Chapter 23
Clock Genes and Cancer

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Abstract Mismatch between the external time and the internal circadian time causes loss of circadian organization and is frequently linked to cancer. This chapter describes the role of the molecular circadian clock in the incidence and progression of cancer. The first section will present the strong association between disrupted clock gene expression in either the host or the tumor tissue with cancer progression. Furthermore, it will be evaluated whether timed clock gene expression is a relevant factor for tumor development. Possible processes that are regulated by the circadian clock and may trigger tumor growth during circadian disruption will be summarized in the second section. The last section will highlight the importance of circadian timing for the development of effective cancer therapies.

23.1 Circadian Disruption Is Associated with Cancer

Changes in environmental conditions can disrupt the molecular circadian clock. For example, abrupt shifts in the day/night cycle, as experienced during jetlag or shift work, result in desynchronization within and between circadian clocks in the suprachiasmatic nucleus (SCN) and in peripheral tissues [1]. Furthermore, increasing evidence links dysfunction of the clockwork with tumor progression [2]. Thus, circadian disruption caused by mismatch of the external time with the internal time is believed to be an underlying factor for the risk of cancer. Indeed, in 2007, an agency of the World Health Organization classified shift work with circadian disruption as "probably carcinogenic" to humans [3] based on results from various experimental and epidemiological studies (reviewed by [4]). For example, a higher incidence of endometrial and colorectal cancer was found in nurses exposed to night shift work compared to their colleagues working on day shifts [5]. Another study indicated an increased risk of people working under night shift conditions to develop non-Hodgkin lymphoma [6].

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Studies in humans were supported by experiments in rodents, e.g., chronic jetlag condition promotes the incidence of lung cancer in rats following injection of tumor cells [7] and enhances the progression of Glasgow osteosarcoma in mice [8]. These mice show circadian disruption on multiple levels, such as disturbed rhythms of clock gene expression in the SCN, body temperature and hormone levels.

Whether circadian disruption is directly linked to cancer occurrence and enhances tumor progression was further investigated by genetic approaches. Results obtained from studies on mice with genetic defects in clock genes, which lead to circadian dysfunction, should therefore match the results obtained from studies on shift workers. Indeed, numerous studies discovered a close association between single nucleotide polymorphisms (SNPs), deletion, deregulation, or epigenetic silencing of circadian genes in humans or targeted gene ablation in animal models; and increased cancer risk (recently reviewed in [9]).

23.1.1 Disturbance of Clock Genes in the Host Is Associated with Cancer

23.1.1.1 Clock Gene Polymorphism in Humans

Recent studies report significant associations between polymorphisms in clock genes and cancer risk, in particular in *PER1*, *PER2*, *PER3*, *CRY1*, *CRY2*, *TIM*, *BMAL1* (or *ARNTL*), *CLOCK*, *NPAS2*, *REV-ERBα* (or *NR1D1*), *DBP*, *DEC1*, *DEC2*, and *CK1ε* [10–12]. For instance, a polymorphism in the *PER3* gene has been associated with prostate and breast cancer risk [13, 14]. However, other studies did not find such an association [10, 15, 16]. The different outcomes of these studies did not verify a clear association between *PER3* and cancer risk; thus, the data have recently been reanalyzed and support an association of *PER3* variants with the incidence of breast cancer, but not with glioma, prostate and colorectal cancer [17].

Breast cancer risk has additionally been associated with variants in either *BMAL1*, *CRY1*, and *NPAS2* [15, 16] or *BMAL1*, *CLOCK*, *CK1ε*, and *NPAS2* [10]. Variants of *NPAS2* have also been associated with prostate cancer risk [14]. Moreover, prostate cancer risk and lymphomagenesis were linked to variants in *CRY2* [18].

Taken together, genetic associations between clock gene variants and cancer risk indicate that variation in clock functions can act as a risk factor for cancer. This is consistent with the possibility that disturbed clock function may enhance the incidence to develop cancer.

23.1.1.2 Clock Gene Mutations in Mice

Apart from spontaneous genetic variation in human clock genes, cancer cell lines were established and various circadian mutants have been identified and designed in rodents. These mutants show circadian phenotypes of different magnitude, from arrhythmicity to minor changes in their behavior. Here we summarize the implication of mutations in specific clock genes in the development and progression of cancer.

Per1 and *Per2* double-knockout (KO) mice lack a functional circadian clock, whereas *Per2* mutants ($Per2^{m/m}$) have a functional but disturbed circadian clock. However, abnormal cell growth was reported in both of these mouse lines and is further reflected in increased radiation-induced tumor occurrence and progression [2, 19]. Accordingly, *Per1* or *Per2* overexpression results in slower tumor growth [20, 21]. Thus, *Per* genes have been characterized as tumor suppressor genes.

