Research progress on circadian clock genes in common abdominal malignant tumors (Review)

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Abstract. The circadian clock refers to the inherent biological rhythm of an organism, which, is accurately regulated by numerous clock genes. Studies in recent years have reported that the abnormal expression of clock genes is ubiquitous in common abdominal malignant tumors, including liver, colorectal, gastric and pancreatic cancer. In addition, the abnormal expression of certain clock genes is closely associated with clinical tumor parameters or patient prognosis. Studies in clock genes may expand the knowledge about the mechanism of occurrence and development of tumors, and may provide a new approach for tumor therapy. The present study summarizes the research progress in this field.

Contents

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1. Introduction

The earliest finding of a circadian clock was the change in position of plant leaves, which spread during the day and droop at night, corresponding to an oscillation with a 24-h period (1,2). Subsequently, circadian clocks were also identified in the form of clear circadian rhythms in the eclosion of insects (3-6), hibernation of animals (7-9), and body temperature, blood pressure and pulse in humans (10-13). The circadian clock is an inherent rhythm developed by life on the earth's surface during the long-term evolutionary process to adapt to ambient and external environments (particularly, to the sunrise and sunset) (14,15).

Multiple clock genes, including circadian locomotor output cycles kaput (CLOCK), brain and muscle arylhydrocarbon receptor nuclear translocator (ARNT)-like 1 (BMAL1), period (Per)1, Per2, Per3, cryptochrome (Cry)1, Cry2, neuronal Per-Arnt-Sim (PAS) domain protein 2 (NPAS2), casein kinase Iε (CKIε), timeless (Tim), nuclear receptor subfamily 1, group D, member 1 (NR1D1, also known as Rev-Erb-α) and differentiated embryo-chondrocyte expressed gene (DEC), accurately regulate the human circadian clock at the molecular level (16-18). These genes constitute two important feedback loops. CLOCK is the core factor of the circadian clock and combines with BMAL1 to form a heterodimer through its basic helix-loop-helix (bHLH)-PAS structural domain. The heterodimer combines with the E-box on the promoter of the Per1-3 and Cry1-2 genes, and activates their transcription. The coding products, the Per1-3 and Cry1-2 proteins, are transported from the cytoplasm to the nucleus, where they directly combine with CLOCK/BMAL1, which inhibits their activities and further blocks the transcription of Per1-3 and Cry1-2. In addition to activating the transcription of Per1-3 and Cry1-2, the CLOCK/BMAL1 heterodimer also activates the transcription of the orphan nuclear receptor Rev-Erb gene (17,19) (Fig. 1). The protein encoded by the Rev-Erb gene can combine with the BMAL1 promoter and block its transcription (17). Since genetic transcription, translation and protein transport from the cytoplasm to the nucleus lasts a certain time, the oscillation of the biological rhythm proceeds with a periodic length of ~24 h via self-induction (18,19). Such a negative feedback cycle of the clock genes forms a precise

