

The Role of Biological Clock in Gynecologic Cancer

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Received: 25 January 2017; **Accepted:** 06 February 2017; **Published:** 10 February 2017

Citation: Karoutsou E, Karoutsos P, Karoutsos D. The Role of Biological Clock in Gynecologic Cancer. Arch Can Res. 2017, 5: 1.

Abstract

Disruption in coordinated co-expression of clock genes, like Period genes mutations, is linked with the increased risk of lung, gastrointestinal, hematologic and gynecologic cancers. Several *clock* genes have been found to functionally interplay with regulators of the cell cycle. It is suggested that abnormal cell cycle function in cancer could also be a consequence of a disrupted biological clock. Chrono-disruption, being studied in different cancer entities, including the gynecologic cancer, could provide time set intervention in cancer therapeutics–chronotherapy in a 24 h–period.

Keywords: Gynecologic cancer; Breast cancer; *Clock* genes

ARNTL (*BMAL*). In turn, *Clock/BMAL* binds in the promoter regions of target genes Period homolog 1, 2 and 3 genes (*PER1*, *PER2*, *PER3*), Cryptochrome genes (*CRY1*, *CRY2*), retinoic acid-related orphan receptor (*Rora*, *Rorb*, *Rorc*) and *Rev-Erb* nuclear orphan receptor (*Rev-Erba*, *Rev-Erbβ*) to activate their transcription [17–19]. Secondly, the negative feedback is achieved by *PER/CRY*, which is commonly seen as the primary generator of the circadian rhythm [20]. Transcription of *Pers* and *Crys* is initiated during the day, and inhibit *Clock/BMAL*-mediated transcription [20,21].

Cryptochrome (CRY) is a blue-light sensor, which regulates neuronal firing rate [22]. The clock that drives behavioral rhythms consists of a feedback loop of the circadian genes Period (PER) and Timeless (TIM). Light acts directly on the clock primarily through CRY; CRY activation causes rapid TIM degradation, resetting the clock both on a daily basis at dawn and on an acute basis following an entraining light pulse during the night hours [23]. TIM and PER in the ovarian follicle cells remain cytoplasmic and do not show daily oscillations in their levels [24].

Regarding the remainder clock components, the ROR/*BMAL/Rev-Erb* (RBR) propels the system [20]. ROR acts as an activator of *BMAL* and *Rev-Erb* as an inhibitor which results in a fine-tuning of *BMAL* transcription [25,26]. Glucocorticoids mediate along with other nuclear ligands the synchronizing effect of this central clock on peripheral tissues [27].

Clock-work dysfunction and cancer

There is increasing evidence that links dysfunction of the clockwork with the pathogenesis of cancer. Cell cycle genes which are affected by the biological clock include C-MYC, Wee1, cyclin D and p21. For example, activation of *PER2* leads to C-MYC overexpression and tumour promotion. Mutations in *CRY 1* and *2* lead to expeditious growth of implanted tumors, in mice [28]. *BMAL1-knockout* mice lose synchronization on the basis of 24 h–period in behavioral or metabolic outputs [29], giving rise to phenotypes of chronic inflammation [30], cancer [31], and diminished sensitivity to anti-cancer drugs such as docetaxel, etoposide, oxaliplatin, and cyclophosphamide [32,33].

Introduction

The endogenous circadian [circa (about) and dies (day)] clock, that is 'tuned' to way– of –life synchronizers, constitutes the synchroscope for all the physiological processes in a 24 h–period [1]. In mammals, the biological clock, so called circadian clock, has a strong effect on sleep-wake cycles, body temperature, renal activity, gastrointestinal tract function and metabolism [1,2]. Further, disturbances of the biological clock trigger off several disorders, including insomnia, depression, hypertension and cancer [3–8].

The Mechanism of the Biological Clock

The biological clock system consists chiefly of the main SCN pacemaker; the molecular basis of SCN (the so-called core *clock* genes such as *PER1-2*, *CRY1-2*, *BMAL1* [9,10]) and the fact that these genes are being rhythmically 'sheltered' in extra-SCN tissues [11–13], flanks this principal oscillator with peripheral clocks [14-16].

