12-2009

Circadian Disruption, *Per3*, and Human Cytokine Secretion

Jaclyn Guess

James B. Burch

Kisito Ogoussan

Cheryl A. Armstead

Hongmei Zhang
University of South Carolina - Columbia, hzhang@mailbox.sc.edu

See next page for additional authors

Publication Info

Published in *Integrative Cancer Therapies*, Volume 8, Issue 4, 2009, pages 329-336.
Guess, J., Burch, J. B., Ogoussan, K., Armstead, C. A., Zhang, H., Wagner, S., ... Hrushesky, W. J. M. (2009). Circadian disruption, *Per3*, and human cytokine secretion. *Integrative Cancer Therapies, 8*(4), 329-336.
DOI: 10.1177/1534735409352029

Author(s)

Jaclyn Guess, James B. Burch, Kisito Ogoussan, Cheryl A. Armstead, Hongmei Zhang, Sara Wagner, James R. Hébert, Patricia Wood, Shawn D. Youngstedt, Lorne J. Hofseth, Udai P. Singh, Dawen Xie, and William J. M. Hrushesky

Published in final edited form as:
Integr Cancer Ther. 2009 December ; 8(4): 329–336. doi:10.1177/1534735409352029.

Circadian Disruption, *Per3*, and Human Cytokine Secretion

Jaclyn Guess, MSPH[1,2], **James B. Burch, MS, PhD**[1,2,3], **Kisito Ogoussan, MD, MPH**[1], **Cheryl A. Armstead, MS(R), PhD**[1], **Hongmei Zhang, PhD**[1], **Sara Wagner, MSPH, PhD**[4], **James R. Hebert, MSPH, ScD**[1,2], **Patricia Wood, MD, PhD**[1,3], **Shawn D. Youngstedt, PhD**[1,3], **Lorne J. Hofseth, PhD**[1], **Udai P. Singh, PhD**[1], **Dawen Xie, MD, PhD**[1], and **William J. M. Hrushesky, MD**[3]

[1]University of South Carolina, Columbia, SC, USA

[2]South Carolina Statewide Cancer Prevention and Control Program, Columbia, SC, USA

[3]WJB Dorn Veteran's Affairs Medical Center, Columbia, SC, USA

[4]University of Georgia, Athens, GA, USA

Abstract

Circadian disruption has been linked with inflammation, an established cancer risk factor. Per3 clock gene polymorphisms have also been associated with circadian disruption and with increased cancer risk. Patients completed a questionnaire and provided a blood sample prior to undergoing a colonoscopy (n = 70). Adjusted mean serum cytokine concentrations (IL-6, TNF-alpha, gamma-INF, IL-I ra, IL-I-beta, VEGF) were compared among patients with high and low scores for fatigue (Multidimensional Fatigue Inventory), depressive symptoms (Beck Depression Inventory II), or sleep disruption (Pittsburgh Sleep Quality Index), or among patients with different Per3 clock gene variants. Poor sleep was associated with elevated VEGF, and fatigue-related reduced activity was associated with elevated TNF-alpha concentrations. Participants with the 4/5 or 5/5 Per3 variable tandem repeat sequence had elevated IL-6 concentrations compared to those with the 4/4 genotype. Biological processes linking circadian disruption with cancer remain to be elucidated. Increased inflammatory cytokine secretion may playa role.

Keywords

circadian rhythm; clock gene; cytokine; inflammation

Corresponding Author: James B. Burch, Department of Epidemiology and Biostatistics, Cancer Prevention and Control Program, University of South Carolina, 915 Greene Street, Room 228, Columbia, SC 29208, USA burch@mailbox.sc.edu.

Declaration of Conflicting Interests
The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding
The authors disclosed receipt of the following financial support for the research and/or authorship of this article: This research was supported by the Arnold School of Public Health and Center for Colorectal Cancer Research, University of South Carolina, Columbia, South Carolina. Dr Burch was supported by a Career Development Award from the US Department of Veterans Affairs, VISN-7, Charleston, South Carolina, by the William Jennings Bryan Dorn Department of Veterans Affairs Medical Center, Columbia, South Carolina. Dr. Hébert was supported by an Established Investigator Award in Cancer Prevention and Control from the Cancer Training Branch of the National Cancer Institute (K05 CA136975). Dr Xie was partially supported by Contract No 212-2008-M-24052 from the Biostatistics and Epidemiology Branch of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia.