Similar to *Per1*/*Per2* double-KO mice, animals lacking both *Cry1* and *Cry2* have a disrupted circadian clock and show increased tumor development following γ-irradiation [2]. However, a different study by Gauger and Sancar reported that animals with targeted disruption of both *Cry* genes did not differ from their wild-type (WT) littermates regarding the frequency of tumor development [22].

Bmal1 gene ablation in mice abolishes circadian rhythmicity [23]. A computational model predicted that perturbation of BMAL1-mediated transcription can generate circadian phenotypes similar to those observed in metastatic cell lines [24]. Indeed, *Bmal1* KO mice show increased radiation-induced tumor development similar to *Per* or *Cry* double-KO mice [2]. Accordingly, knockdown of *Bmal1* promoted tumor growth in mice [25], whereas its overexpression inhibited colorectal cancer cell proliferation [26].

Comparable to *Bmal1* KO mice, mice with a *Clock* gene mutation ($Clock^{\Delta 19}$) were unable to maintain circadian rhythms in constant darkness [27]. However, these mice only show a moderate cancer-related phenotype [28]. In particular, $Clock^{\Delta 19}$ mice do only exhibit increased tumor formation when exposed to long-term γ-irradiation.

Abolished circadian rhythmicity is not necessarily needed to promote cancer. Indeed, deregulation of a single clock gene may be sufficient to change the overall level of other clock genes and account for the cancer-related phenotype. Mice lacking the clock kinases CK1δ, which have an abnormal period, were reported to have an increased incidence to develop mammary carcinogenesis and a shorter life span [29]. Also, as mentioned above, *Per2* mutant mice exhibit a cancer phenotype comparable to arrhythmic *Per1*/*Per2* double-KO mice [19].

Taken together, it is necessary to address the role of each clock component within the molecular clockwork at the systems level, especially in relation to an increased risk to develop cancer. In this regard, the expression levels of clock genes have been correlated with the magnitude and prognosis of cancer. In the next section, results are summarized showing a correlation between the gene expression level for specific clock genes and the incidence of cancer.

23.1.2 Clock Gene Expression Within Tumor Cells Correlates with Cancer Progression

23.1.2.1 Tumor-Intrinsic Clock Gene Expression Is Associated with Cancer

In humans or WT animals, multiple recurring changes in the light-dark cycle disrupt the molecular clock in the SCN and in peripheral tissues [1]. Aside of circadian disorganization, animals undergoing repeated jetlag exhibit increased tumor frequency and faster tumor growth [7, 8]. Interestingly, circadian disruption has been reported, not only in the host's tissues, but also in various tumor cell lines and tumor tissues. However, the direction and magnitude of the changes in clock gene expression can be in opposite directions for different cancer types. For example, *CRY1* levels are decreased in pancreatic cancer [30], but increased in ovarian cancer [31]. Furthermore, changes in the same clock gene can have opposite effects on tumor growth and prognosis, e.g., *PER2* suppression in human pancreatic cancer cells results in reduced proliferation [32], whereas the overall survival in patients with low *PER2* levels in pancreatic tumors was found to be reduced [30]. In conclusion, the relationship between clock gene expression and cancer incidence seems to be gene and cancer type specific and does not always match between cancer cell lines and their related tumor tissues. Table 23.1 lists the studies in humans and rodents reporting significant correlations of clock gene expression with the incidence of cancer and overall prognosis.

23.1.2.2 Circadian Rhythm Disruption in the Tumor Is Associated with Cancer

Various associations of clock gene expression with cancer have been reported (see Table 23.1). However, only an analysis of the time-dependent – circadian – effect of clock genes can allow addressing the role of the circadian rhythms in tumor development and progression. In this regard, disturbed circadian rhythms of clock genes have been documented in various cancer cells and tumor tissue (Table 23.2). Interestingly, circadian rhythmicity of *Per1*, *Per2*, *Rev-Erbα*, and *Dbp* was significantly reduced in colon tumor tissue, but not in healthy colon tissue surrounding the tumor [33], while the rhythmicity of *Bmal1* was abolished on both sides, indicating that tumor-intrinsic circadian rhythms may play a more pronounced role in cancer progression and development than rhythms in the host. Indeed, timed manipulation of circadian rhythms in the tumor was shown to accelerate tumor growth and strongly influence the magnitude of symptoms and prognosis. For example, lack of *Per1* or *Per2* increases tumor growth only at times when their intrinsic expression levels were high [34]. Thus, tumor-intrinsic circadian rhythms may represent a new target for cancer-related therapies. Indeed, since various tumor

