

Does “Clock” Matter in Prostate Cancer?

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Abstract

The ancient adaptation of a 24-hour circadian clock has profound effect on our daily biochemical, physiologic, and behavioral processes, including the monitoring of sex hormone levels. Although the disruption of the circadian cycle has been implicated in the etiology of hormone-related female breast cancer, few studies have been undertaken to determine if a link exists in the development of the most common cancer type among men whose etiology remains largely unknown: hormone-related prostate cancer. Here, we hypothesize that both altered-lighted environments and genetic variations in

genes responsible for maintaining circadian rhythms may result in deregulation of clock-associated biological processes, such as androgen expression, and consequently influence an individual's risk of prostate cancer. There is also a potential for the interaction of genetic variants and exposures, such as evening shift work. Confirmation of this hypothesis will add to our understanding of the role of the circadian clock in prostate tumorigenesis and further facilitate the development of novel risk and prognostic biomarkers for prostate cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(1):3–5)

Introduction

Prostate cancer is the most common and the second most fatal cancer among men in the industrialized world. Despite tremendous efforts to improve our understanding of this cancer, its etiology remains largely unknown. To date, the only well-established risk factors for prostate cancer are older age, family history of the disease, and race. Recent studies of another hormone-related cancer, breast cancer, have supported the hypothesis that circadian disruption may be a novel risk factor and that genetic determinants for circadian rhythms may play a role in breast tumorigenesis (1, 2). It is from these encouraging findings that we postulate that both circadian disturbance and genetic variations that alter functions of circadian genes are involved in hormone-related prostate tumorigenesis among men.

Clock-Cancer Connection: Epidemiologic Evidence

One fundamental component of our biological world is a universal 24-hour oscillation in biochemical, physiologic, and behavioral processes, which occurs in almost all living organisms due to an ancient adaptation to the rotation of the earth. Disruptions of these critical rhythms may have a profound influence on our health and might be involved in tumorigenesis (3).

The clock-cancer connection has been investigated in studies of pilots, flight attendants, and shift workers who are more likely to have disrupted circadian cycles due to abnormal work hours. Observations from a Nordic pilots cohort showed that the relative risk of prostate cancer increased as the number of flight hours in long distance aircraft increased (4). A similar cohort of men working for Air Canada showed a significantly

increased incidence rate of prostate cancer when compared with the respective Canadian population rates (5). These increased rates of prostate cancer may be due to circadian hormonal disturbances associated with long distance flights although confounding by other factors cannot be ruled out. Similarly, an increased risk of breast cancer among female flight attendants has also been observed (6). In addition, recent studies in female shift workers who work predominantly at night have reported an elevated risk for breast cancer (reviewed in ref. 7). This hypothesis was based on a light-at-night suppression of melatonin (8). Analogously, melatonin has been reported to be lower in men with prostate cancer than those without (9) and melatonin has been shown *in vitro* to modulate prostate cancer cell growth via androgen receptor interactions (10). The clock-cancer connection has gained some very limited but consistent support from previous studies.

Other findings that may 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 (11). In studies of totally blind men, prostate cancer risk was reduced (12, 13) although this reduction was not significant. A reduced risk of breast cancer has also been reported among blind women (13-15).

These observations have suggested a role for circadian rhythms in the etiology of both prostate and breast cancers as previously proposed (16), which implies some common etiologic features shared by these two hormone-related cancer types. However, little is known about possible molecular mechanisms underlying this clock-cancer connection.

Circadian Genes and Their Roles in Tumorigenesis

Emerging areas of interest for circadian and cancer research are the roles of several newly identified clock genes in the master circadian pacemaker of the suprachiasmatic nucleus and in the peripheral cells and tissues, and how this clock apparatus controls the expression of a wide variety of genes. 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*), and *Period3* (*Per3*), *Bmal1*, and *NPAS2*. A model of transcription-translation feedback loops

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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% to 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 ref. 3). 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 the core circadian regulator, casein kinase I, functions in promoting cell proliferation and tumorigenesis (20). A recent study showed that γ radiation-induced apoptosis is circadian time dependent and disruption of the *Per2* gene stops the response of all core circadian genes to γ radiation (21). Moreover, disruption of circadian rhythms in mice is 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).