Introduction

Factors that disrupt circadian rhythms, for example, shift work or altered clock gene expression, are emerging as novel cancer risk factors. [1-4] Circadian rhythm disruption can lead to the development of fatigue, sleep disruption, and depressive symptoms, and these psychological perturbations have been associated with increased secretion of inflammatory cytokines.[5-10] Chronic inflammation is an established risk factor for several types of cancer.[11,12] Thus, the putative association between circadian disruption and cancer may be driven, at least in part, by immune system dysregulation and changes in the secretion of cytokines that influence inflammation or tumor development. Similarly, polymorphic variation in certain clock genes can result in the phenotypic expression of symptoms related to circadian rhythm disruption, including disrupted sleep and altered mood. [13,14] More recently, certain clock gene polymorphisms have been associated with increased cancer risk [15-18] The human *Per3* clock gene is a putative tumor suppressor gene that contains a polymorphic domain expressing 4 or 5 copies of a 54-bp tandem repeat sequence. Variation in this sequence has been associated with circadian preference, sleep and mood disorders, and increased breast cancer risk among premenopausal women. [18-24] Rhythmic changes in *Per* and other clock genes have been linked with regulation of the innate immune system.[25-27] However, no study has examined whether variation in the human *Per3* clock gene is associated with altered cytokine secretion. The objective of this study was to test the hypothesis that individuals with fatigue, poor sleep, depressive symptoms or a *Per3* clock gene variant genotype have altered serum concentrations of cytokines that can influence inflammation or growth regulation.

Materials and Methods

The study population consisted of male veterans scheduled for a screening or diagnostic colonoscopy at the Dom Veterans Affairs Medical Center (DVAMC) in Columbia, South Carolina. Data were collected from March through November 2007 between 9:00 AM and 5:00 PM. Following informed consent, participation included a personal interview and collection of a peripheral blood sample for recovery of serum and DNA. All data were collected prior to completion of the colonoscopy; therefore, participants had no knowledge of the procedure outcome at the time of interview. The questionnaire included information on sociodemographic characteristics, lifestyle, diet, employment and shift work, health status, medications and supplements, mental and physical well-being, physical activity,[28] time spent outdoors, risk factors for colorectal polyps and cancer, sleep habits, major life events, stress and coping strategies, social support, and validated instruments targeting sleep disruption (Pittsburgh Sleep Quality Index [PSQI]),[29] fatigue (Multidimensional Fatigue Inventory [MFI]),[30] and depressive symptoms (Beck Depression Inventory II [BDI]).[31,32] The study was approved by the institutional review boards of the DVAMC and University of South Carolina.

Whole blood samples used for DNA recovery were collected in ethylenediaminetetraacetic acid–preserved vacutainers and stored in 0.5-mL aliquots at –80°C prior to analysis. Genomic DNA was extracted using the DrGentle protocol (Takara, Japan). After extraction, genomic DNA pellets (50-100 μg) were dissolved in 100 to 200 μL of TE buffer, of which about 200 ng was subjected to polymerase chain reaction (PCR) using a Perkin Elmer GeneAmp System 9700 (Waltham, MA) according to the manufacturer's protocol. The *Per3* variable number tandem repeat (VNTR) DNA sequence was amplified using the following primers (forward) 5′-CAAAATTTTATGACACTACCAGAATGGCTGAC-3′ and (reverse) 5′-AACCTTGTACTTCCACATCAGTGCCTGG-3′, with a reaction mixture consisting of 25 μL standard PCR buffer, 5% DMSO, 1.0 mM $MgC1_2$, 0.2 mM dNTP, 1 unit Taq polymerase (Gibco-Invitrogen, Carlsbad, CA), and 0.4 μM of each oligonucleotide primer. The reactions were heated to 94°C for 2 minutes followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds. Finally, the reactions were extended for 7 minutes at 72°C.

PCR products were then separated by electrophoresis on a 3% agarose gel to identify homozygous (4-repeat: 4/4), 5-repeat (5/5), or heterozygous (4/5) individuals. Laboratory personnel were blinded to the identity and characteristics of participants, and a 10% random sample was reanalyzed to assess genotyping concordance.

Following collection of a nonfasting venous whole blood sample in a red-top vacutainer, serum samples were clotted at room temperature for 15 to 30 minutes, centrifuged (5000 × *g* for 5 minutes at 4°C), and 0.5-mL serum aliquots were stored at –80°C until analysis. Serum cytokine concentrations (interferon [IFN]-γ, tumor necrosis factor [TNF]-α, interleukin [IL]-6, IL-1ra, IL-1-β, vascular endothelial growth factor [VEGF]) were determined using a Beadlyte human multicytokine detection system kit (Bio-Rad, Hercules, CA). Filter bottom ELISA plates were rinsed with 100 μL of Bio-plex assay buffer. The assay buffer was then removed using a Millipore Multiscreen Separation Vacuum Manifold System (Bedford, MA) set at 5 mm Hg. Analyte beads in assay buffer were then added to the wells followed by 50 μL of serum or standard solution. The plates were incubated for 30 minutes at room temperature with continuous shaking using a Lab-Line Instrument Titer Plate Shaker (Melrose, IL). The filter bottom plates were washed and centrifuged as before at 300 × *g* for 30 seconds. Subsequently, 50 μL of antihuman IFN-γ, TNF-α, IL-6, IL-1ra, IL-1-β, or VEGF antibody–biotin reporter solution was added to each reaction well, after which the plates were incubated with continuous shaking for 30 minutes followed by centrifugation and washing. Following this, 50 μL streptavidin–phycoerythrin solution was added, and the plates were incubated with continuous shaking for 10 minutes at room temperature. Next, 125 μL of Bio-plex assay buffer was added, and Beadlyte readings were measured using a Luminex System (Austin, TX) and calculated using Bioplex software. Samples below the limit of detection were assigned a value of one half the limit of detection (0.25) pg/mL for data analyses.

