	Gene	Expression in CRC	Observations	(Refs.)
Hasakova, 2018	Cry1	Upregulated	High expression of Cry1 was associated with unfavorable survival in females. Higher expression of Cry1 in the right-sided colon tumor tissue tended to be associated with unfavorable survival in females	(50)
He, 2022	Cry1	Upregulated	Not mentioned	(37)
Yu, 2013	Cry1	Upregulated	High Cry1 expression was associated with lymph node metastasis and TNM stage III-IV and tended to be associated with poor OS in patients with CRC	(73)
Aroca-Siendones, 2021	Cry1	Not mentioned	High expression of Cry1 was associated with lower OS and DFS at five years in patients with CRC	(49)
Lu, 2015	Cry1	No differences	Not specified	(46)
Mazzocchi, 2011	Cry1	No differences	In patients with CRC, elevated Cry1 expression was associated with poorer survival. Cry1 was particularly decreased in elderly female patients with CRC and in tumors located in the transverse colon tend to exhibit lower Cry expression level.	(39)
Lu, 2015	Cry2	Not mentioned	Cry2 expression was higher in tissue with pathological complete regression	(46)
Hasakova, 2018	Cry2	Decreased	Not specified	(50)
He, 2022	Cry2	Decreased	Patients with CRC with high Cry2 expression had a tendency of unfavorable survival prognosis	(37)
Mazzocchi, 2011	Cry2	Decreased	Patients with CRC with high Cry2 expression had an unfavorable survival prognosis	(39)

CRC, colorectal cancer; Cry1, cryptochrome 1; OS, overall survival; DFS, disease-free survival.

Momma *et al* (56) indicated that colon tumours with positive CLOCK expression were generally more advanced and invasive. In addition, Mostafaie *et al* (40) reported that CLOCK expression was significantly higher in G(2) tumours of male patients than that in female patients. Another study suggested that the CLOCK gene was expressed in all CRC tissue samples and colon tumours with higher expression levels of CLOCK were associated with lymph node metastasis and tended to be more advanced (37). Accordingly, higher CLOCK expression was found in CRC cell lines with greater metastatic potential (80,81). However, the CLOCK expression levels were significantly elevated in CRC tissue samples from patients who achieved a pCR compared to those from non-pCR patients (46). Karantanos *et al* (38) determined that CLOCK gene expression levels were higher in colorectal cancerous tissues. They also performed a case-control study (82) and found that a polymorphism in the CLOCK1 gene significantly increased CRC susceptibility. Mutations in DNA MMR system genes, such as MutL homolog1, MutS homolog 2 (MSH2) and MSH6 result in dysfunctional DNA repair, leading to MSI. MSI accounts for ~15% of CRCs and is thus the main hallmark of CRC (83). Alhopuro *et al* (84) demonstrated that CLOCK mutations occurred in nearly half of MSI CRCs (total of 101 MSI CRCs), and restoring CLOCK expression in cells enhanced the protective activity against UV-induced apoptosis.

Clockwork genes drive the metabolism and the cell cycle. Fuhr *et al* (85) identified a reciprocal connection between the circadian clock and glycolysis. Disruption of BMAL1 impacts hexokinase domain containing 1, a glycolysis gene, and leads to increased glycolytic activity. A Japanese study implanted colon tumours in mice and found that the growth of CLOCK-mutant colon tumours was slower than that of wild-type colon tumours. They further indicated that the BMAL1 and CLOCK genes control the time-dependent variations in cellular iron levels by promoting the mRNA expression of iron regulatory protein 2 and transferrin receptor 1. High levels of iron uptake may be associated with an increased risk of CRC (86,87) and thus promote tumour proliferation (88). However, certain studies indicated that both the BMAL1 and CLOCK genes may act as tumour suppressors. CLOCK-binding elements were identified near p21 and BRCA1, which were indicated to interfere with the DNA repair response and cell cycle (84). Sakamoto and Takenoshita (89) observed that overexpression of CLOCK and BMAL1 prevented CRC cells from entering the G1 to S phase by suppressing CyclinD1 to inhibit cell growth. In CLOCK knockout mice, the mRNA levels of WEE1 decreased significantly. Another study demonstrated that inhibiting BMAL1 expressions in murine colon cancer cells decreased DNA damage (90). Zhang *et al* (91) found that under different P53 backgrounds, BMAL1 knock-down in CRC cells induced a relatively equal activation of



Table III. Studies exploring the expression and clinical significance of BMAL1/CLOCK in patients with CRC.