Table 23.1 Clock gene expression levels associated with cancer

Clock gene	Species	Expression/ manipulation	Tissue	Outcome	References
Per1	Human	Decreased	Pancreatic cancer	Negative	[30, 64]
	Human	Increased	Pancreatic cancer cells	Negative	[65]
	Human	Knockdown	Pancreatic cancer cells	Positive	[32, 65]
	Human	Decreased	Colorectal cancer		[66]
	Human	Decreased	Colorectal cancer	Negative	[21, 67–70]
	Human	Overexpression	Colorectal cancer cells	Positive	[21]
	Human	Decreased	Gastric cancer	Negative	[71]
	Human	Decreased	Breast cancer		[72, 73]
	Mouse	Decreased	Breast cancer		[74]
	Human	Enhanced	Breast cancer	Positive	[72]
	Human	Decreased	Liver cancer		[75]
	Human	Knockdown	Liver cancer cells	Positive	[65]
	Mouse	Increased	Lung cancer		[76]
	Human	Decreased	Lung cancer/cells		[77]
	Human	Overexpression	Lung cancer cells	Positive	[77]
	Human	Decreased	Glioma cells		[78]
	Rat	Overexpression	Mammary adenocarcinoma	Positive	[79]
	Mouse	Mutated	Mammary cancer	Negative	[34]
	Human	Decreased	Prostate cancer cells		[80]
	Human	Decreased	Ovarian cancer		[31]
	Human	Decreased	Endometrial carcinoma		[81, 82]
	Human	Decreased	Skin melanoma		[83]
	Human	Decreased	Squamous cell carcinoma		[84]
	Human	Decreased	Pleural mesothelioma		[85]
Per2	Human	Decreased	Pancreatic cancer	Negative	[30]
	Human	Suppression	Pancreatic cancer cells	Positive	[32]
	Human	Overexpression	Pancreatic cancer cells	Positive	[86]
	Human	Decreased	Colorectal cancer		[70]
	Human	High	Colorectal cancer	Positive	[68]
	Mouse	Mutation	Colon cancer	Negative	[87]
	Mouse	Suppression	Colon cancer cells	Negative	[87]
	Human	Decreased	Gastric cancer	Negative	[71]
	Human	Decreased	Breast cancer		[73]
	Mouse	Decreased	Breast cancer		[74]
	Mouse	Suppression	Breast cancer	Negative	[88]
	Human	Increased	Breast cancer	Positive	[72]
	Human	Restored	Lung cancer	Positive	[89]
	Human	Decreased	Liver cancer		[75]
	Human	Decreased	Kidney cancer		[90]
	Human	Decreased	Glioma cells		[78, 91]

(continued)

Table 23.1 (continued)

Clock gene	Species	Expression/ manipulation	Tissue	Outcome	References
	Mouse	Mutated	Mammary cancer	Negative	[34]
	Human	Decreased	Skin melanoma		[83]
	Human	Decreased	Squamous cell carcinoma		[84]
	Mice	Overexpression	Human sarcoma cancer	Positive	[92]
	Human	Decreased	Ovarian cancer		[31]
	Human	Overexpression	Osteosarcoma cells	Positive	[93]
Per3	Human	Decreased	Pancreatic cancer	Negative	[30]
	Human	Suppression	Pancreatic cancer cells	Positive	[32]
	Human	Decreased	Colorectal cancer		[66]
	Human	Decreased	Colorectal cancer	Negative	[68, 70]
	Human	Enhanced	Breast cancer	Positive	[72]
	Human	Decreased	Liver cancer		[75]
	Human	Increased	Ovarian cancer		[31]
	Human	Decreased	Squamous cell carcinoma		[84]
	Human	Decreased	Pleural mesothelioma		[85]
Cry1	Human	Decreased	Pancreatic cancer		[30]
	Human	Overexpression	Colorectal cancer	Negative	[94]
	Human	Increased	Ovarian cancer		[31]
	Human	Decreased	Mucinous cancer		[31]
	Human	Decreased	Skin melanoma		[83]
	Human	Decreased	Squamous cell carcinoma		[84]
Cry2	Human	Decreased	Pancreatic cancer		[30]
	Human	Decreased	Colorectal cancer		[70]
	Human	Enhanced	Breast cancer	Positive	[72]
	Human	Decreased	Liver cancer		[75]
	Human	Decreased	Ovarian cancer		[31]
	Human	Decreased	Squamous cell carcinoma		[84]
	Human	Decreased	Pleural mesothelioma		[85]
Bmal1	Human	Decreased	Pancreatic cancer	Negative	[30]
	Human	Decreased	Colorectal cancer		[70]
	Human	Increased	Colorectal cancer		[66]
	Human	Increased	Colorectal cancer	Positive	[26]
	Mouse	Knockdown	Colon cancer cells	Negative	[25]
	Human	Increased	Ovarian cancer		[31]
	Human	Suppressed	Ovarian cancer		[43]