Data analyses were performed using the SAS statistical software package (Version 9.l, SAS Institute Inc, Cary, NC). Values for IL-6, TNF-α, INF-γ, IL-1ra, and IL-l-β were log-transformed prior to analysis, and mean concentrations were transformed back to original units for presentation. VEGF and psychosocial measures (BDI, MFI, and PSQI scores) were analyzed without transformation. One participant had elevated TNF-α and INF-γ concentrations, which were evaluated as potential outliers using the method described by Cook.[33] These concentrations met the criteria for Cook's *d* statistic[33] and were within a physiological range; thus, the values were retained in the analyses. A 2-step variable selection procedure was used to identify potential confounding factors associated with each cytokine and psychometric (MFI, BDI, PSQI) variable. First, each independent variable obtained from the questionnaire was compared with each dependent variable using the generalized linear models (GLM) procedure in SAS. Second, screened variables ($P < .05$) were combined in another GLM model, and factors that were no longer associated with the dependent variable ($P > .10$) were removed sequentially from further analysis. This step was repeated until a set of statistically significant ($P < .05$) covariates was identified for each dependent variable, and these were included in subsequent hypothesis testing analyses. To test the study hypotheses, the GLM procedure was used to determine whether mean serum cytokine concentrations (IL-6, TNF-α, IFN-γ, IL-1-β, IL-1ra, and VEGF) differed among individuals grouped according to different circadian disruption symptoms (using MFI, BDI, or PSQI scores) or among those with different *Per3* genotypes. Adjusted (least squares [LS]) mean cytokine concentrations were calculated, and differences among symptom categories or genotype were determined using the least significant differences (LSD) statistic, after adjustment for selected potentially confounding factors. BDI scores were used to group participants into categories of normal (≥10), mild (11-20), or moderate to severe (≥21) depressive symtoms.[32,34] Poor sleepers were defined as those with PSQI scores ≥5,[29,35] and MFI cutpoints were chosen to obtain a distribution of 10 to 15 individuals in several fatigue categories. The relationship between cytokine concentrations and circadian disruption symptoms was also evaluated using each score as a continuous variable

in separate GLM analyses. The GLM procedure was used to determine whether circadian disruption symptoms were associated with different *Per3* variants by comparing adjusted mean MFI, BDI, or PSQI scores among the *Per3* genotypes. Inclusion of polyp status (no polyp detected, benign or hyperplastic polyp, adenoma, or unknown—colonoscopy not yet performed), time of day, or month of sample collection in the statistical analyses did not affect the results obtained.

Results

A total of 83 eligible subjects participated (participation rate = 45%), and complete data were available for 70 men (n = 13 with no blood sample). The average age was 57 ± 9 years, and the racial composition of African Americans and European Americans was approximately equal (51 % and 49%, respectively). Summary scores for general fatigue (mean ± standard deviation = 12 ± 2) and depressive symptoms (14 ± 11) among participants were generally consistent with published normative data (MFI[30]: 15 ± 7, BDI[36]: 11 ± 8). The mean global PSQI score in this population (10 ± 5) was elevated, and a bimodal distribution was suggested, with scores centering on approximately 5 and 13 (data not shown).

There were no statistically significant differences in adjusted mean inflammatory cytokine concentrations between those with adequate and poor quality sleep (Table 1). However, poor sleep was associated with a statistically significant increase in circulating VEGF concentrations ($P = .04$, Table 1). There was no association between serum cytokine concentrations and scores for depressive symptoms, reduced motivation, or general, physical, or mental fatigue (data not shown). Participants with higher MFI subscale scores for fatigue-related reductions in activity tended to have higher TNF-α concentrations compared with those with lower scores ($P = .05$, Table 2), although the results were of borderline statistical significance.

Genotyping for the *Per3* VNTR was in Hardy–Weinberg equilibrium ($P = .81$), and there was 100% concordance with independently genotyped quality control samples. The overall frequency of the homozygous 5/5 VNTR (10%) or the combined genotype frequency (4/5 and 5/5, 51%) agreed well with previous reports (approximately 7% to 10% and 40% to 54%, respectively).[18,37] Evening circadian preference was reported among 67%, 54%, and 57% of participants with the 5/5, 4/5, and 4/4 *Per3* tandem repeat sequences, respectively. Table 3 presents adjusted mean cytokine concentrations among individuals with different *Per3* VNTR genotypes. Mean IL-6 concentrations were elevated among individuals with the 4/5 or 5/5 VNTR sequence compared with those homozygous for the common (4/4) genotype. Table 4 presents mean scores for sleep, fatigue, and depressive symptoms among those with different *Per3* variant genotypes. A tendency toward reduced motivation and a modest increase in depressive symptoms was suggested among subjects with the 4/5 or 5/5 genotype, whereas physical fatigue and sleep disruption tended to be reduced among these individuals compared with those with the 4/4 genotype (Table 4).