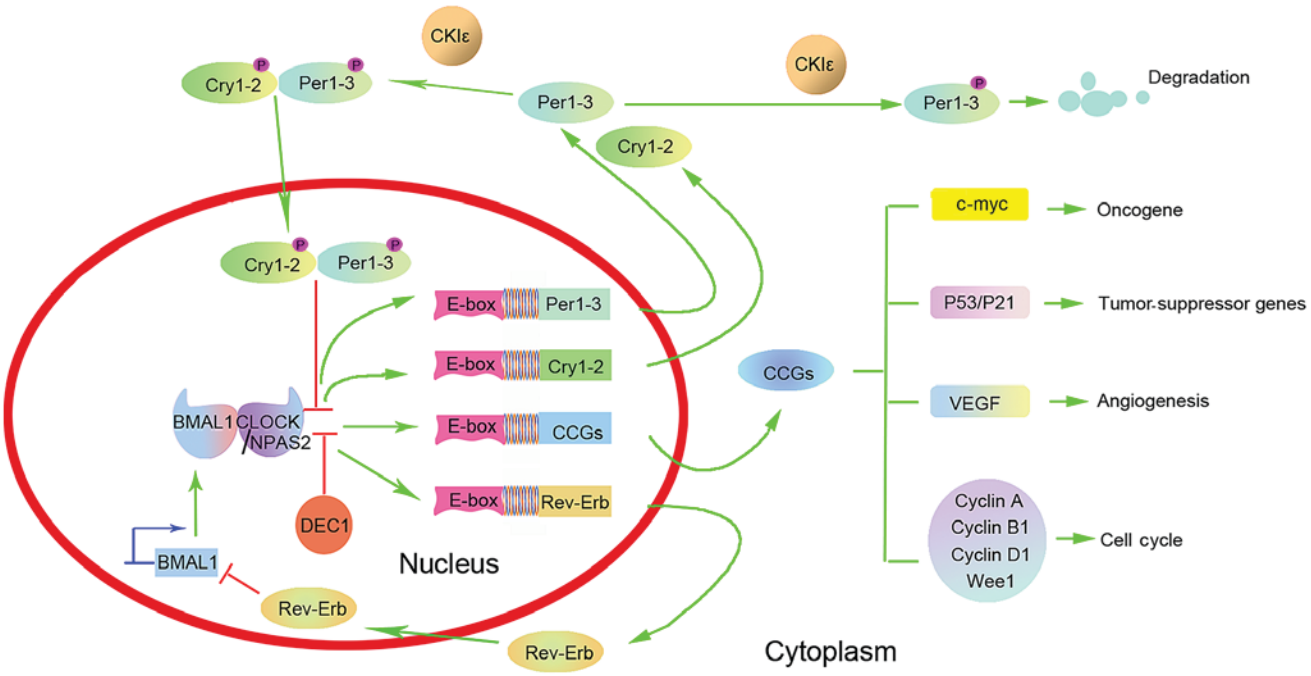

Figure 1. Representation of the circadian clock network. CLOCK or NPAS2 combines with BMAL1 to form a core CLOCK/BMAL1 or NPAS2/BMAL1 transcriptional complex, which subsequently activates the transcription of Per1-3 and Cry1-2 via E-box elements on their promoters. DEC1 can compete with CLOCK/BMAL1 or NPAS2/BMAL1 heterodimers for E-box binding and therefore inhibit CLOCK/BMAL1-mediated transactivation. The coding products, the Per1-3 and Cry1-2 proteins, form a multimeric complex, translocate from the cytoplasm to the nucleus and inhibit CLOCK/BMAL1-mediated transcription. Degradation of Per1-3 and Cry1-2 proteins prompts a new circadian cycle whereby CLOCK/BMAL1 transcription is reinitiated. The CLOCK/BMAL1 heterodimer also activates the transcription of the orphan nuclear receptor Rev-Erb gene. The protein encoded by the Rev-Erb gene can combine with the BMAL1 promoter and block its transcription. Besides transcriptional regulation, post-translational modifications are also involved in the modulation of circadian proteins. CKIε can phosphorylate Per1-3 and Cry1-2 proteins, and enable Per1-3 and Cry1-2 proteins to be translocated from the cytoplasm to the nucleus. In addition, CKIε-mediated phosphorylation can also destabilize Per1-3 proteins. Finally, the CLOCK/BMAL1 complex regulates the expression of CCGs, including oncogenes (c-myc), tumor-suppressor genes (P53 and P21), genes involved in the regulation of the cell cycle (cyclins A, B1 and D1, and WEE1 G2 checkpoint kinase) and VEGF. These target genes regulated by the biological clock genes are involved in DNA repair, cell proliferation and apoptosis. Therefore, circadian clock disorders may lead to uncontrolled cell growth and malignant transformation. CLOCK, circadian locomotor output cycles kaput; NPAS2, neuronal Per-Arnt-Sim domain-containing protein 2; BMAL1, brain and muscle arylhydrocarbon receptor nuclear translocator-like 1; Per, period; Cry, cryptochrome; DEC, differentiated embryo-chondrocyte expressed gene; CKIε, casein kinase Iε; CCG, circadian-clock-controlled gene; VEGF, vascular endothelial growth factor.

endogenous 'molecular clock' in the body. Clock genes output the rhythm signal of a circadian clock through downstream clock controlled genes (CCGs). Thereby, molecular activity within the cell also exhibits a temporal rhythm (18,19).

