Circadian Disruption and Breast Cancer
From Melatonin to Clock Genes
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Abstract: The global impact of breast cancer is large and growing. It seems clear that something about modern life is the culprit, yet there is thus far a lack of satisfactory explanations for most of the increases in risk as societies industrialize. Support has developed for a possible role of “circadian disruption,” particularly from an altered-lighted environment (such as light at night). Lighting during the night of sufficient intensity can disrupt circadian rhythms, including reduction of circulating melatonin levels and resetting of the circadian pacemaker of the suprachiasmatic nuclei. Reduced melatonin may increase breast cancer risk through several mechanisms, including increased estrogen production and altered estrogen receptor function. The genes that drive the circadian rhythm are emerging as central players in gene regulation throughout the organism, particularly for cell-cycle regulatory genes and the genes of apoptosis. Aspects of modern life that can disrupt circadian rhythms during the key developmental periods (eg, in utero and during adolescence) may be particularly harmful. Epidemiologic studies should consider gene and environment interactions such as circadian gene variants and shift work requirements on the job.

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Breast cancer is the leading cause of cancer death among women worldwide. Risk of breast cancer varies 5-fold among countries, is increasing everywhere, and is highest in the industrialized nations. Less than half the risk in industrialized areas can be explained by changes in the known risk factors for breast cancer. As societies industrialize, there are changes in many aspects of life, prominent among which is the opportunity for circadian disruption from use of artificial lighting. Circadian rhythms are controlled by a core of 8 genes, which also exert control over key cell-cycle checkpoint genes and genes of apoptosis. These rhythms can be altered by ill-timed artificial lighting and by lack of sunlight. In modern industrialized societies where people live most of their lives inside buildings, people receive very little sunlight. There is also evidence that sleep patterns and melatonin production have been altered in people by the lighting practices in the modern urban environment.

CIRCADIAN DISRUPTION HYPOTHESIS

According to the circadian disruption hypothesis, factors in the environment (eg, light at night) that might disrupt the endogenous circadian rhythm, and that specifically suppress nocturnal melatonin production, would increase the risk of breast cancer. Originally, it was further argued that reduced melatonin might result in elevated estrogen, but the extent to which melatonin and estrogen are related in humans remains unclear. Light during the night can suppress melatonin in humans as well as alter circadian phase in a wavelength-dependent manner. Melatonin, in turn, has been shown to have a strong inhibitory effect on chemically induced mammary tumorigenesis in rodents. Most experiments on the effect of constant light on mammary tumorigenesis in rats have shown a strong stimulation, although a recent study has instead found an inhibition. The reason for the different tumor response appears to be differences in the age of the rat at first exposure to constant light, which resulted in profound differences in mammary tissue development. This, in turn, would alter tumor susceptibility. Constant light began in utero in Shah et al., but began at age 26 days in Anderson et al.

Blask et al. implanted a human breast cancer xenograft (from MCF7) into a nude rat model. Constant light dramatically increased the growth of the xenograft. This is the first evidence that nocturnal illumination can affect growth of human-derived breast cancer in a whole animal model.

EPIDEMIOLOGIC EVIDENCE

The possible role of circadian disruption (principally from light at night) in risk of breast cancer has gained limited,
but so far consistent, epidemiologic support. Predictions based on the hypothesis include the idea that women who work evening or overnight shifts would be at higher risk, and profoundly blind women would be at lower risk. Consistent with these predictions, risk has been reported to be elevated in shift workers, and reduced in blind women.

Hansen conducted a large case-control study (over 7000 cases and 7000 population controls) of night-work occupations and risk of breast cancer in women age 30 to 54 years in Denmark. The overall estimate of the odds ratio (OR) was 1.5 (95% confidence interval [CI] = 1.3–1.7). The largest job entailing work at night was “catering” (restaurant waiter, 300 cases) with the same odds ratio estimate of 1.5 (1.2–1.7). Another night-work occupation was “air transport worker” (flight attendant), which had 54 cases and an odds ratio of 1.9 (1.2–3.0). This estimate is the same as from the cohort study by Pukkala et al., which focused on breast cancer risk in flight attendants in Finland (standardized incidence ratio = 1.9; 95% CI = 1.1–2.2).

In a case-control study of women age 20 to 74 years in the Seattle area, Davis et al. tested the hypothesis that increased light at night is associated with increased risk. The authors used 3 surrogates for exposure: shift work occupation, self-reported ambient light level in the bedroom at night, and degree of nonpeak sleep (awake between 1 and 2 AM). There was a modest increased risk associated with shift work (OR = 1.6; 95% CI = 1.0–2.5) and with nonpeak sleep (OR = 1.4; 1.0–2.0). These 2 groups overlap, but the association with nonpeak sleep was still elevated after controlling for shift work. For self-reported ambient bedroom light level, the odds ratio for the highest of 3 light-level categories compared with the lowest was 1.4 (0.8–2.6).