Author, year	Gene	Expression in CRC	Observations	(Refs.)
Karantanos, 2013	BMAL1	Upregulated	No correlation was found between BMAL1 gene expression and clinical significance	(38)
Burgermeister, 2019	BMAL1	Decreased	Patients with CRC with low expression of BMAL1 tended to have a good response to bevacizumab	(78)
Oshima, 2011	BMAL1	Not mentioned	High expression of BMAL1 was associated with liver metastasis in patients with CRC	(42)
Zhang, 2021	BMAL1	Not mentioned	Patients with CRC with high expression of BMAL1 had enrichment of EMT and invasive gene signatures	(79)
He, 2022	BMAL1	Decreased	Not specified	(37)
Aroca-Siendones, 2021	BMAL1	Not mentioned	Low expression of BMAL1 was associated with metastasis	(49)
Karantanos, 2013	CLOCK	Upregulated	No correlation was found between CLOCK gene expression and clinical significance	(38)
Momma, 2017	CLOCK	Not mentioned	Colon tumors with positive CLOCK expression were generally more advanced and invasive	(56)
Mostafaie, 2009	CLOCK	Not mentioned	CLOCK was significantly higher in G(2) tumors of male patients	(40)
He, 2022	CLOCK	Upregulated	Patients with CRC with higher expression of CLOCK tended to have lymph node metastasis and tended to be more advanced	(37)
Lu, 2015	CLOCK	Not mentioned	CLOCK expression levels were elevated in CRC tissue samples from patients with pCR vs. non-pCR	(46)
Karantanos, 2013	CLOCK	Upregulated	Polymorphism in the CLOCK1 gene significantly increased the risk of CRC susceptibility	(38)
Alhopuro, 2010	CLOCK	Not mentioned	CLOCK mutations occurred in nearly half of MSI CRCs	(84)

CRC, colorectal cancer; CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like-1; EMT, epithelial to mesenchymal transition; MSI, microsatellite instability; pCR, pathological complete regression.

AKT/mTOR but ultimately resulted in different cell fates. Under a wild-type P53 background, BMAL1 knockdown cells rapidly underwent apoptosis, while the surviving cells contained less P53.

Stokes *et al* (36) indicated that CRC tumours only have a weak CLOCK function *in vivo*. Loss of BMAL1 increases tumour initiation. Intestinal organoid assays indicated that loss of BMAL1 increases self-renewal in a Yes-associated protein 1-dependent manner. BMAL1 was also reported to affect the invasion and metastasis of CRC by promoting EMT. BMAL1 is involved in maintaining the epithelial-mesenchymal equilibrium of CRC cells and influences migration, invasion, adhesion and chemoresistance. BMAL1 knockdown increased the expression of epithelial markers, such as E-cadherin, epithelial cell adhesion molecule and cytokeratin 20, but decreased the expression of Twist and mesenchymal markers (e.g., vimentin and N-cadherin) in CRC cells. In addition, the invasion and drug resistance capacities also decreased in BMAL1-knockdown CRC cells (79). In addition, BMAL1 was reported to promote CRC metastasis by stimulating exosome secretion (92).

Although the BMAL1 and CLOCK expression levels were observed to be related to advanced pathological clinical features, certain experiments have indicated that these two genes may inhibit CRC cell growth. This inconsistency requires to be clarified by further research.

*Other clock genes.* Altered expression levels of other circadian clock genes were also detected in CRC tissue samples (Table IV). Compared to adjacent normal colon tissues, the SIRT1 and RORA expression levels decreased in CRC tumour tissues, while TIM was reported to be increased (37,39,93,94). High TIM levels were an unfavourable prognostic factor significantly associated with microsatellite instability and proximal lymph nodes and were prevalent in TNM stages III-IV (39). However, the most recent study indicated that loss of TIM expression is associated with advanced tumour stage, metastasis and microsatellite stability status, and TIM expression levels are inversely correlated with a set of gene signatures of EMT markers (95). In addition, NPAS2 expression was significantly downregulated in CRC tumour tissues. Low NPAS2 expression was associated with tumour size, TNM stage and metastases (96).

TIM has no apparent circadian clock function in mammals, but research has identified it as being important for CRC progression (97). Recent data revealed that TIM depletion increases  $\gamma$ H2AX, a marker of DNA damage, and increases CHK1 and CDK1 phosphorylation (98). A report also demonstrated that overexpression of TIM in colon cancer cells suppressed G2/M arrest, while TIM depletion increased CHK1 and CDK1 phosphorylation and triggered G2/M arrest (98). Another study suggested that TIM inhibited

Table IV. Studies exploring the expression and clinical significance of SIRT1, RORA and TIM in patients with CRC.