Polymorphisms in Circadian Genes: Phenotypic Effects

Several 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 located in the 3' flanking region of the human *clock* gene was reported to be a predictor of diurnal preference in a population-based randomly selected subjects (23). In addition, associations of *Per3* polymorphisms with delayed sleep phase syndrome or diurnal preference have recently been 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 (1). Findings from this study and other association studies with other circadian gene variants require much more work to be confirmed. If associations of circadian gene variants with cancer risk exist, then an obvious question becomes how these variants might interact with exposures such as shift work to modify their effects on risk. For example, a variant which predisposes to morning preference may be particularly detrimental for night workers.

Possible Mechanisms: Clock-Controlled Androgen Expression?

The prostate is an androgen-dependent organ because androgens are essential for its normal growth and maintenance. Androgen may act as a carcinogen by increasing cellular proliferation and thereby increase the chance of random DNA copy errors. Evidence from animal studies has shown that large amounts of androgens given to rodents can induce prostate cancer (26). A human study also reported that long-term exposure to high levels of androgens is associated with prostate cancer risk (27). Levels of testosterone, a principle circulating androgen, have further been shown to play a pivotal role in the differentiation and maintenance of selected prostate cancer cells lines both *in vitro* and *in vivo* (28).

The effect of circadian rhythms on the expression of androgens has been previously documented in animals (29). In men, a circadian pattern, with regard to the levels of serum total testosterone, has also been observed and this circadian rhythm becomes blunted with normal aging (30). Circadian rhythms in the plasma levels of cortisol, dehydroepiandrosterone, δ 4-androstenedione, testosterone, and dihydrotestosterone have also been detected in healthy young men (31). These observations suggest that expression of these androgens might be controlled by circadian rhythms. Moreover, circadian genes are directly involved in the regulation of tumor-related genes in prostate tumorigenesis. For example, the expression of cyclin D1 is deregulated in *Per2* mutant mice (32). Cyclin D1 activates the cyclin-dependent kinase 6 that may bind to the androgen receptor and stimulate its transcriptional activity in the presence of dihydrotestosterone (33).

The above findings suggest that androgens might play a role in prostate cancer development. Given the possible effect of the circadian rhythm on androgen expression, we postulate that circadian disruptions, caused by both genetic and environmental factors, may affect androgen expression patterns and consequently contribute to prostate cancer development, offering a potential mechanism for prostate tumorigenesis (Fig. 1).

Hypothesis

We hypothesize that circadian disruption may explain some etiology of prostate cancer and may provide a novel panel of biomarkers to determine an individual's associated risk.