Discussion

Circadian rhythm disruption is emerging as a novel and important cancer risk factor. In experimental animals, circadian disruption induced by simulated jet lag or via light exposure at night results in accelerated tumor formation and increased cancer mortality.[38-45] Shift work can have significant impacts on workers; it has been associated with sleep disruption, fatigue, and, in some cases, depression.[46-50] More recently, shift work has been identified as a risk factor for cancers of the colon, breast, prostate, endometrium, and lymphatic system,[1,51-58] and the International Agency for Research on Cancer classified shift work as a probable human carcinogen.[4] The biological mechanisms underlying the association between circadian

disruption and cancer remain to be fully elucidated but may involve changes in the secretion of cytokines that control proinflammatory or proliferative processes.

Results from the present study provide some support for this hypothesis. Poor sleep was associated with increased serum VEGF concentrations, and mean PSQI scores among our participants (10 ± 5) were similar to those observed among cancer patients (11 ± 5).[35] VEGF is important among cancer patients because its angiogenic properties support tumor progression. Additionally, it may affect risk among cancer-free individuals because it can participate in the development of premalignant lesions such as colonic adenomas.[59,60] It has been suggested that VEGF levels may be elevated in depression,[61] a condition frequently accompanied by poor sleep,[62] although there was no association between VEGF and depressive symptoms in this study. Sleep deprivation has been linked with increased circulating concentrations of several inflammation mediators, including TNF-α, IFN-γ, IL-6, and IL-l in humans.[7,8,63-66] Results from our study did not support an association between sleep disruption and circulating inflammatory cytokines, although fatigue-related inactivity was linked with increased TNF-α concentrations. TNF-α is an established mediator offatigue,[9 10] and therapeutic strategies targeting this cytokine for fatigue reduction are under development.[67, 68] Reasons for the inconsistencies among the cytokines measured in this study and results from the existing literature may have been due to the inherent variability of the cytokines measured or due to the cross-sectional nature of the study design. However, we used validated instruments, and our psychometric measures were generally consistent with normative data. We were also able to rigorously evaluate and control for numerous potential confounding factors in the analysis. It may be that multiple, severe, and/or prolonged circadian disruption symptoms are needed to elicit immune dysfunction and robust changes in circulating cytokine concentrations. More detailed longitudinal studies with larger samples and quantitative sleep measures, such as actigraphy or polysomnography, would help in clarifying these issues and in characterizing potential thresholds of biological dysfunction and disease risk that may be linked with circadian disruption.

The molecular clock responsible for human circadian rhythm generation consists of 9 core clock genes, and their expression has been characterized in virtually every tissue investigated.[2,69-71] Clock genes regulate the timing of DNA repair, apoptosis, and cell proliferation, processes that are hallmarks of carcinogenesis.[2,72,73] About 5% to 15% of genome-wide mRNA expression exhibits a circadian rhythm that is driven by the clock genes, including some established tumor suppressor genes and oncogenes.[74,75] Mutation or dysregulation of the *Period* or other clock genes has been associated with increased cancer susceptibility in several animal models as well as in human populations.[15,17,18,76-80] The *Per3* gene has a polymorphic repeat region with 4 to 5 copies of a 54-bp repetitive sequence in exon 18. This variation results in an insertion/deletion of 18 amino acids, and it has been linked with sleep and mood disorders and circadian preference in humans.[19-24] A recent breast cancer study found that the 4/5 or 5/5 *Per3* VNTR was more common among premenopausal cases (63%) than controls (49%; odds ratio = 1.7; 95% confidence interval = 1.0-3.0).[18] In the present study, individuals with the 4/5 or 5/5 genotype had higher adjusted mean IL-6 cytokine concentrations compared with those with the 4/4 genotype. IL-6 is a pro inflammatory cytokine involved in tumor progression[81] that can induce expression of the *Perl* clock gene.[82] Although the function of human *Per3* in conjunction with IL-6 is currently not known, results from the present study suggest a need for additional research to determine whether the cancer risks associated with the *Per3* VNTR variant may be related to elevated IL-6 or other inflammatory cytokine concentrations.

In summary, chronic inflammation is a known risk factor for cancer, and symptoms of depression, fatigue, and sleep disruption are associated with inflammatory cytokine secretion. However, few studies have attempted to determine whether the association between circadian disruption and cancer is driven by the secretion of inflammatory cytokines. Results from this

study suggest that individuals with poor sleep had higher VEGF concentrations compared with good sleepers, participants with greater fatigue-related inactivity had higher TNF-α concentrations, and those with a 4/5 or 5/5 *Per3* variant genotype had higher mean IL-6 concentrations compared with referents. The results provide some support for the hypothesis that fatigue, poor sleep, or the *Per3* VNTR polymorphism is associated with elevated serum cytokine concentrations. If a link between circadian disruption and inflammation is established, therapeutic strategies targeting improved circadian hygiene may provide a benefit by ameliorating inflammation and reducing cancer risks.