Author, year	Gene	Expression in CRC	Observations	(Refs.)
Pazienza, 2012	SIRT1	Decreased	Not specified	(94)
He, 2022	RORA	Decreased	Not specified	(37)
Gu, 2018	RORA	Decreased	Not specified	(93)
Mazzocchi, 2011	TIM	Upregulated	High TIM levels were associated with MSI and proximal lymph nodes, and were prevalent in TNM stages III-IV	(39)
Colangelo T, 2022	TIM	Not mentioned	Loss of TIM expression is associated with advanced tumor stage, metastasis and microsatellite stability status; TIM expression was inversely correlated with a set of EMT marker genes	(95)
Xue, 2014	NPAS2	Decreased	Low expression of NPAS2 was associated with tumor size, TNM stage and metastasis	(96)

CRC, colorectal cancer; MSI, microsatellite instability; EMT, epithelial to mesenchymal transition; NPAS2, neuronal PAS domain protein 2; TIM, timeless; SIRT1, sirtuin1; ROR, retinoic acid receptor-related orphan receptor.

EMT, and its ectopic silencing promoted invasion, migration and stemness in CRC cells (95). Overexpression of SIRT1 in Apc(Min/+) mice resulted in reduced neoplasia, while SIRT1-knockout mice had an increased tumour incidence (99). NPAS2 increased the G0/G1 phase population in CRC cells and inhibited proliferation, invasion and migration *in vitro* (96).

#### 4. Influence of the circadian clock in CRC treatment

Circadian scheduling produced advantages in chemotherapy treatments of patients with CRC. A 24-h programmable time ambulatory pump was used to administer oxaliplatin by venous infusion against mCRC. This type of treatment increased the maximum tolerated doses and decreased the toxicities of oxaliplatin (100). Another previous phase II trial also proved that if chemo-drug delivery was chronomodulated rather than constant over time, chemotherapy was more effective and less toxic (101). The addition of cetuximab to chronotherapy in patients with mCRC increased the chance of complete resection compared to conventional chemotherapy (102). However, an international randomized trial reported that chronomodulated FLO4 provided no survival benefit compared with ordinary FOLFOX2 in patients with mCRC (103). Of note, Innominato *et al* (104) chronomodulated irinotecan, oxaliplatin, 5-fluorouracil and leucovorin dosing times against mCRC and found encouraging activity in second-line treatments, with limited haematological toxicity. Another study indicated sex differences in the advantages of chronomodulated irinotecan delivery to minimize adverse events; it is better to administer irinotecan in the morning for males and in the afternoon for females (105).

Fluoropyrimidine-related toxicity is strongly affected by the activity of dihydropyrimidine dehydrogenase (DPD), a 5-fluorouracil-metabolizing enzyme (106). Krugluger *et al* (45) revealed that disturbed transcription of

Per1 may be a cause of disrupted daily DPD oscillation in CRC cells. Fang *et al* (107) demonstrated that the expression levels of Cry2 are elevated in chemoresistant CRC samples and knockdown of Cry2 increased oxaliplatin sensitivity in CRC cells. Circadian delivery of irinotecan to Caco-2 colon cancer cells indicated that chronomodulated chemotherapy may be an optimized option (108). Hesse *et al* (109) established a mathematical model to predict the impact of various parameters (e.g., BMAL1 degradation rates, cytosolic BMAL1 degradation rates and CLOCK activation rates) on irinotecan toxicity, but clinical evidence is required to prove this hypothesis.

These findings indicated that the circadian clock system may guide oncological treatments of CRC.

#### 5. Conclusion

The present review outlines the connections between the circadian clock and CRC progression (Fig. 3). Bowel physiology indicates a circadian rhythm and molecular clockwork dysregulation may host the colorectal carcinogenesis process. According to previously published reports, circadian clock genes are related to CRC progression, patient survival and response to chemotherapy. Although the findings are not all consistent, it may be hypothesized that this largely resulted from individual differences, such as different ethnicities, gender and age. Furthermore, multiple detection methods may also lead to different findings. Based on the circadian rhythm system, chronotherapy may be an optimal method for effective treatment, particularly to increase the maximum tolerated doses and alleviate the toxicity of chemotherapy agents. In addition, the oscillating circadian clock regulates multiple cellular activities related to colorectal carcinogenesis, such as metabolism, the cell cycle, DNA damage response, EMT processes and stemness. Finally, it may be concluded that the circadian clock is tightly correlated with CRC prognosis and allows further advances in modern therapeutic approaches.