2. CLOCK

In May 1997, the Takahashi research group of Northwestern University (Evanston, USA) successfully cloned the murine CLOCK gene (20). This represented a milestone in the study of the molecular mechanism of circadian clocks in mammals. In 1999, this group reported the cloning of the human CLOCK gene, which is located on the long arm of chromosome 4 (4q12) and comprises a protein-coding sequence of 2,538 bp. The CLOCK gene belongs to the bHLH-PAS family of transcriptional regulatory factors (21). The containing bHLH domain participates in protein-protein interactions for the formation of protein dimers (21). Two PAS structural domains (PAS-A and PAS-B) mediate the combination of the protein with DNA. Furthermore, the glutamine-rich C-terminus of the CLOCK protein also participates in transcriptional activation (21). The CLOCK gene is a necessary regulator of the circadian rhythm and serves a central role in the circadian clock system. Homozygote mice with CLOCK mutations develop both circadian clock rhythm and feeding rhythm disorders (22,23).

3. BMAL1

BMAL1, also called ARNT3, was identified by Ikeda and Nomura in 1997 (24). BMAL1 is 32-kb long and its coding product belongs to the bHLH-PAS family (24). A clear circadian rhythm was observed in the expression pattern of BMAL1 in suprachiasmatic nuclei (SCN) of mice (25). BMAL1 knockout mice completely lose their circadian rhythm in constant darkness (25). In addition to participating in the regulation of the circadian clock, BMAL1 is also associated with glucose metabolism (26-28), energy conservation (26-28) and aging (29,30).

4. Per1, Per2 and Per3

In 1971, Konopka and Benzer located the Per gene in the X chromosome of *Drosophila* when observing the influence of gene mutations on the circadian rhythm (31). The Per gene of *Drosophila* has three mutant types: PerO, PerL and PerS. These mutant phenotypes exhibit circadian rhythm disappearance, extension and shortening, respectively. Subsequently, similar

genes to the Per gene of *Drosophila* with genotypes Per1 Per2 and Per3 were also identified in mice and humans (31). The Per1-3 genes not only participate in the regulation of the circadian clock, but also inhibit the growth and proliferation of tumor cells, and induce apoptosis, thus being considered as potential tumor-suppressor genes (32-36).

5. Cry1 and Cry2

The Cry gene was initially discovered in plants (37). It encodes the photoreception molecule of blue light and participates in the circadian rhythm reaction guided by blue light in plants. Although this gene also is present in mammals in the mutant forms Cry1 and Cry2, it is unable to act as a photoreceptor in mammals (38). The mutation of mouse (m)Cry2 leads to a 1-h extension of free-motion period. However, the Cry1 mutant manifests the reverse phenotype. The mutant of both mCry1 and mCry2 manifests circadian rhythm disorders, which indicates that mCry1 and mCry2 are core elements of the circadian rhythm (38).

6. NPAS2

NPAS2, also known as member of PAS protein 4 (MOP4), is located on the human chromosome 2p11.2-2q13. Similar to CLOCK, NPAS2 also belongs to the bHLH-PAS family. NPAS2 exhibits the bHLH structural domain at its N-terminus, and two PAS structural domains (PAS-A and PAS-B) in addition to a nuclear receptor-joining region at its C-terminus (39). NPAS2 can regulate the circadian clock rhythm by forming an NPAS/BMAL1 heterodimer with BMAL1, combining with the target gene promoter E-box, and regulating the expression of the Per and Cry genes (40). NPAS2 is an essential gene to maintain a normal biological rhythm. Disorders of the circadian rhythm could be caused by mutation or deletion of NPAS2 (40). In addition, NPAS2 also regulates and interferes with oncogenes, tumor-suppressor genes, and genes associated with the cell cycle, cell proliferation and apoptosis (41-43). Furthermore, NPAS2 is important in cell cycle regulation, DNA damage repair response and tumor growth inhibition, and may also act as a tumor-suppressor gene (41-43).

