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Clock-Cancer Connection in Non-Hodgkin's Lymphoma

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Abstract

The universal 24-hour circadian clock has a profound impact on many daily biological processes. Disturbance of circadian rhythms has been implicated in the etiologies of many chronic illnesses, including cancer; with correlations most profoundly found among hormone-related breast and prostate cancers. Given the fact that circadian disruption can cause immune deregulation, which is so far the only established risk factor for non-Hodgkin's lymphoma (NHL), we hypothesize that altered circadian rhythms due to environmental factors and/or genetic variations in genes responsible for maintaining circadian rhythms may result in the deregulation of clock-associated biological processes such as immune responses and activities, and consequently influence an individual's risk of developing NHL. Our recent findings provided the first molecular epidemiological evidence linking a major circadian gene to NHL and warrant further investigation. Confirmation of this hypothesis will further add to our understanding of the role of the circadian clock in lymphomagenesis and facilitate the development of novel risk and prognostic biomarkers for NHL.

Keywords

Circadian Clock; Cancer; NHL

Introduction

Currently, Non-Hodgkin's lymphomas (NHL) is among the most rapidly increasing malignancies, increasing to a point where it accounted for 4% of all new cancer cases in 2005 in the United States [1]. Despite tremendous efforts to improve our understanding of this cancer, its etiology remains largely unknown with the only well-established risk factor for NHL being immune deregulation [2]. Given that several essential components of the innate immune system are influenced by the 24-hour circadian rhythm [3–5], we speculate that circadian disruption might play a role in lymphomagenesis by detrimentally affecting immune activity. Furthermore, we hypothesize that genetic variation in genes responsible for maintaining circadian rhythm might act as a novel panel of biomarkers associated with an individual's susceptibility to NHL.

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Clock-cancer connection: epidemiological evidence

The vast majority of organisms experience a 24-hour oscillation of many essential biochemical, physiological and behavioral processes. This oscillation arises from an ancient adaptation to the rotation of the earth and is one of the fundamental components of our biological world. Disruptions of this critical rhythm have the potential to significantly affect our health, and may play a significant role in tumor development [6].

A connection between cancer development and the circadian cycle has been investigated mainly in hormone-related breast and prostate cancers through the study of pilots, flight attendants, and shift workers who are more likely to have disrupted circadian cycles due to their abnormal work hours. In many epidemiological studies of human shift workers, associations between circadian disruption and breast cancer have been observed [7–10]. Recently a similar association has also been reported in prostate cancer [11,12]. Other findings that support a connection between circadian rhythms and cancer risk come from studies of the blind, who have a reduced or abolished sensitivity to environmental light exposures [13]. A reduced risk, due to decreased light sensitivity, was observed in both blind men and women, and was equally pronounced in hormone-dependent tumors as in other types of cancer [14].

Though there is increasing evidence supporting a role of circadian factors in several cancer types, the role of these factors in NHL development has not been widely explored. Currently, a cohort of men working for Air Canada, however, showed a significantly increased incidence rate of acute myeloid leukemia (SIR = 4.72, 90% CI 2.05–9.31) when compared to the respective Canadian population rates [15]. There is also a case report showing that a patient with primary cerebral B-cell non-Hodgkin's lymphoma lost circadian control of sleep [16].

Clock-cancer connection: genetic evidence

To date, there have been nine core circadian genes identified: *Clock*, *casein kinase 1 ϵ* (CK1 ϵ), *cryptochrome 1* (*Cry1*), *cryptochrome 2* (*Cry2*), *Period1* (*Per1*), *Period2* (*Per2*), *Period3* (*Per3*), *Bmal1*, and NPAS2. A model of transcription-translation feedback loops of these circadian genes has been proposed to explain the molecular clockwork [17] in which cell functions are regulated through the expression of clock-controlled genes. It has been estimated that 2–10% of all mammalian genes are clock-controlled genes, indicating an extensive circadian gene regulation [18].

Recent findings suggest that circadian genes may function as tumor suppressors at the systemic, cellular and molecular levels due to their involvement in cell proliferation, apoptosis, cell cycle control, and DNA damage response (reviewed in [6]). In humans, the rhythmic expression of several cyclins and tumor suppressor p53 is regulated by the circadian clock [19]. It has also been shown that a core circadian regulator, CK1 ϵ , functions in promoting cell proliferation and tumorigenesis [20]. A recent study demonstrated that γ -radiation-induced apoptosis is circadian time-dependent and the disruption of the *Per2* gene stops the response of all core circadian genes to γ -radiation [21]. Moreover, disruption of circadian rhythms in mice has been shown to be associated with accelerated growth of malignant tumors, suggesting that the host circadian clock may play an important role in endogenous control of tumor progression [22].