The report from Schernhammer et al. relied on a single question on the 1988 Nurses’ Health Study questionnaire, asking how many years the nurse had worked rotating shifts in addition to days and evening shifts. Among nurses who reported working 30 or more years of rotating night shifts, the relative risk was 1.36 (95% CI = 1.04–1.78) compared with nurses who had no history of rotating shift work. This may underestimate the effect of shift work because, as the authors point out, nurses working permanent night shifts would have responded “no” to this question and been included in the baseline comparison group who did not ever work rotating shifts. In addition, Schernhammer et al. have recently reported reduced melatonin and elevated estrogen in nurses with a long history of rotating night shifts.

Not all evidence is consistent in supporting the hypothesis. A recent nested case-control study of the association of urinary concentrations of 6-sulfatoxymelatonin in the morning (a good indicator of nocturnal melatonin production) among breast cancer cases and controls found no differences. The authors stress the limitations of their analysis (eg, limited size, 127 cases, and only 1 urinary melatonin determination).

MECHANISM FOR CIRCADIAN PHOTOTRANSDUCTION

The timing of circadian rhythms and nocturnal melatonin suppression in humans appears to be dependent on light on the retina. The mechanism of phototransduction for the circadian system is not identical to the visual system, although the visual system may also be involved. For example, a very bright light in the middle of the night suppressed nocturnal melatonin in some but not all (3 of 11 subjects tested) subjects who were totally visually blind. In addition, mice with congenital absence of rods and cones are reported to retain circadian sensitivity (behavioral phases shifting and melatonin suppression) to light of a particular wavelength (480–509 nm) during the night. However, artificial light during the night does not affect melatonin production in enucleated mice. This observation is consistent with studies in blind subjects; all bilaterally enucleated humans so far studied have free-running rhythms and no circadian light response. These observations indicate that the retina is required for a melatonin response to light at night.

The leading candidates for the photopigment for the circadian system have been melanopsin and cryptochrome (reviewed by Van Gelder). However, it has been shown by use of knockout mice that neither the absence of melanopsin nor the congenital lack of vitamin A obliterates light sensitivity. Absence of cryptochromes also does not completely suppress light sensitivity. Mice lacking rods, cones, and melanopsin have no light sensitivity, suggesting that there is not just 1 photopigment that entirely determines circadian light response. The discovery of a new photoreceptive ganglion cell in the rat retina adds further complexity to our understanding of the biologic relationships.

The spectral irradiance of daylight is very different from electric light sources inside buildings, both in spectrum and amplitude. As can be seen in Figure 1, the peak irradiance of daylight is approximately 460 to 480 nm. The biologic evolution of a photoreceptive system that would signal daylight to the organism might be expected to be maximally sensitive, therefore, to these wavelengths. In fact, recent estimates for maximal human melatonin responsiveness (approximately 464 nm) and for maximal light response of the newly discovered photoreceptive retinal ganglion cell in the rat (approximately 484 nm) are both close to this daylight peak.

There may be unrecognized polymorphisms for aspects of the phototransduction pathway that alter the ability of light to influence circadian rhythmicity and thereby alter breast cancer risk in women in the modern, artificially lit world.

CIRCADIAN RHYTHM GENES

The original light-at-night idea was based on alterations in melatonin production and action. However, an emerging area of interest to the circadian and cancer research communi-
ties is the relation of the circadian genes\textsuperscript{37} in the master circadian pacemaker of the suprachiasmatic nuclei to the peripheral clock mechanism in cells and tissues, and how this circadian apparatus controls expression of a wide variety of genes, in particular those for cell-cycle regulation and for apoptosis.\textsuperscript{5}

Zheng et al.\textsuperscript{38} constructed a mouse with a deletion mutation in Per2, resulting in a truncated transcript and a shorter circadian period (approximately 22 hours). Fu et al.\textsuperscript{39} reported that this mouse was more susceptible to radiation-induced malignant lymphoma and showed reduced apoptosis in thymocytes. In addition, this mouse showed temporal deregulation of cyclin D1, cyclin A, and c-myc. Circadian rhythm disruption may also play an important role in progression of cancer.\textsuperscript{40}

So far, 8 core circadian genes have been identified\textsuperscript{5}: Clock, casein kinase 1e (CK1e), cryptochrome 1 (Cry1) and cryptochrome 2 (Cry2), Period1 (Per1), Period2 (Per2) and Period3 (Per3), and Bmal1. Functional effects of mutations in most of these genes have been found in mice.\textsuperscript{5} A limited number of epidemiologic studies have examined polymorphisms in clock-related genes and phenotypes such as morning/evening preference and depressive symptoms.\textsuperscript{41} A polymorphism in the Clock gene was reported by Katzenberg et al.\textsuperscript{42} to be associated with morning/evening preference as assessed by the Horne-Östberg scale,\textsuperscript{43} a validated and widely used questionnaire to determine diurnal preference.