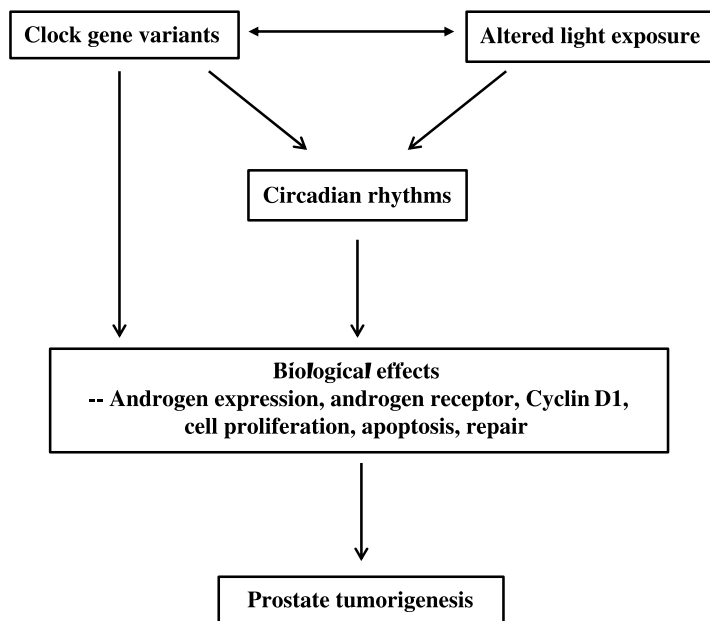


Figure 1. Proposed role of the circadian clock in prostate tumorigenesis. The circadian rhythms can be affected by either genetic variants in circadian genes or altered light exposure. Consequently, androgen expression and biological pathways regulated by circadian rhythms may contribute to prostate cancer development and progression. In addition, circadian genes are directly involved in the regulation of tumor-related genes (such as cyclin D1) in prostate tumorigenesis.

Specifically, increased exposure to light at night will disrupt circadian rhythms and consequently elevate the risk of prostate cancer. In addition, genetic and epigenetic variations in circadian genes may result in deregulation of circadian rhythms and other biological processes regulated by these genes; we propose that genetic variations and methylation changes altering the expression and functionality of circadian genes will be potential biomarkers associated with an individual's risk of prostate cancer.

Testing the Hypothesis

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

Genetic and epigenetic analyses of the circadian genes will further illustrate the role of circadian genes in prostate tumorigenesis. Three approaches could be applied in this regard: (a) candidate genetic variation approach, to examine associations between genotypes and haplotypes of predicted functional single nucleotide polymorphisms in the circadian genes and prostate cancer risk; (b) whole gene approach, to investigate associations between haplotypes estimated from haplotype-tagging single nucleotide polymorphisms in the circadian genes and prostate cancer risk; and (c) epigenetic approach, to explore changes of methylation status in CpG islands of the circadian genes as biomarkers for prostate cancer.

Analysis on 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 prostate cancer for individuals with the putative high-risk genotypes and haplotypes of circadian genes. Furthermore, determining possible associations between shift work and level of androgens, such as testosterone, may help to illustrate potential effects of aberrant light exposures on androgen expressions and provide a likely molecular mechanism should an increased risk be observed between shift work and prostate cancer.

Conclusions

We propose that some prostate cancer 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 androgen expressions might be one potential mechanism involved in prostate tumorigenesis. These hypotheses are testable in a molecular epidemiologic study and could lead to the development of novel risk and prognostic biomarkers.

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References

- Zhu Y, Brown HN, Zhang Y, Stevens RG, Zheng T. Period3 structural variation: a circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol Biomarkers Prev* 2005;14:268–70.