(continued)

Table 23.1 (continued)

Clock gene	Species	Expression/ manipulation	Tissue	Outcome	References
	Human	Overexpression	Ovarian cancer	Positive	[43]
	Human	Decreased	Mucinous adenocarcinomas		[31]
	Human	Decreased	Squamous cell carcinoma		[84]
	Human	Increased	Pleural mesothelioma		[85]
	Human	Increased	Mesothelioma cells		[41]
	Human	Knockdown	Mesothelioma cells	Positive	[41]
	Human	Overexpression	Glioma cancer cells	Positive	[95]
Clock	Human	Reduced	Pancreatic cancer	Negative	[30]
	Human	Increased	Colorectal cancer		[66]
	Human	Increased	Colorectal cancer		[67, 68]
	Human	Mutated	Colorectal cancer		[45]
	Human	Restored	Colorectal carcinoma cells	Positive	[45]
	Human	Enhanced	Breast cancer	Positive	[72]
	Human	Knockdown	Glioma cells	Positive	[49]
	Human	Decreased	Ovarian cancer		[31]
	Human	Decreased	Skin melanoma		[83]
Npas2	Human	Decreased	Colorectal cancer		[96]
	Human	Knockdown	Colorectal cancer	Negative	[96]
	Human	Enhanced	Breast cancer	Positive	[72, 97]
	Human	Increased	Glioma	Negative	[98]
	Human	Increased	Pleural mesothelioma		[85]
Dec1	Human	Decreased	Pancreatic cancer		[64]
	Human	Increased	Pancreatic cancer cells		[99]
	Human	Increased	Breast cancer		[100]
	Human	Decreased	Lung cancer		[47]
	Human	Knockdown	Lung cancer cells	Negative	[47]
	Human	Overexpression	Lung cancer cells	Positive	[47]
	Human	Increased	Liver cancer		[101]
	Human	Reduced	Liver cancer	Positive	[101]
	Human	Variant	Kidney cancer	Negative	[102]
	Human	Decreased	Esophageal cancer cells		[103]
	Human	Restoration	Esophageal cancer cells	Positive	[103]
	Human	Decreased	Lymph node metastasis		[104]
	Human	Increased	Gastric cancer		[105]
Dec2	Human	Increased	Endometrial carcinogenesis		[106]

(continued)

Table 23.1 (continued)

Clock gene	Species	Expression/ manipulation	Tissue	Outcome	References
CK1ε	Human	Decreased	Pancreatic cancer		[30]
	Human	Reduced	Pancreatic cancer	Negative	[30]
	Human	Increased	Colorectal cancer		[68]
	Human	Decreased	Ovarian cancer		[31]
	Human	Decreased	Endometrial carcinoma		[82]
	Human	Decreased	Squamous cell carcinoma		[84]
Rev-Erbα	Human	Decreased	Breast cancer		[107]
	Human	Enhanced	Breast cancer	Negative	[108, 109]
	Human	Increased	Breast cancer cells		[110]
	Human	Knockdown	Breast cancer cells	Positive	[110]
	Human	Agonist	Breast cancer cells	Positive	[48]
	Human	Increased	Papillary carcinoma		[111]
	Human	Decreased	Pleural mesothelioma		[85]
Rorα	Human	Decreased	Colorectal cancer		[112]
	Human	Decreased	Breast cancer cells		[113]
	Human	Enhanced	Breast cancer	Negative	[114]
	Human	Increased	Prostate cancer cells	Positive	[115]
	Human	Knockdown	Breast cancer cells	Positive	[114]
	Human	Decreased	Breast cancer/cells	Negative	[116]
	Human	Restored	Breast cancer cells	Positive	[116]
	Rat	Agonist	Pituitary cancer	Positive	[117]
	Rat	Antagonist	Pituitary cancer	Negative	[117]
Rorγ	Human	Enhanced	Breast cancer	Positive	[72]
	Human	Decreased	Thyroid cancer		[111]
Tim	Human	Decreased	Pancreatic cancer		[30]
	Human	Increased	Colorectal cancer		[70]
	Human	Decreased	Liver cancer		[75, 82]
	Human	Decreased	Kidney cancer		[90]

tissues and human cancer cell lines harbor a dysfunctional circadian clock, the strategy to improve circadian rhythms in those cells becomes obvious.