Several recent molecular epidemiologic studies have examined genetic polymorphisms in clock-related genes and corresponding phenotypes, such as morning/evening preference and depressive symptoms. A single nucleotide polymorphism (SNP) located in the 3' flanking region of the human *clock* gene was reported to be a predictor of diurnal preference in a population-based study of randomly selected subjects [23]. In addition, a possible association of *Per3* polymorphisms with delayed sleep phase syndrome or diurnal preference has also been recently reported [24,25]. In the first study of a circadian gene variant and risk of cancer, we

reported that the variant *Per3* genotype was significantly associated with an increased risk of breast cancer among premenopausal women [26]. Our recent results also demonstrated a robust association of the variant 394Thr genotypes (Ala/Thr and Thr/Thr) with reduced risk of NHL (OR = 0.66, 95% CI: 0.51–0.85, $p = 0.001$), especially B-cell lymphoma (OR = 0.61, 95% CI: 0.47–0.80, $p < 0.0001$) [27]. These findings provide the first molecular epidemiological evidence supporting a role of circadian genes in lymphomagenesis.

Overall, the clock-cancer connection has gained some limited but consistent support from previous studies. These observations have suggested a role for circadian rhythms in the etiology of human cancers. The impact of circadian rhythms on hormone regulation could explain the observed role of circadian factors in hormone-related breast and prostate cancers [28,29]. However, little is known about possible molecular mechanisms underlying this clock-cancer connection in NHL.

Possible mechanisms: clock-controlled immune function

Circadian rhythms have been observed in immune activities, including in Natural Killer (NK) cells, which are an essential component of the innate immune system that responds to infections and cancerous growth [3]. The expression of NK cells oscillates throughout a 24 hour period, with peak expression occurring in the early morning [4]. Numerous other components of the immune system, such as T-helper, T-suppressor and splenic B-cells, also display a 24-hour oscillation similar to that of the NK cells [5]. These findings support a potential role for the circadian cycle in immune responses.

A disruption of the circadian cycle may result in disordered immune responses such as aberrant immune cell trafficking and abnormal cell proliferation cycles [30–32]. Sleep disorders, which result in an altered circadian rhythm, have been shown to affect the expression of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a major component of the neuroendocrine system that specifically controls the body's reaction to stress, and plays an important role in regulating the immune system [30]. A change in the HPA axis's expression consequently affects the expression of the pro-inflammatory cytokine IL-6, which is secreted by T-cells and macrophages of the immune system, and/or TNF-alpha, which is involved in the immune system's acute response. Such a disruption in the circadian rhythm of NK cells and their phagocytic activity has been observed in malignant melanoma cells, resulting in a discoordination between the two components of immune system, which is not observed in healthy humans [33].

Findings from clinical studies have shown that circadian disruptions of interleukin-2 receptors, serum thymidine kinase and beta-2-microglobulin in blood samples were detected in NHL patients and might be related to therapeutic outcome [34]. At the cellular level, significant circadian cell cycle variations in S-phase have been found in pathological lymph nodes from NHL patients [35]. It has been shown that a significant number of *PER2* mutant mice died from spontaneous lymphomas before the age of 16 months [21]. Results from a mouse model study have indicated that circadian gene *CK1delta* plays a role in lymphocyte physiology [36]. Observations from a human study have also demonstrated that expression levels of the circadian gene *PER2* were reduced in lymphoma cell lines and in acute myeloid leukemia (AML) patient samples [37].

Hypothesis

Given the established association between immune deregulation and non-Hodgkin's lymphoma (NHL), we speculate that circadian rhythm might play a role in lymphomagenesis by affecting immune activity. For example, increased exposure to light at night will disrupt circadian rhythms and consequently elevate the risk of NHL. Furthermore, genetic and epigenetic

variations in circadian genes may result in deregulation of circadian rhythms and other biological processes regulated by these genes. We hypothesize that genetic variations and methylation changes resulting in altered expression patterns and functionality of the circadian genes will be potential biomarkers for an individual's susceptibility to NHL.

Testing the hypothesis

From an epidemiological perspective, a well-designed population-based case-control study would provide evidence for the role of circadian disruption in NHL. In this type of study, the questionnaire for assessing environmental light exposures and sleeping patterns must be carefully designed in order to ensure meaningful results. Additional studies involving nocturnal occupational groups, such as shift workers (e.g. nurses), would also test this hypothesis in NHL.

Genetic and epigenetic analyses of the circadian genes will further elucidate the role of circadian genes in lymphomagenesis. Three approaches could be applied in this regard: 1) the candidate genetic variation approach: to examine associations between genotypes and haplotypes of predicted functional single nucleotide polymorphisms (SNPs) in circadian genes and NHL risk; 2) the whole gene approach: to investigate associations between haplotypes estimated from haplotype tagging SNPs (htSNPs) in circadian genes and NHL risk; 3) the epigenetic approach: to explore changes of methylation status in CpG islands of circadian genes as biomarkers for NHL.

Analysis of the joint-effect of circadian biomarkers and environmental factors would also test the hypothesis that increased exposure to light at night may elevate the risk of NHL for individuals with the putative high-risk genotypes and haplotypes of circadian genes. Furthermore, relating genetic/epigenetic variations in circadian genes with the progression and treatment outcomes of NHL patients will shed a light on the role of circadian genes as a prognostic factor in NHL.

Conclusions

We propose that some NHL cases could be explained by light-induced circadian disruptions and/or genetic/epigenetic variations in circadian genes and/or gene-environment interactions between these two factors. We also propose that the clock-regulated immune activities might be one potential mechanism involved in lymphomagenesis. These hypotheses are testable in a molecular epidemiological study and could lead to the development of novel circadian risk and prognostic biomarkers for NHL.

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