References

1. Davis S, Mirick DK, Stevens RG. Night shift work, light at night, and risk of breast cancer. J Natl Cancer Inst 2001;93:1557–1562. [PubMed: 11604479]
2. Fu L, Lee CC. The circadian clock: pacemaker and tumor suppressor. Nat Rev Cancer 2003;3:350–361. [PubMed: 12724733]
3. Stevens RG. Circadian disruption and breast cancer: from melatonin to clock genes. Epidemiology 2005;16:254–258. [PubMed: 15703542]
4. Straif K, Baan R, Grosse Y, et al. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol 2007;8:1065–1066. [PubMed: 19271347]
5. Antoni MH, Lutgendorf SK, Cole SW, et al. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. Nat Rev Cancer 2006;6:240–248. [PubMed: 16498446]
6. Reiche EM, Morimoto HK, Nunes SM. Stress and depression-induced immune dysfunction: implications for the development and progression of cancer. Int Rev Psychiatry 2005;17:515–527. [PubMed: 16401550]
7. McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. Metabolism 2006;55(10, supp1 2):S20–S23. [PubMed: 16979422]
8. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab 2004;89:2119–2126. [PubMed: 15126529]
9. Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 2007;21:153–160. [PubMed: 17088043]
10. Opp MR. Cytokines and sleep. Sleep Med Rev 2005;9:355–364. [PubMed: 16102986]
11. Rhodes JM, Campbell BJ. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. Trends Mol Med 2002;8:1O–16. [PubMed: 11796254]
12. Perwez Hussain S, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007;121:2373–2380. [PubMed: 17893866]
13. McClung CA. Circadian genes, rhythms and the biology of mood disorders. Pharmacol Ther 2007;114:222–232. [PubMed: 17395264]
14. Ebisawa T. Circadian rhythms in the CNS and peripheral clock disorders: human sleep disorders and clock genes. J Pharmacol Sci 2007;103:150–154. [PubMed: 17299246]
15. Zhu Y, Stevens RG, Leaderer D, et al. Non-synonymous polymorphisms in the circadian gene NPAS2 and breast cancer risk. Breast Cancer Res Treat 2008;107:421–425. [PubMed: 17453337]
16. Chu LW, Zhu Y, Yu K, et al. Variants in circadian genes and prostate cancer risk: a population-based study in China. Prostate Cancer Prostatic Dis 2008;11:342–348. [PubMed: 17984998]
17. Zhu Y, Leaderer D, Guss C, et al. Ala394Thr polymorphism in the clock gene NPAS2: a circadian modifier for the risk of non-Hodgkin's lymphoma. Int J Cancer 2007;120:432–435. [PubMed: 17096334]
18. 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;14:268–270. [PubMed: 15668506]
19. 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;2:342–346. [PubMed: 11306557]

20. 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;26:413–415. [PubMed: 12841365]
21. Johansson C, Willeit M, Smedh C, et al. Circadian clock related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. Neuropsychopharmacology 2003;28:734–739. [PubMed: 12655319]
22. Mansour H, Wood J, Devlin B, Logue T, Chowdari K, Kupfer D. Family based and case-control association analysis of circadian gene polymorphisms in bipolar I disorder. Am J Hum Genet 2003;73:504.
23. Nievergelt CM, Kripke DF, Barrett TB, et al. Suggestive evidence for association of the circadian genes PERIOD3 and ARNTL with bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 2006;141:234–241. [PubMed: 16528748]
24. Viola AU, Archer SN, James LM, et al. PER3 polymorphism predicts sleep structure and waking performance. Curr Biol 2007;17:613–618. [PubMed: 17346965]
25. Arjona A, Sarkar DK. The circadian gene mPer2 regulates the daily rhythm of IFN-gamma. J Interferon Cytokine Res 2006;26:645–649. [PubMed: 16978068]
26. Arjona A, Sarkar DK. Evidence supporting a circadian control of natural killer cell function. Brain Behav Immun 2006;20:469–476. [PubMed: 16309885]
27. Arjona A, Sarkar DK. Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. J Immunol 2005;174:7618–7624. [PubMed: 15944262]
28. Topolski TD, LoGerfo J, Patrick DL, Williams B, Walwick J, Patrick MB. The Rapid Assessment of Physical Activity (RAPA) among older adults. Prev Chronic Dis 2006;3:A118. [PubMed: 16978493]
29. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213. [PubMed: 2748771]
30. Smets EM, Grassen B, Bonke B, De Haes J. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995;39:315–325. [PubMed: 7636775]
31. Beck, AT.; Steer, RA.; Brown, GG. Manual for Beck Depression Inventory-II. Psychological Corporation; San Antonio, TX: 1996.
32. Vandeputte M, Weerd A. Sleep disorders and depressive feelings: a global survey with the Beck Depression Scale. Sleep Med Rev 2003;4:343–345.
33. Kleinbaum, DG. Applied Regression Analysis and Other Multivariable Methods. PWS-Kent; Boston, MA: 1988.
34. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561–571. [PubMed: 13688369]
35. Beck SL, Schwartz AL, Towsley G, Dudley W, Barsevick A. Psychometric evaluation of the Pittsburgh Sleep Quality Index in cancer patients. J Pain Symptom Manage 2004;27:140–148. [PubMed: 15157038]
36. Creamer M, Foran J, Bell R. The Beck Anxiety Inventory in a non-clinical sample. Behav Res Ther 1995;33:477–485. [PubMed: 7755538]
37. Pereira DS, Tufik S, Louzada FM, et al. Association of the length polymorphism in the human PER3 gene with the delayed sleep-phase syndrome: does latitude have an influence upon it? Sleep 2005;28:29–32. [PubMed: 15700718]
38. Filipski E, Delaunay F, King VM, et al. Effects of chronic jet lag on tumor progression in mice. Cancer Res 2004;64:7879–7885. [PubMed: 15520194]
39. Filipski E, King VM, Li X, et al. Disruption of circadian coordination accelerates malignant growth in mice. Pathol Biol (Paris) 2003;51:216–219. [PubMed: 12852994]
40. Filipski E, King VM, Li X, et al. Host circadian clock as a control point in tumor progression. J Natl Cancer Inst 2002;94:690–697. [PubMed: 11983758]
41. Shah PN, Mhatre MC, Kothari LS. Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rats in varying photoperiods. Cancer Res 1984;44:3403–3410. [PubMed: 6430548]