23.1.2.3 Restoring Circadian Rhythm in the Tumor Inhibits Tumor Growth

Only a limited number of studies have addressed whether tumor-intrinsic clock manipulations may become important for cancer prevention or therapy. For example, the inhibitory effect of the drug seliciclib on tumor growth was enhanced when administration was done at times of the day when it stimulates a high amplitude of

Table 23.2 Disturbed circadian rhythms in cancer cells and tumor tissue

Clock gene	Species	Tissue	Circadian expression	References
Per1	Human	Breast cancer	Disturbed	[118]
	Human	Breast cancer	Dampened	[73]
	Human	Neuroblastoma	Dampened	[119]
	Human	Astrocytoma	Arrhythmic	[119]
	Human	Hepatoma cells	Arrhythmic	[119]
	Human	Myeloid leukemia	Disrupted	[120]
	Hamster	Buccal mucosa cancer	Decreased	[121]
	Mouse	Colorectal cancer	Decreased	[33]
Per2	Human	Breast cancer	Disturbed	[118]
	Human	Breast tumors	Dampened	[73]
	Rat	Human breast cancer cells	Arrhythmic	[122]
	Rat	Human breast cancer	Repressed/disrupted	[122]
	Human	Myeloid leukemia	Disrupted	[120]
	Mouse	Colorectal cancer	Decreased	[33]
Per3	Human	Breast cancer	Disturbed	[118]
	Human	Myeloid leukemia	Disrupted	[120]
Cry1	Human	Myeloid leukemia	Disrupted	[120]
Cry2	Human	Myeloid leukemia	Disrupted	[120]
Rev-Erbα	Mouse	Colorectal cancer	Decreased	[33]
Dbp	Mouse	Colorectal cancer	Decreased	[33]
Bmal1	Rat	Human breast cancer	Repressed/disrupted	[122]
	Rat	Human breast cancer cells	Arrhythmic	[122]
	Human	Myeloid leukemia	Disrupted	[120]
	Mouse	Colorectal cancer	Abolished	[33]
CK1ε	Human	Myeloid leukemia	Disrupted	[120]

clock gene expression in the tumor [35]. These results match observations by Li et al. indicating reduced tumor growth when circadian rhythms were restored by time-restricted food access [36]. Both studies are limited by the possible side effects of the drug and the feeding schedule on other peripheral circadian clocks, such as the liver. Whether restoration of circadian oscillations specifically in the tumor is sufficient to inhibit tumor growth needs to be validated in future studies.

Taken together, changes in overall clock gene expression as well as disruption of their rhythmic expression have been documented in the host and in cancer cells. However, a correlation between clock gene alterations, circadian disruption, and their role in delaying cancer development is an indication but not evidence for a causal relationship in either one or both directions. Thus, two distinct hypotheses can be made. A circadian clock gene may be influencing cancer incidence or tumor development (1) due to their gene-specific activities on target genes involved in cancer-related pathways or (2) through their involvement in circadian clock functions regulating cancer-related pathways. In line with the first hypothesis, clock gene alterations or mutations do not necessarily lead to disruption of the circadian system, either centrally or peripherally. Nevertheless, changes in clock gene

expression have been correlated to the incidence to develop cancer. In contrast, the best indication for the second hypothesis is that WT mice with an environmentally disrupted circadian system show enhanced tumor progression. Moreover, deregulated clock genes are frequent in human cancer cells and tumor tissue.

23.2 Possible Mechanisms Linking the Circadian Clock with Cancer

Cancer development may be pronounced when tumor suppressor functions of either the circadian clock or specific clock genes are lacking. Transcriptome analysis has revealed that many important genes involved in cancer-related pathways, such as the cell cycle, cell proliferation, apoptosis, and DNA damage, are targets of the circadian clock [37]. This section will describe the current knowledge regarding the involvement of the circadian clock in processes which are dysregulated during tumorigenesis. Another possible mechanism involves the circadian control of immune responses, in particular NK cell cytotoxicity. The reader is referred to Chap. 22 for more details.

23.2.1 The Circadian Clock Controls the Cell Cycle

The cell cycle is the process by which a cell prepares for and accomplishes its division into two daughter cells. This occurs through different phases: G1, where the cell prepares for DNA replication; S, where DNA replication occurs; G2, where the cell prepares for mitosis; and M (mitosis), where the cell divides to give rise to the two daughter cells. The cell cycle involves a network of cyclin-dependent kinases (CDKs) forming complexes with cyclins, which regulate each phase of the cell cycle by controlling major checkpoints. This gene network is coupled to the circadian clock through circadian variations in the levels of major players regulating cell cycle checkpoints, such as WEE1, c-MYC, p21, and cyclin E [19, 38] (see Fig. 23.1).