- Stevens RG, Rea MS. Light in the built environment: potential role of circadian disruption in endocrine disruption and breast cancer. *Cancer Causes and Control* 2001;12:279–87.
- Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer* 2003;3:350–61.
- Pukkala E, Aspholm R, Auvinen A, et al. Cancer incidence among 10,211 airline pilots: a Nordic study. *Aviat Space Environ Med* 2003;74:699–706.
- Band PR, Le ND, Fang R, et al. Cohort study of Air Canada pilots: mortality, cancer incidence, and leukemia risk. *Am J Epidemiol* 1996;143:137–43.
- Rafnsson V, Tulinius H, Jonasson JG, Hrafnkelsson J. Risk of breast cancer in female flight attendants: a population-based study (Iceland). *Cancer Causes Control* 2001;12:95–101.
- Stevens RG. Circadian disruption and breast cancer: from melatonin to clock genes. *Epidemiology* 2005;16:254–8.
- Blasko DE, Dauchy RT, Sauer LA, Krause JA, Brainard GC. Growth and fatty acid metabolism of human breast cancer (MCF-7) xenografts in nude rats: impact of constant light-induced nocturnal melatonin suppression. *Breast Cancer Res Treat* 2003;79:313–20.
- Bartsch C, Bartsch H, Schmidt A, Ilg S, Bichler KH, Fluchter SH. Melatonin and 6-sulfatoxymelatonin circadian rhythms in serum and urine of primary prostate cancer patients: evidence for reduced pineal activity and relevance of urinary determinations. *Clin Chim Acta* 1992;209:153–67.
- Zisapel N. Benign and tumor prostate cells as melatonin target sites. The pineal gland and cancer: neuroimmunoendocrine mechanisms in malignancy. Berlin: Springer; 2001.
- Lockley SW, Skene DJ, Arendt J, Tabandeh H, Bird AC, DeFrance R. Relationship between melatonin rhythms and visual loss in the blind. *J Clin Endocrinol Metab* 1997;82:3763–70.
- Feychting M, Osterlund B, Ahlborn A. Reduced cancer incidence among the blind. *Epidemiology* 1998;9:490–4.
- Pukkala E, Ojamo M, Rudanko SL, Stevens RG, Verkasalo PK. Does incidence of breast cancer and prostate cancer decrease with increasing degree of visual impairment. *Cancer Causes Control*. In press.
- Verkasalo PK, Pukkala E, Stevens RG, Ojamo M, Rudanko SL. Inverse association between breast cancer incidence and degree of visual impairment in Finland. *Br J Cancer* 1999;80:1459–60.
- Hahn RA. Profound bilateral blindness and the incidence of breast cancer. *Epidemiology* 1991;2:208–10.
- Stevens RG, Davis S, Thomas DB, Anderson LE, Wilson BW. Electric power, pineal function, and the risk of breast cancer. *FASEB J* 1992;6:853–60.
- Young MW, Kay SA. Time zones: a comparative genetics of circadian clocks. *Nat Rev Genet* 2001;2:702–15.
- Storch KF, Lipan O, Leykin I, et al. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002;417:78–83.
- Bjarnason GA, Jordan RC, Sothorn RB. Circadian variation in the expression of cell-cycle proteins in human oral epithelium. *Am J Pathol* 1999;154:613–22.
- Morin PJ. β -Catenin signaling and cancer. *BioEssays* 1999;21:1021–30.
- Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene *Period2* plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell* 2002;111:41–50.
- Filipinski E, King VM, Li X, et al. Host circadian clock as a control point in tumor progression. *J Natl Cancer Inst* 2002;94:690–7.
- Katzenberg D, Young T, Finn L, et al. A CLOCK polymorphism associated with human diurnal preference. *Sleep* 1998;21:569–76.
- Ebisawa T, Uchiyama M, Kajimura N, et al. Association of structural polymorphisms in the human *period3* gene with delayed sleep phase syndrome. *EMBO Rep* 2001;2:342–6.
- Archer SN, Robilliard DL, Skene DJ, et al. A length polymorphism in the circadian clock gene *Per3* is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 2003;26:413–5.
- Brown CE, Warren S, Chute RN, Ryan KJ, Todd RB. Hormonally induced tumors of the reproductive system of parabiosed male rats. *Cancer Res* 1979;39:3971–6.
- Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. *J Natl Cancer Inst* 1996;88:1118–26.
- Soronen P, Laiti M, Torn S, et al. Sex steroid hormone metabolism and prostate cancer. *J Steroid Biochem Mol Biol* 2004;92:281–6.
- Francavilla A, Eagon PK, DiLeo A, et al. Circadian rhythm of hepatic cytosolic and nuclear estrogen and androgen receptors. *Gastroenterology* 1986;91:182–8.
- Plymate SR, Tenover JS, Bremner WJ. Circadian variation in testosterone, sex hormone-binding globulin, and calculated non-sex hormone-binding globulin bound testosterone in healthy young and elderly men. *J Androl* 1989;10:366–71.
- Guignard MM, Pesquies PC, Serrurier BD, Merino DB, Reinberg AE. Circadian rhythms in plasma levels of cortisol, dehydroepiandrosterone, δ -androstenedione, testosterone and dihydrotestosterone of healthy young men. *Acta Endocrinol* 1980;94:536–45.
- Lee CC. The circadian clock and tumor suppression by Mammalian period genes. *Methods Enzymol* 2005;393:852–61.
- Lim JT, Mansukhani M, Weinstein IB. Cyclin-dependent kinase 6 associates with the androgen receptor and enhances its transcriptional activity in prostate cancer cells. *Proc Natl Acad Sci U S A* 2005;102:5156–61.