42. Blask DE, Brainard GC, Dauchy RT, et al. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. Cancer Res 2005;65:11174–11184. [PubMed: 16322268]
43. Blask 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;79:313–320. [PubMed: 12846415]
44. Blask DE, Sauer LA, Dauchy RT, Holowachuk EW, Ruhoff MS, Kopff HS. Melatonin inhibition of cancer growth in vivo involves suppression of tumor fatty acid metabolism via melatonin receptor-mediated signal transduction events. Cancer Res 1999;59:4693–4701. [PubMed: 10493527]
45. Blask DE, Wilson ST, Zalatan F. Physiological melatonin inhibition of human breast cancer cell growth in vitro: evidence for a glutathione-mediated pathway. Cancer Res 1997;57:1909–1914. [PubMed: 9157984]
46. Burch JB, Tom J, Zhai Y, Criswell L, Leo E, Ogoussan K. Shiftwork impacts and adaptation among health care workers. Occup Med 2009;59:159–166.
47. Burch JB, Yost MG, Johnson W, Allen E. Melatonin, sleep, and shift work adaptation. J Occup Environ Med 2005;47:893–901. [PubMed: 16155474]
48. Violanti JM, Charles LE, Hartley TA, et al. Shift-work and suicide ideation among police officers. Am J Ind Med 2008;51:758–768. [PubMed: 18704914]
49. Woo JM, Postolache TT. The impact of work environment on mood disorders and suicide: evidence and implications. Int J Disabil Hum Dev 2008;7:185–200. [PubMed: 18836547]
50. Saijo Y, Deno T, Hashimoto Y. Twenty-four-hour shift work, depressive symptoms, and job dissatisfaction among Japanese firefighters. Am J Ind Med 2008;51:380–391. [PubMed: 18286600]
51. Conlon M, Lightfoot N, Kreiger N. Rotating shift work and risk of prostate cancer. Epidemiology 2007;18:182–183. [PubMed: 17179764]
52. Kubo T, Ozasa K, Mikami K, Wakai K. Prospective cohort of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. Am J Epidemiol 2006;164:549–555. [PubMed: 16829554]
53. Hansen J. Increased breast cancer risk among women who work predominantly at night. Epidemiology 2001;12:74–77. [PubMed: 11138824]
54. Schernhammer ES, Laden F, Speizer FE, et al. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst 2001;93:1563–1568. [PubMed: 11604480]
55. Schernhammer ES, Laden F, Speizer FE, et al. Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst 2003;95:825–828. [PubMed: 12783938]
56. Schernhammer ES, Kroenke CH, Laden F, Hankinson SE. Night work and risk of breast cancer. Epidemiology 2006;17:108–111. [PubMed: 16357603]
57. Viswanathan AN, Hankinson SE, Schernhammer ES. Night shift work and the risk of endometrial cancer. Cancer Res 2007;67:10618–10622. [PubMed: 17975006]
58. Lahti TA, Partonen T, Kyyronen P, Kauppinen T, Pukkala E. Night-time work predisposes to non-Hodgkin lymphoma. Int J Cancer 2008;123:2148–2151. [PubMed: 18697199]
59. Wong MP, Cheung N, Yuen ST, Leung SY, Chung LP. Vascular endothelial growth factor is up-regulated in the early pre-malignant stage of colorectal tumor progression. Int J Cancer 1999;81:845–850. [PubMed: 10362127]
60. Zhang X, Gaspard JP, Chung DC. Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. Cancer Res 2001;61:6050–6054. [PubMed: 11507052]
61. Warner-Schmidt JL, Duman RS. VEGF as a potential target for therapeutic intervention in depression. Curr Opin Pharmacol 2008;8:14–19. [PubMed: 18061540]
62. Motivala SJ, Levin MJ, Oxman MN, Irwin MR. Impairments in health functioning and sleep quality in older adults with a history of depression. J Am Geriatr Soc 2006;54:1184–1191. [PubMed: 16913983]
63. Vgontzas AN, Chrousos GP. Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. Endocrinol Metab Clin North Am 2002;31:15–36. [PubMed: 12055986]