The important checkpoint kinase Wee1 shows rhythmic expression, established through gene activation by CLOCK/BMAL1 and repression by PERs/CRYs [21, 38]. WEE1 inhibits the entry into the M phase by suppression of CDK1 and CDK2 activity [38, 39]. Consequently, reduced synthesis of WEE1 favors the entry into mitosis and may even shorten its duration. Dysregulated circadian expression of WEE1 in turn could induce disruption of the cell cycle, which may result in uncontrolled fast proliferation. Indeed, low and arrhythmic levels of *Wee1* have been reported in *Clock* mutant mice [40] and after *Bmal1* deletion in cancer cells [41]. In contrast, loss of *Cry* genes increased *Wee1* expression, which inhibited the G2/M transition and may account for slower liver regeneration [38].

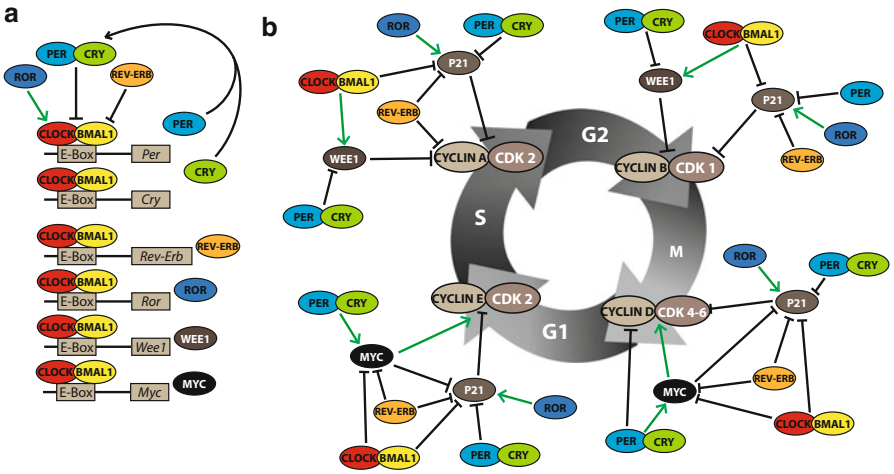

Fig. 23.1 The circadian clock controls the cell cycle on multiple levels. The circadian clock (**a**) interacts with all cell cycle phases by controlling major cell cycle factors, such as WEE1, MYC, p21, cyclin A, and cyclin D (**b**). The cell cycle network consists of cyclin-dependent kinases (CDKs) forming complexes with cyclins and regulating each phase of the cell cycle by controlling major checkpoints: G1 and G1/S phase transition are regulated by cyclin D/CDK4–6 and cyclin E/CDK2, respectively; entry into S phase and thereby DNA replication as well as S-G2 transition are controlled by cyclin A/CDK1; cyclin B/CDK1 finally elicits G2/M phase transition [21, 38, 39]

Another important cell cycle regulator, the oncogene *c-Myc*, is rhythmically regulated via promoter elements for CLOCK/BMAL1 [19]. In contrast to *Wee1*, *c-Myc* expression is repressed by CLOCK/BMAL1 and REV-ERBα but elevated by PER1 [21]. Cell proliferation is regulated by c-MYC via activation of cyclin E/CDK2 and cyclin D/CDK4–6 in parallel, to inhibition of cell cycle inhibitors p21 and p27 [39]. Importantly, *c-Myc* expression was found to be highly elevated in many human tumors [42] and mouse mutants [19]. For example, enhanced expression of *c-Myc* correlates with increased γ-irradiation-induced cell proliferation and tumor development in *Per2* mutant mice, which exhibit suppressed *Bmal1* expression. Accordingly, overexpression of *BMAL1* can suppress elevated *c-MYC* levels and restore its rhythmic activity in ovarian cancer cells [43]. c-MYC is required for G0/G1 transition and elicits S-phase entry, and thus overexpression in cancer cells may be a crucial link for enhanced tumor development [42].

Circadian rhythmicity of the CDK inhibitor *p21* is achieved via inhibition by REV-ERB factors and activation by ROR factors [44]. Additionally, *p21* harbors CLOCK-binding elements [45], and *Per1* overexpression was reported to repress *p21* [21]. P21 inactivates various cyclin/CDK complexes, which induce cell cycle phase entries, such as S-phase transition by cyclin E/CDK2. Other than *Wee1*, *p21* is upregulated and arrhythmic in *Clock* mutant and *Bmal1* KO mice [40, 44]. Consequently, decreased proliferation rate was observed in *Bmall* KO hepatocytes, which can be rescued by *p21* knockdown. In contrast and despite enhanced *p21* levels, increased cell proliferation was found in the epidermis of *Bmal1* KO mice [46], and

enhanced tumor development after tissue-specific ablation of *Bmal1* has been documented [25]. An explanation might be that the regulatory function of p21 in clock gene KO mice may be masked by other cell cycle regulators also regulated by CLOCK/BMAL1, such as c-MYC or WEE1. This is seen, for example, in pleural mesothelioma cells, where *P21* and *WEE1* and *CYCLIN E* levels were altered upon knockdown of *BMAL1* [41].