The clock network is constituted initially by the heterodimer complex, *Clock/BMAL*, formed by the product of the genes circadian locomotor output cycles kaput (Clock) and brain and muscle aryl hydrocarbon receptor nuclear translocator like –

Recently, environmental disruption of molecular clocks promoted liver carcinogenesis and accelerated cancer progression in rodents. Mteyrek assessed liver histopathology, and determined molecular and physiology circadian patterns in mice on chronic diethylnitrosamine exposure, according to constitutive *PER2* mutation [34]; *PER2* mutation severely deregulated liver gene or protein expressions related to three cancer endpoints, including uncontrolled proliferation, genomic instability, and tumor promoting inflammation, and accelerated liver carcinogenesis several-fold [34]. Previously, it was demonstrated that mutation or knockout of *clock* genes *PER2*, *CRY1/CRY2* or *BMAL1* also accelerated the development of lymphomas following whole body exposure to γ radiations [35,36]. *CRY* mutation renders p53 mutant cells susceptible to tumor necrosis factor α (TNF α)-initiated apoptosis by interfacing with the NF- κ B signaling pathway. These findings provide a mechanistic foundation for the delayed onset of tumorigenesis in clock-disrupted p53 mutant mice, giving a note of optimism in treating cancers associated with p53 mutation [37].

In humans, the expression level of *TIM* was higher in the tumor tissue of colorectal cancer patients, while the overexpression of the circadian clock gene *BMAL1* increases sensitivity to oxaliplatin in patients with colorectal cancer [38,39]. Mazzocchi evaluated chronodisruption in lung cancer; actually, he found that in patients with lung cancer, GH, IGF1, TRH, TSH, FT4, cortisol and IL2 values did not show rhythmic variation [40]. In addition, *PER3* structural variation has been found to increase breast cancer risk twofold in young women [41], while a functional polymorphism in the circadian gene *NPAS2* modifies an individual's susceptibility to non-Hodgkin's lymphoma, suggesting a role for circadian biomarker in this particular disease [42].

Clock genes in gynecologic cancer

The expression rhythms of *PER1/2* and *BMAL1* are modulated by the levels of ovarian steroid hormones in both reproductive and nonreproductive tissues [43]. Progesterone likely causes increases of *PER1*, *PER2* and *BMAL1* expression in human breast cancer MCF-7 cells [43]. Single nucleotide polymorphisms of the clock are significantly associated with estrogen receptor/progesterone receptor (*ER/PR*)-negative cases of breast cancer [44-46] (**Figure 1**); pairwise correlations between functionally-related *clock* genes (e.g., *PER2-PER3* and *CRY2-PER3*) were stronger in ER+, HER2- and low-grade carcinomas; whereas, weaker correlation coefficients were observed in ER- and HER2+ tumors, high-grade tumors and tumors that progressed to metastatic disease [47].

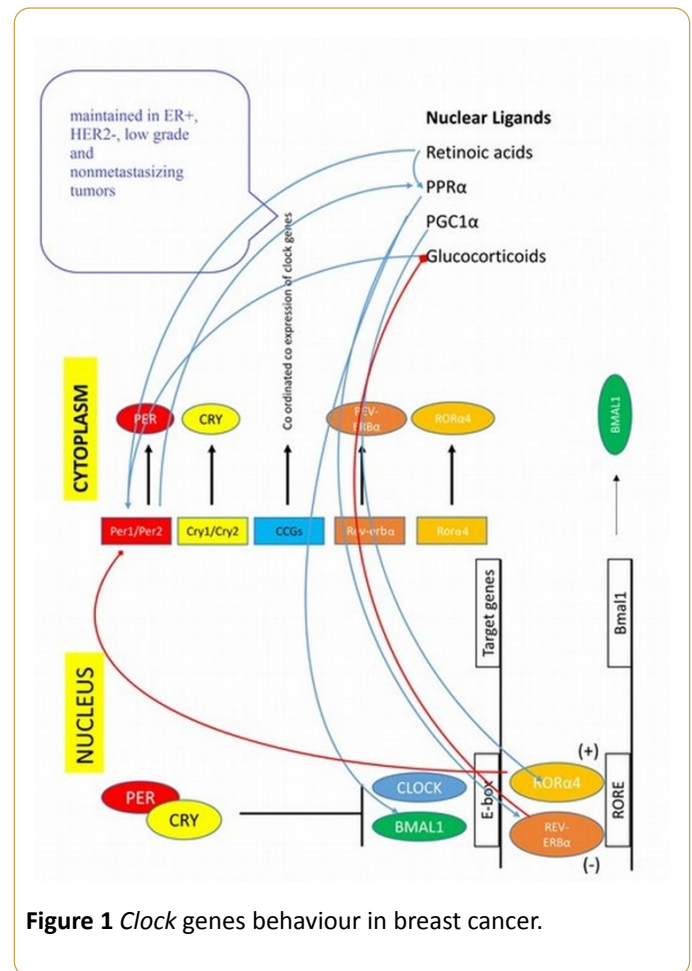


Figure 1 Clock genes behaviour in breast cancer.