64. Vgontzas AN, Zoumakis M, Bixler EO, et al. Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. J Clin Endocrinol Metab 2003;88:2087–2095. [PubMed: 12727959]
65. Zheng H, Patel M, Hryniewicz K, Katz SD. Association of extended work shifts, vascular function, and inflammatory markers in internal medicine residents: a randomized crossover trial. JAMA 2006;296:1049–1050. [PubMed: 16954481]
66. Mullington JM, Hinze-Selch D, Pollmiicher T. Mediators of inflammation and their interaction with sleep: relevance for chronic fatigue syndrome and related conditions. Ann N Y Acad Sci 2001;933:201–210. [PubMed: 12000021]
67. Weinblatt ME, Keystone EC, Furst DE, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003;48:35–45. [PubMed: 12528101]
68. Wolfe F, Michaud K. Fatigue, rheumatoid arthritis, and antitumor necrosis factor therapy: an investigation in 24,831 patients. J Rheumatol 2004;31:2115–2120. [PubMed: 15517621]
69. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. Science 2003;302:255–259. [PubMed: 12934012]
70. Shearman LP, Sriram S, Weaver DR, et al. Interacting molecular loops in the mammalian circadian clock. Science 2000;288:1013–1019. [PubMed: 10807566]
71. Albrecht U, Zheng B, Larkin D, Sun ZS, Lee CC. Mper1 and mper2 are essential for normal resetting of the circadian clock. J Biol Rhythms 2001;16:100–104. [PubMed: 11302552]
72. Reddy AB, Wong GK, O'Neill J, Maywood ES, Hastings MH. Circadian clocks: neural and peripheral pacemakers that impact upon the cell division cycle. Mutat Res 2005;574:76–91. [PubMed: 15914209]
73. Kondratov RY, Gorbacheva VY, Antoch MP. The role of mammalian circadian proteins in normal physiology and genotoxic stress responses. Curr Top Dev Biol 2007;78:173–216. [PubMed: 17338917]
74. Canaple L, Kakizawa T, Laudet V. The days and nights of cancer cells. Cancer Res 2003;63:7545–7552. [PubMed: 14633665]
75. Schibler U. The daily timing of gene expression and physiology in mammals. Dialogues Clin Neurosci 2007;9:257–272. [PubMed: 17969863]
76. 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. [PubMed: 12372299]
77. You S, Wood PA, Xiong Y, Kobayashi M, Du-Quiton J, Hrushesky WJ. Daily coordination of cancer growth and circadian clock gene expression. Breast Cancer Res Treat 2005;91:47–60. [PubMed: 15868431]
78. Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis 2005;26:1241–1246. [PubMed: 15790588]
79. Lee CC. Tumor suppression by the mamalian period genes. Cancer Causes Control 2006;17:525–530. [PubMed: 16596306]
80. Krugluger W, Brandstaetter A, Kállay E, et al. Regulation of genes of the circadian clock in human colon cancer: reduced period-l and dihydropyrimidine dehydrogenase transcription correlates in high-grade tumors. Cancer Res 2007;67:7917–7922. [PubMed: 17699798]
81. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5:749–759. [PubMed: 16175180]
82. Motzkus D, Albrecht U, Maronde E. The human PER1 gene is inducible by interleukin-6. J Mol Neurosci 2002;18:105–109. [PubMed: 11931340]

Table 1

Cytokine Concentrations by Sleep Quality[a]

Cytokine Concentration (pg/mL)	Good Sleep[b] (n = 7)	Poor Sleep[c] (n = 58)	Good Versus Poor Sleep Difference	Good Versus Poor Sleep *P* Value	Continuous Variable *P* Value
IL-6	37.6	34.2	−9%	.78	.48
TNF-α	0.8	1.2	+51%	.40	.72
IFN-γ	3.3	2.1	−37%	.25	.12
IL-Ira	6.8	3.1	−55%	.15	.06
IL-I-β	1.7	3.2	−86%	.28	.82
VEGF	664.0	934.0	+41%	.17	.04

NOTES: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ra, receptor antagonist; VEGF, vascular endothelial growth factor; PSQI, Pittsburgh Sleep Quality Index.

[a]Least squares means adjusted for covariates as follows: IL-6: family history of colon cancer, how often person feels stress, prior stressful event.TNF-α: fruit juice intake per week. IFN-γ: family history of colon cancer, fruit juice intake per week. IL-Ira: body mass index, diarrhea in the past 6 months, fruit juice intake per week. IL-I-β: body mass index, circadian type.VEGF: use of nonsteroidal anti-inflammatory drugs, unable to visit doctor due to cost, packs of cigarettes smoked per day.

[b]PSQI score <5.

[c]PSQI score ≥5.