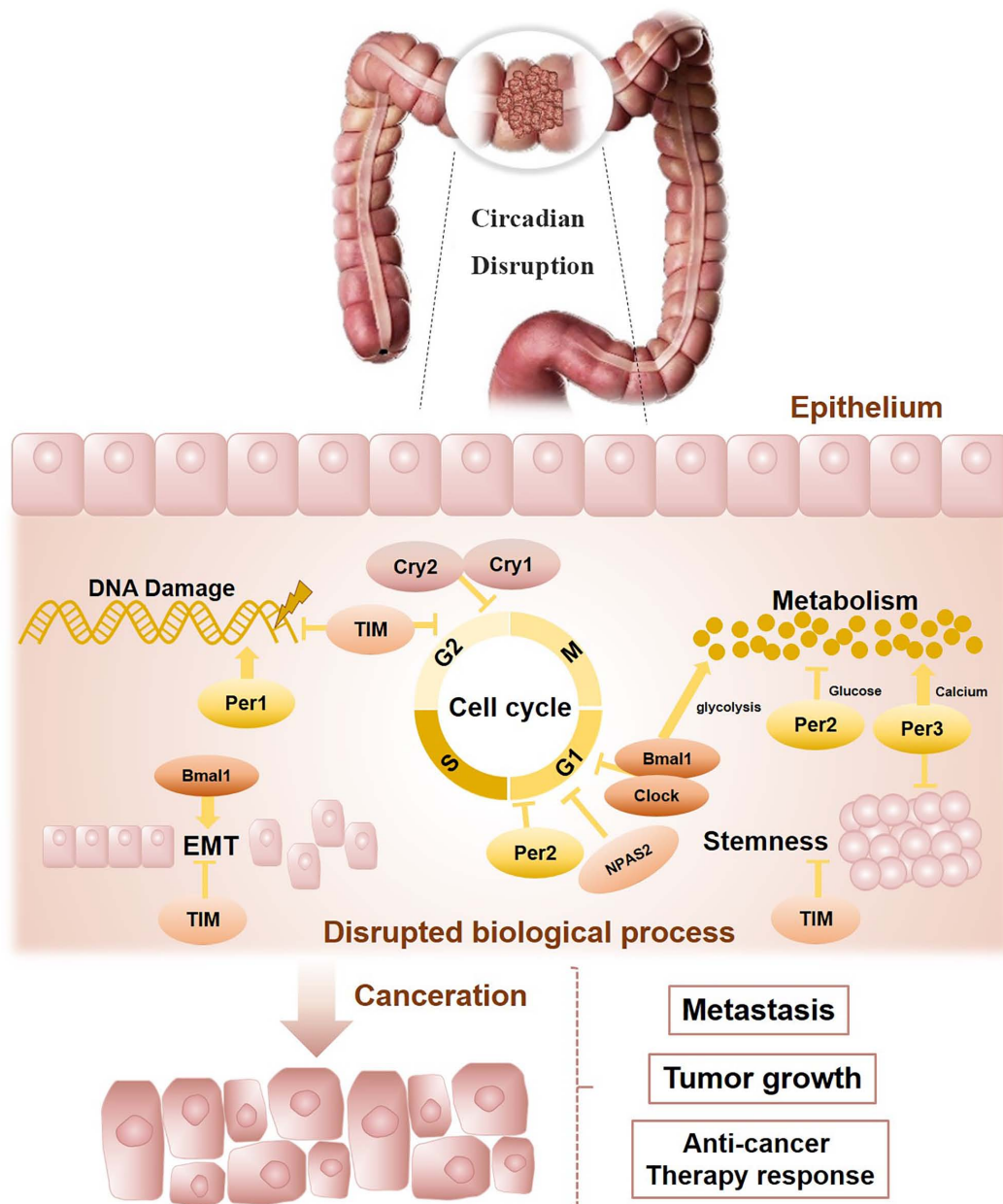


Figure 3. Circadian disruption promotes the progression of colorectal cancer. CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like-1; Per1, 2, 3 period1; 2, 3; Cry1, 2, cryptochrome 1, 2; TIM, timeless; EMT, epithelial-mesenchymal transition; NPAS2, neuronal PAS domain protein 2.

The relationship between the circadian clock and CRC warrants further investigation.

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#### Availability of data and materials

Not applicable.

#### Authors' contributions

LL was involved in the conception and design of the structure of the manuscript. XR then performed the literature search, generated the figures and wrote the manuscript.

#### Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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