Further, breast cancer etiology and prognosis-associated *PERs*, *CRYs*, *Clock* downregulation, and *TIM* upregulation, may be related to relevant gene methylation in tumor tissue [48]. Yang et al. found that *PER1*, mutated in human breast cancers, suppresses cancer cell proliferation and tumour growth [49] at certain time points during the course of the day.

On the other hand, Jim HS examined single nucleotide polymorphisms in *clock* genes *BMAL1*, *CRY2*, *CSNK1E*, *NPAS2*, *PER3*, *REV1* and *TIM* and downstream transcription factors *KLF10* and *SEN3*, as prognostic biomarkers of epithelial ovarian cancer [50]. Risk of overall and serous epithelial ovarian cancer was associated with variants in *KLF10*, while downregulation of *BMAL1* when *C-MYC* was overexpressed, resulted in increasing ovarian epithelial cell transformation [50]. Previously, it was also shown that *ARNTL* (*BMAL*) expression is downregulated in ovarian cancer cell lines [51]; Enhancement of apoptosis by cisplatin was also found in *ARNTL* (*BMAL*) overexpressing ovarian cells [51].

In a study performed by Tokunaga, *CRY1* expression was highest among the eight examined clock genes, followed by *PER3* and *BMAL1*; interestingly, it was suggested that the combination of *CRY1* and *BMAL1* expression might become a possible prognostic marker in epithelial ovarian cancer [52]. Upon studying of a sample population of American women over 28 years, the association between circadian disruption and the risk of fatal ovarian cancer was investigated; it was indicated that the elevated risk of fatal ovarian cancer has an

important association with a rotating work schedule [53]. Similarly, rotating night-shift work increased breast cancer morbidity [54]. It is supposed that 'latent' mutations in clockwork function consist the genetic background that 'sets off' the morbidity of breast cancer or the increased risk of fatal ovarian cancer in night-shift workers.

Moreover, the clock protein *PER2* synchronizes mitotic expansion and decidual transformation of human endometrial stromal cells [55]. In Taiwan, promoter methylation in the *PER1*, *PER2*, or *CRY1* clock genes was detected in about one-third of endometrial cancers and one-fifth of noncancerous endometrial tissues of thirty-five paired specimens indicating disruption of the biological clock in the development of endometrial cancer [56]. Expression levels of *PER1* were significantly decreased in endometrial cancer, and mutational analysis of the coding regions was implemented by Yeh and colleagues [57]. Results suggest that the down-regulation of *PER1* expression in endometrial cancer was partly due to inactivation of the *PER1* gene by DNA methylation of the promoter and partly due to other factors, since the analyses detected four single nucleotide polymorphisms in both tumour and non-tumour tissues, which had no relationship with the expression of *PER1* [57]. Of note is that, analysis of *PER2* levels in the lung and endometrial samples show a less profound difference between tumor and normal samples [17,57]. On collating studies with endometrial cancer and rotating night schedule, it was found, similarly to breast or ovarian cancer risk levels, that the risk of endometrial cancer was elevated in those women on shift work for a period longer than twenty years.

Future Goals

Analyses of the biological clock genetic variability, will amplify the data correlating night-shift work and the incidence of gynecologic cancer, i.e., findings demonstrate that a single night of wakefulness can alter the epigenetic and transcriptional profile of core circadian clock genes in key metabolic tissues [58-60]. The mutations in clock genes underpin the disturbance of clockwork function associated with shift work; this derangement triggers the molecular gears affecting inflammatory and immune responses, giving thus 'room' to cancer appearance [60].

PER3 expression in leukocytes represents a useful molecular marker of the circadian processes governing sleep-wake timing [61]. Withal, disruption of *PER3* function may serve as an indicator of probability of tumor recurrence in patients with ER-positive tumors [52,62].

Conclusion

Circadian disruptions induced by genetic modifications in clock genes and interactions between genes and environment form a set of data, suggesting that genes implicated in cancerogenesis go far beyond the cell cycle. Results also demonstrate that chronodisruption is important for the progression of gynecologic cancer and that restoring on the

balance of clockwork function, lends cancer chronotherapy the present tense.

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