Table 2

Cytokine Concentrations by Reduced Activity Scores[a]

Cytokine Concentration (pg/mL)	Groups of Reduced Activity[b]				Group 1 Versus 4 Difference	Group 1 Versus 4 *P* Value	Continuous Variable *P* Value
	1 (10 ± 1), n = 11	2 (11 ± 0), n = 16	3 (12 ± 0), n = 27	4 (14 ± 1), n = 15			
IL-6	3.72	3.69	3.59	4.26	+15%	.17	.22
TNF-α	0.79	1.69	0.91	2.46	+211%	.05	.24
IFN-γ	1.57	2.49	1.78	2.13	+36%	.56	.71
IL-Ira	3.31	2.99	3.60	4.25	+28%	.65	.94
IL-I-β	3.03	3.69	2.22	5.26	+74%	.39	.61
VEGF	1050	970	853	919	−12%	.53	.15

NOTES: IL, interleukin;TNF, tumor necrosis factor; IFN, interferon; ra, receptor antagonist; VEGF, vascular endothelial growth factor.

[a]Least squares means adjusted for covariates as follows: IL-6: family history of colon cancer, how often person feels stress, prior stressful event.TNF-α: fruit juice intake per week. IFN-γ: family history of colon cancer, fruit juice intake per week. IL-Ira: body mass index, diarrhea in the past 6 months, fruit juice intake per week. IL-I-β: body mass index, circadian type. VEGF: use of nonsteroidal anti-inflammatory drugs, unable to visit doctor due to cost, packs of cigarettes smoked per day.

[b]Multidimensional Fatigue Inventory reduced activity subscale. Mean ± standard deviation of reduced activity scores in parentheses (higher scores represent lower activity).

Integr Cancer Ther. Author manuscript; available in PMC 2010 December 1.

Integr Cancer Ther. Author manuscript; available in PMC 2010 December 1.

Table 3

Cytokine Concentrations by *Per3* VNTR Genotype[a]

	***Per3* VNTR Genotype**						
Cytokine Concentration (pg/mL)	**4/4 (n = 35)**	**4/5 (n = 27)**	**5/5 (n = 6)**	**4/5 and 5/5 (n = 33)**	**4/4 Versus 4/5 *P* Value**	**4/4 Versus 5/5 *P* Value**	**4/4 Versus 4/5 and 5/5 *P* Value**
IL-6	34.1	52.8	66.7	54.8	.09	.13	.05
TNF-α	1.3	0.9	2.7	1.1	.32	.23	.66
IFN-γ	2.1	1.5	2.9	1.7	.29	.57	.46
IL-Ira	3.5	3.0	6.5	3.4	.70	.33	.95
IL-I-β	3.1	3.0	5.5	3.3	.95	.45	.90
VEGF	904	880	1002	901	.85	.65	.99

NOTES: VNTR, variable number tandem repeat; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ra, receptor antagonist; VEGA, vascular endothelial growth factor.

[a]Least squares means adjusted for covariates as follows: IL-6: family history of colon cancer, how often person feels stress, prior stressful event. TNF-α: fruit juice intake per week. IFN-γ: family history of colon cancer, fruit juice intake per week. IL-Ira: body mass index, diarrhea in the past 6 months, fruit juice intake per week. IL-I-β: body mass index, circadian type. VEGF: use of nonsteroidal anti-inflammatory drugs, unable to visit doctor due to cost, packs of cigarettes smoked per day.

Table 4

Circadian Disruption Symptoms by *Per3* VNTR Genotype[a]

	***Per3* VNTR Genotype**						
Symptom	**4/4 (n = 35)**	**4/5 (n = 27)**	**5/5 (n = 6)**	**4/5 and 5/5 (n = 33)**	**4/4 Versus 4/5 *P* Value**	**4/4 Versus 5/5 *P* Value**	**4/4 Versus 4/5 and 5/5 *P* Value**
Depressive symptoms	15.3	19.5	18.6	19.3	.08	.38	.07
Sleep quality	9.3	7.4	9.8	7.9	.06	.79	.11
General fatigue	12.2	12.6	11.4	12.4	.38	.36	.65
Physical fatigue	12.0	11.0	11.4	11.1	.03	.43	.03
Mental fatigue	12.5	12.4	11.6	12.2	.83	.15	.52
Reduced motivation	11.7	12.5	12.6	12.5	.15	.39	.13
Reduced activity	11.9	11.5	11.2	11.5	.37	.34	.29

NOTES: *Per*, *Period*; VNTR, variable number tandem repeat.

[a]Least squares means adjusted for covariates as follows: Beck Depression Inventory II: diarrhea in the past 6 months, how often person feels stress, posttraumatic stress disorder. Pittsburgh Sleep Quality Index: personal cancer history, spouse kicks or moves during sleep, how often person feels stress, soft drink intake per week. Multidimensional Fatigue Inventory: General fatigue—personal cancer history, diarrhea in the past 6 months, tea intake per week. Physical fatigue—calcium supplement intake per day, bacon intake per week. Mental fatigue—number of dependents, personal cancer history, cigarettes smoked per day. Reduced motivation—diagnosed inflammatory illness, months reported being tired, frequency of stress, weekly intake of French fries. Reduced activity—age, number of months feeling like a failure.