7. CKIε

CKIε was cloned in 1995 (44). Its protein product, CKIε, belongs to the serine/threonine kinase family, has a relative molecular weight of 43.7 kDa and is widely distributed in its monomeric form (44). CKIε can phosphorylate BMAL1, Per1, Per2, Per3, Cry1 and Cry2 proteins, thus regulating their activity and stability. In addition, CKIε can regulate clock genes at the post-translational level (45-47).

8. Rev-Erb-α and Rev-Erb-β

Rev-Erb-α (identified in 1989) and Rev-Erb-β (identified in 1994) are members of the nuclear receptor superfamily of ligand-inducible transcription factors (48,49). Both receptors possess a DNA-binding domain with a conservative zinc finger and a ligand-binding domain. The DNA-binding domain contains the sequence coding the nuclear localization signal. Depending on the circadian rhythm, these domains are expressed in the human supraoptic nuclei, liver and heart (48,49). Rev-Erb-α can inhibit the expression of CLOCK (50), BMAL1 (51) and NPAS2 (52). The SCN of Rev-Erb-α knockout mice do not periodically express BMAL1 and their active phase is shortened. This indicates that Rev-Erb-α is required for maintaining the accuracy of the circadian clock (53). A previous study indicated that Rev-Erb-α and Rev-Erb-β coordinated to protect against major perturbations in circadian and metabolic physiology (54). The periodic expression of the core circadian clock and the lipid metabolism network were observed to be markedly dysregulated in Rev-Erb-α and Rev-Erb-β knockout mice, which indicates that Rev-Erb-α and Rev-Erb-β are also important components of the circadian clock core mechanism (17,55,56).

9. DEC1 and DEC2

The genes DEC1 and DEC2 were identified in 1997 (57) and 2001 (58), respectively. Both transcription factors contain the bHLH structure, but not the PAS domain. The level of homology of DEC1 and DEC2 in the bHLH region is 97%, while that in the orange region (a motif of ~35 amino acids located C-terminally of the bHLH domain, providing an additional protein-protein interaction interface) is only 52% (59). In contrast to DEC1, the DEC2 transcription factor is rich in alanine and glycine, which may be one of the main reasons for their functional difference (59,60). DEC1 is widely expressed in multiple tissues, while the expression of DEC2 is highly tissue-dependent (59,60). DEC1 can downregulate and inhibit the activity of DEC2. Following combination with E-box functional elements (CACGTG) located on the clock gene promoter, DEC1 and DEC2 regulate the circadian clock rhythm through inhibiting the transcriptional activation process mediated by the CLOCK/BMAL1 heterodimer (61,62). Both transcription factors, particularly DEC2, are closely associated with sleep disorders (61). In addition, DEC1 and DEC2 also participate in regulating the expression of factors associated with tumor growth and apoptosis, and are linked to tumor occurrence and development (63-66).

10. Tim

In 1994, Sehgal *et al* screened a new mutant influencing the biological rhythm of *Drosophila* in a similar manner than PerO. The corresponding wild-type gene of this mutant gene was named Tim (67). Since the identification of the Tim gene occurred on the 1990s, in-depth studies are still required at present to elucidate its role in the regulation of the human circadian clock (68).

11. Conclusions

In recent years, due to the accelerated pace of life and an increased pressure for competition, a large number of people stay awake until late, lose sleep and miss meals, causing a circadian clock disorder and an increase in circadian clock disorder-related diseases (69-71). Epidemiologic studies revealed that circadian rhythm disorders (mainly caused by the influence of light) are correlated with breast, ovarian and

Table I. Expression of clock genes in liver, colorectal, gastric and pancreatic cancer.