Adding to this picture, other cell cycle genes were identified as targets of the circadian clock, such as cyclin D1 and A [19, 47, 48].

Taken together, the clock-controlled cell cycle proteins have different sometimes even antagonistic effects on cell cycle progression. This may explain why different clock gene mutants exhibit different cell cycle- and cancer-related phenotypes. Nevertheless, this complex interaction between the circadian clock and cell cycle events probably underlies at least in part the key role for circadian rhythm alterations in carcinogenesis.

23.2.2 DNA Damage Response

Errors during repair of damaged DNA can cause mutations [49]. Genomic instability or accumulation of mutations within the genome is a hallmark of cancer. When damaged DNA is detected by sensor kinases, such as ATM or ATR, cells activate the cell cycle checkpoint kinases CHK1 and CHK2, which stabilize the oncogene p53. P53 in turn activates a series of genes that restrict cell cycle progression and stimulates DNA repair or, in the case of irreparable damage, triggers apoptosis [50]. Important p53 targets are *Mdm2*, *Gadd45α*, and the CDK inhibitor *p21*, a major cell cycle player which mediates G1 arrest and is controlled by the circadian clock (see above). Additionally, p53 and its targets *Mdm2* and *Gadd45α* are regulated by the circadian clock [19]. MDM2 activates cell cycle arrest, but it also feeds back on p53 and inhibits its functions [37, 38, 51]. GADD45 inhibits the G2/M transition and triggers apoptosis [37].

Daily oscillation of DNA excision repair was documented in mouse skin [52]. At the clock gene level, BMAL1 was found to suppress p53 functions in human fibroblast cells and thus to induce the release from cell cycle arrest [51]. Accordingly, overexpression of *BMAL1* inhibited DNA damage sensitivity [26], whereas tissue-specific ablation of *Bmal1* increased the risk of genomic instability and cell cycle arrest in the epidermis [46].

In *Per2* mutant mice, *p53* induction and *Gadd45α* and *Mdm2* rhythmicity were deregulated [19]. Accordingly, daytime-dependent DNA damage-induced apoptosis was perturbed in thymocytes of *Per2* mutant mice [53]. Recent studies demonstrated that PER proteins modulate apoptosis and cell cycle arrest by controlling ATM and CHK2 [21, 54]. In this regard, overexpression of *PER1* triggered DNA damage-induced apoptosis, whereas inhibition of *PER1* blocked apoptosis in human cancer cells [21]. In contrast, *CLOCK* knockdown increased γ-irradiation-induced cell cycle arrest and apoptosis in human glioma cells [49].

Interestingly, *p53*, *Gadd45α*, and *Mdm2* are additionally controlled by *c-Myc*, which is in turn regulated by BMAL1 [37]. Thus, clock-controlled *c-Myc* expression may be another important factor for tumorigenesis by integrating the cell cycle and DNA damage response with the circadian clock. Indeed, jetlag in mice was sufficient to uncouple p53 and c-MYC signaling in the thymus and induce tumor development [2].

Taken together, the circadian clock has been implicated in both DNA damage-induced apoptosis and DNA repair. Consequently, in cases of circadian deregulation, the occurrence of DNA mutations may increase. Moreover, mutated cells may bypass their apoptosis, which would result in accumulation of mutated cells and thereby inducing cancer. Consequently, a reasonable explanation for how clock gene manipulation leads to enhanced cancer incidence or growth rate could be their interaction with transcriptional regulators controlling cell proliferation, DNA repair, and apoptosis.

23.3 Does the Tumor Disrupt Circadian Rhythms?

The interconnection between the circadian clock and the cell cycle does not allow conclusions about the cause of cell cycle deregulation and circadian perturbation in cases of cancer. An intriguing hypothesis is that disturbance of one of the cycles during cancerogenesis, either the circadian clock or the cell cycle, can in turn disrupt the function of the other one. In this section, we address the possible mechanisms for the disruption of clock function in tumors.