Associations of Per3 polymorphisms with delayed sleep-phase syndrome or diurnal preference have recently been reported.\textsuperscript{41,44} Archer et al.\textsuperscript{44} used the Horne-Östberg scale to examine a Per3 length polymorphism in which the longer allele was associated with morning preference and the shorter allele with evening preference; the shorter allele was also strongly related to delayed sleep-phase syndrome; they reported allele frequencies of 68% for the shorter allele and 32% for the longer allele. Johansson et al.\textsuperscript{41} examined a single nucleotide polymorphism and also found an association with diurnal preference.

Figure 2 depicts a possible cascade of signaling pathways from retinal light perception to effects on mammary tissue. Polymorphic variants at each stage of this process could alter the impact of light on the breast.

Diurnal preference (morning or evening) has been reported to predict tolerance to evening or overnight shift work\textsuperscript{45} and to be related to melatonin level\textsuperscript{46}; people considered to be “morning types” were less tolerant and more likely to stop such work for medical reasons. Time-of-day requirements of the work schedule may confer a different risk of breast cancer for a woman with a morning diurnal preference as assessed by the Horne-Östberg scale, or with a genetic polymorphic variant associated with morning preference, compared with women with evening preference.

**EARLY LIFE EXPOSURES**

The idea that early life experience has a large and lifelong impact on risk comes in part from the longstanding observation that there is a strong cohort pattern to breast cancer risk; that is,
successive birth cohorts of women have higher levels of risk throughout their lives\textsuperscript{47,48} beginning early in life.

In utero exposures may affect lifetime risk of breast cancer\textsuperscript{49,50} by altering normal breast development. Childhood experience may also influence risk of breast cancer later in life. The adolescent period of development may play a particularly strong role.\textsuperscript{51} In addition, factors that might raise pregnancy estrogens may be expected to increase risk in the short term, whereas factors that lower estrogen may reduce risk.\textsuperscript{52} These developmental periods may be times when the female is particularly vulnerable to circadian disruption.

CONCLUSION

Knowledge of the biology of the circadian system and of melatonin action is essential in the design and conduct of future studies. There are a number of potential mechanisms by which circadian disruption might increase risk of breast cancer. It is unknown which, if any, of these mechanisms may be acting, and they are not mutually exclusive. The original proposal that light at night from use of electric lighting might increase risk\textsuperscript{3} was based on the idea that reduced melatonin would lead to increased estrogen.\textsuperscript{5} Other potential mechanisms are: 1) melatonin may disrupt the signaling of estrogen without affecting its production\textsuperscript{53,54}; 2) melatonin may be directly oncostatic, independent of any effects on estrogen, or interactions with estrogen\textsuperscript{18,55}; 3) other attributes of melatonin such as effects on immune function\textsuperscript{56} or free radicals\textsuperscript{57} may play a role; and 4) apart from effects on melatonin, ill-timed artificial light exposure may disrupt circadian gene function in the suprachiasmatic nuclei, which then alters cell-cycle regulation in mammary tissue cells.\textsuperscript{4}

This impact on the master circadian pacemaker of the suprachiasmatic nuclei might be modified by genetic variants in aspects of the phototransduction system of the retina or by genetic polymorphisms of 1 or more of the core circadian genes. In fact, Zhu et al.\textsuperscript{58} have reported an elevated risk of breast cancer in young women associated with the length polymorphism of Per3.

Depending on melatonin’s primary physiological action in humans, its suppression may affect other diseases in addition to breast cancer. Recently described polymorphisms of the melatonin 1a receptor gene should be examined for these possible disease associations.\textsuperscript{59} Disruption of circadian gene function may have many biologic consequences, some of which are just now being investigated. Finally, epidemiologic studies of altered light exposure should take account of circadian gene polymorphisms as well as other factors such as diet (eg, alcohol\textsuperscript{60}) with which it might interact. Case-control studies are efficient for examining gene polymorphism associations with cancer, and have yielded information on shift work and nighttime light exposures at home. The methodology for assessing altered light exposure is evolving; some factors are obvious and easily estimable such as shift work occupation, whereas others are more challenging such as ambient light levels in the bedroom at night. A large prospective study, although expensive, with archived blood samples and morning urine samples would also advance this field.

REFERENCES