Author, year	Cancer type	Country	Carcinoma/peritumoral tissue cases, n	Detection method	Clock gene expression									
					CLOCK	BMAL1	Per1	Per2	Per3	Cry1	Cry2	CKIε	Tim	(Refs.)
Lin *et al*, 2008	Liver	Taiwan	46/46	RT-qPCR and IHC	NS	NS	↓	↓	↓	NS	↓	NS	↓	(82)
Yang *et al*, 2014	Liver	China	30/30	RT-qPCR	NS	NS	↓	↓	↓	NS	↓	NS	ND	(83)
Krugluger, *et al* 2007	Colorectal	Austria	30/30	RT-qPCR	NS	ND	↓	NS	ND	ND	ND	ND	ND	(84)
Wang *et al*, 2011	Colorectal	China	38/38	RT-qPCR and IHC	ND	ND	ND	↓	ND	ND	ND	ND	ND	(85)
Mazzoccoli *et al*, 2011	Colorectal	Italy	19/19	RT-qPCR	NS	ND	↓	↓	↓	NS	↓	NS	↑	(86)
Oshima *et al*, 2011	Colorectal	Japan	202/202	RT-qPCR	↑	NS	↓	NS	↓	NS	NS	↑	ND	(87)
Wang *et al*, 2012	Colorectal	China	203/203	RT-qPCR and IHC	ND	ND	ND	ND	↓	ND	ND	ND	ND	(88)
Karantanos *et al*, 2013	Colorectal	China	30/30	RT-qPCR and IF	↑	ND	ND	ND	ND	ND	ND	ND	ND	(89)
Wang *et al*, 2013	Colorectal	China	168/10	RT-qPCR and IHC	ND	ND	ND	ND	ND	↑	ND	ND	ND	(90)
Yu *et al*, 2013	Colorectal	Greece	42/42	RT-qPCR	↑	↑	↓	NS	↓	ND	ND	ND	ND	(91)
Wang *et al*, 2015	Colorectal	China	203/203	RT-qPCR and IHC	ND	ND	↓	ND	ND	ND	ND	ND	ND	(92)
Zhao *et al*, 2014	Gastric	China	246/45	IHC	ND	ND	↓	ND	↓	ND	ND	ND	ND	(93)
Hu *et al*, 2014	Gastric	Taiwan	29/29	RT-qPCR and IHC	NS	NS	NS	↑	NS	NS	NS	NS	NS	(94)
Relles *et al*, 2013	Pancreatic	USA	65/50	RT-qPCR	NS	NS	↓	↓	↓	NS	↓	↓	NS	(95)

CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle arylhydrocarbon receptor nuclear translocator-like 1; Per, period; Cry, cryptochrome; CKIε, casein kinase Iε; CCG, circadian-clock-controlled gene; Tim, timeless; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; IHC, immunohistochemistry; IF, immunofluorescence; NS, no significant difference between expression in carcinoma and para-carcinoma tissues; ↓, decreased expression in carcinoma tissue; ↑, increased expression in carcinoma tissue; ND, not detected.

Table II. Association between expression of clock genes and clinical parameters of liver, colorectal, gastric and pancreatic cancer.

Author, year	Cancer type	Country	Association	(Refs.)
Lin *et al*, 2008	Liver	Taiwan	Per2 and Per3 were correlated with the size of liver cancer; Tim was correlated with the pathological grade of liver cancer	(82)
Yang *et al*, 2014	Liver	China	Association of clock genes with clinical parameters was not analyzed	(83)
Krugluger *et al*, 2007	Colorectal	Austria	Per1 expression in poorly differentiated tumors was significantly decreased, particularly in female patients	(84)
Wang *et al*, 2011	Colorectal	China	Per2 expression was correlated with the degree of tumor differentiation, TNM stage and depth of infiltration	(85)
Mazzoccoli *et al*, 2011	Colorectal	Italy	Per1 and Per3 were correlated with patients' survival time; Tim was correlated with TNM stage	(86)
Oshima *et al*, 2011	Colorectal	Japan	High expression of BMAL1 and low expression of Per1 were correlated with liver metastasis; patients with high Per2 expression had a better prognosis	(87)
Wang *et al*, 2012	Colorectal	China	Per3 was correlated with tumor differentiation, degree of differentiation and TNM stage; Per3-negative patients exhibited a higher mortality and shorter survival time	(88)
Karantanos *et al*, 2013	Colorectal	China	High CLOCK expression was positively correlated with poor tumor differentiation, advanced stage and lymphatic metastasis	(89)
Wang *et al*, 2013	Colorectal	China	High Cry1 expression was correlated with lymphatic metastasis, TNM stage and poor prognosis	(90)
Yu *et al*, 2013	Colorectal	Greece	No association was observed between CLOCK, BMAL1 or Per1-3 and clinical parameters of colorectal cancer	(91)
Wang *et al*, 2015	Colorectal	China	The expression of Per1 was significantly associated with distant metastasis, but not with patients' prognosis	(92)
Zhao *et al*, 2014	Gastric	China	Per1 and Per2 were correlated with the clinical stage of gastric cancer and depth of infiltration; Per1 was also correlated with lymphatic metastasis and degree of pathological differentiation; patients with high Per2 expression had a better prognosis	(93)
Hu *et al*, 2014	Gastric	Taiwan	High Cry1 expression was positively correlated with advanced gastric cancer	(94)
Relles *et al*, 2013	Pancreatic	USA	Low expression of clock genes was associated with short survival time	(95)