23.3.1 Cancer-Related Genes

Interestingly, recent evidence supports the idea that cancer-related signals may interfere with the circadian clock machinery. The clock-controlled major cell cycle regulator MYC can induce circadian malfunction by indirectly repressing *Bmal1* through upregulation of REV-ERBα and PER2 [55]. Importantly, previous studies mentioned an upregulation of *Myc* in human tumor tissues [42] and circadian mouse mutants [19]. Consequently, MYC is an even more important candidate for cancerogenesis by integrating disturbed circadian rhythms and cell cycle dysfunctions and thus is considered as an important target for the development of cancer therapies.

Perturbation of another oncogene, RAS, has been reported to cause circadian clock disruption. RAS transformation induced major phase shifts of *Bmal1* promoter-driven luminescence in human keratinocytes, mouse fibroblasts, and human colorectal cancer cells [24]. Moreover, decreased *PER2* levels and upregulated *CRY1* expression were observed after RAS transformation, supporting the possibility that the activity of RAS might modulate the circadian disruption in cancer cells by influencing CLOCK/BMAL1.

23.3.2 Epigenetic and Posttranscriptional Modifications

DNA methylation plays an important role in modifying gene expression posttranscriptionally, and promoter hypermethylation is a hallmark of cancer. Most core clock genes are predominantly downregulated in various cancer cell lines and tumor tissues (see Table 23.1). Thus, improper DNA methylation may suppress clock gene expression, contributing to the development and progression of cancer. Interestingly, methylated DNA immunoprecipitation microarray identified BMAL1 among the genes that are differentially methylated in ovarian cancer cells [43]. Cancer cell growth could be restored by rescuing *BMAL1* expression, indicating that DNA methylation may be an important mechanism to suppress the circadian clock in cancer cells and induce cancer proliferation. Indeed, hypermethylation has been found on the promoters of core clock genes, such as *PER1*, *PER2*, *CRY1*, and *BMAL1* in breast cancer tissue [56].

Another tumor-intrinsic mechanism which may disrupt circadian rhythms is ubiquitination. For example, transfection with the oncogenes E6/E7 in mouse fibroblasts led to BMAL1 ubiquitination and degradation after the action of the UBE3A ubiquitin ligase on this clock protein and suppression of circadian rhythms in these cells [57].

Histone modifications such as acetylation/deacetylation can also control gene expression and in turn underlie circadian clock control [58]. Interestingly, the histone deacetylase sirtuin 1 (SIRT1) was identified as a circadian clock component, as it deacetylates BMAL1 and PER2 [59]. Low levels of *SIRT1* were documented in various colorectal cancer cell lines and tumor tissues [60], and a correlation was found between expression levels of *SIRT1*, altered clock gene expression and the outcome of pancreatic adenocarcinoma in patients [61]. Collectively, these data indicate that tumor components may direct epigenetic modifications, leading to disruption of circadian rhythms.

23.4 Conclusions and Perspectives

In conclusion, circadian disruption within tumor tissues and in the host enhances cancer progression, and a poorer prognosis was documented in cancer patients with altered circadian rhythms. Thus, improving circadian rhythms in the host and in the tumor may be an important strategy to address cancer therapy.

Also important is cancer chronotherapy – the timed administration of anticancer drugs. The circadian regulation of physiological processes, such as metabolism or detoxification, has severe consequences on the outcome of anticancer therapies [62]. For example, the treatment efficacy and patient survival were improved by rhythmic delivery of the therapeutic into colorectal cancer [63]. Studying the timing of anticancer therapies will allow maximal therapeutic effect with minimal cytotoxic side effects, which may dramatically enhance the life quality of cancer patients.

However, the molecular mechanism linking circadian disruption and cancer should be examined based on specific cancer subtypes. The alteration of clock gene expression differs between cancer subtypes and thus does not allow generalizations about the function of circadian clock genes or the overall circadian system on the development of cancer. Precise characterization of specific cancer subtypes could be used to develop therapeutic approaches involving circadian control, tailored for each cancer type.

Key Questions of Interest and Suggested Readings

- Is circadian clock disruption the cause of cancerogenesis or does cancer induce circadian clock disruption? Hints exist to support both hypotheses, but further studies are required to address this key question.
- How could circadian clock disruption enhance tumor growth? Dysregulation of the cell cycle by altered expression of cell cycle regulators such as WEE1 or c-MYC in circadian clock mutant mice affects the speed of the cell cycle and thus may regulate cancer progression [19, 38].
- What tumor-intrinsic mechanism could downregulate clock genes? Possible factors are DNA methylation [43], ubiquitination [57], or histone modifications [59].
- How can we take advantage of the link between the circadian clock and cancer? Improving circadian rhythms in the host and tumor tissue may reduce cancer progression [35, 36]. Cancer chronotherapy [63] uses the circadian time to treat cancer most effectively.

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