Per, period; TNM, tumor-node-metastasis; Tim, timeless; BMAL1, brain and muscle arylhydrocarbon receptor nuclear translocator-like 1; CLOCK, circadian locomotor output cycles kaput; Cry, cryptochrome.

prostate cancer. Working on night or rotating shifts is linked to a greatly increased risk for women to develop breast and ovarian cancer, and for men to develop prostate cancer (69-71). Clock genes contribute to the occurrence and development of tumors by regulating and interfering with oncogenes (c-myc), tumor-suppressor genes (P53 and P21), genes involved in the regulation of the cell cycle (cyclins A, B1 and D1, and WEE1 G2 checkpoint kinase) and vascular endothelial growth factor, as well as affecting the internal secretion pathway (72-81) (Fig. 1). These target genes regulated by the biological clock genes are involved in DNA damage repair, cell proliferation and apoptosis. Thus, biological clock disorders are likely to lead to uncontrolled cell growth and malignant transformation (73).

Although the exact association between clock genes and common abdominal malignant tumors, including liver cancer (82,83), colorectal cancer (84-92), gastric cancer (93,94) and pancreatic cancer (95), is not clear yet, it has been demonstrated that an abnormal expression of clock genes is ubiquitous in these tumors. Abnormal expression of the CLOCK gene may be one of the important reasons for occurrence and development of these tumors. The relevant articles are summarized in Tables I and II.

As shown in these tables, only a few articles focus on clock genes in abdominal tumors. The majority of them are single-center and small-sample studies, mainly focusing on colon cancer and genes such as CLOCK, BMAL1, Per1, Per2, Per3, Cry1, Cry2, CKIε and Tim, whereas only a few studies focus on NPAS2, Rev-Erb and DEC (83-91,93-95). Low expression of Per1 and Per3 in liver, colon and pancreatic cancer has been observed, and Per1 and Per3 are closely associated with prognosis (83-91,93-95) (Tables I and II).

Currently, the reason and mechanism of low expression of clock genes in abdominal tumors are not clear. Preliminary studies indicate that, in liver cancer, hypoxia, hypoxia inducible factor (HIF)-1α, HIF-2α and hepatitis B virus X protein (HBx) can disrupt the expression of circadian clock genes (83,96). Besides HBx, hepatitis C virus can also modulate the hepatic clock gene machinery (97). Therefore, it can be hypothesized that the tumor microenvironment and virus infections may contribute to circadian clock disorders in hepatocellular carcinoma cells (83,96).

The new interdiscipline generated by the integration of chronobiology and onco-molecular biology is expected to expand the knowledge about tumor occurrence and development, and may provide a new approach for tumor therapy (98-102). Tumor chronotherapy, which is the selection of the optimum treatment time to achieve the maximum curative effect and the minimum toxic and side effects based on the rhythm characteristics of tumor growth, has achieved satisfactory results in clinical practice (98-102). However, the association between clock genes and tumors remains to be fully understood. The circadian clock system of *Drosophila* is well understood, but this knowledge cannot be completely transferred to the human circadian clock, as this is more complex than that of *Drosophila* and large individual differences exist. Numerous factors in the natural and social environments that can influence the human circadian clock and the formation of tumors have not yet been fully elucidated. However, future findings in this field will lead to an increased knowledge in the disciplines of tumor and circadian clock research.

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