Review

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Artificial light-at-night – a novel lifestyle risk factor for metabolic disorder and cancer morbidity

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Abstract: Both obesity and breast cancer are already recognized worldwide as the most common syndromes in our modern society. Currently, there is accumulating evidence from epidemiological and experimental studies suggesting that these syndromes are closely associated with circadian disruption. It has been suggested that melatonin (MLT) and the circadian clock genes both play an important role in the development of these syndromes. However, we still poorly understand the molecular mechanism underlying the association between circadian disruption and the modern health syndromes. One promising candidate is epigenetic modifications of various genes, including clock genes, circadian-related genes, oncogenes, and metabolic genes. DNA methylation is the most prominent epigenetic signaling tool for gene expression regulation induced by environmental exposures, such as artificial light-at-night (ALAN). In this review, we first provide an overview on the molecular feedback loops that generate the circadian regulation and how circadian disruption by ALAN can impose adverse impacts on public health, particularly metabolic disorders and breast cancer development. We then focus on the relation between ALAN-induced circadian disruption and both global DNA methylation and specific loci methylation in relation to obesity and breast cancer morbidities. DNA hypo-methylation and DNA hyper-methylation, are suggested as the most studied epigenetic tools for the activation and silencing of genes that regulate metabolic and monostatic responses. Finally, we discuss the potential clinical and therapeutic roles of MLT suppression and DNA methylation patterns as novel biomarkers for the early detection of metabolic disorders and breast cancer development.

Keywords: artificial light-at-night; biological rhythms; breast cancer; circadian disruption; light-emitting diodes; light pollution; melatonin; obesity.

Introduction

Since the development of the incandescent bulb by Thomas Alva Edison during the second half of the 19th century, light technology has experienced an accelerated advancement towards more energy-efficient technologies, such as the modern light-emitting diodes (LEDs). These improvements in light technology have led to a significant increase in illumination for both indoor and outdoor lighting, particularly during the night period. Accordingly, artificial light-at-night (ALAN) has become a defining feature of our modern lifestyle; it has also been increasingly recognized as a novel source of pollution and environmental risk factor for human health risks, including obesity and breast cancer [1]. The negative impacts of ALAN are mediated by disrupting the endogenous circadian clock, which is responsible for anticipating and tracking environmental temporal changes and accordingly regulates behavioral and physiological responses to meet environmental challenges [2]. At the cellular level, the mammalian circadian regulation is driven by a complex machinery of gene and protein crosstalk, through negative and positive transcription and translation feedback loops [3]. The circadian system is most sensitive to short wavelengths, such as the blue light (dominant at daytime) emitted from the LED lighting at nighttime [4]. Following modernization, humans have interrupted the natural light–dark cycle (the most reliable cue for entraining the biological clock) by introducing ALAN of short wavelengths, which is increasingly suggested as a possible public health problem [1, 5].

In the current paper, we first review the molecular machinery of the biological clock, which is responsible for generating circadian rhythms, and how ALAN interacts

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with the core machinery to disrupt these rhythms. Thereafter, we consider the association between ALAN and both obesity and cancer by discussing a possible epigenetic mechanism for mediating the adverse effects of the environmental exposure. Finally, biomarkers for obesity and cancer are suggested as a novel tool for monitoring and detecting the ALAN-induced adverse effects at the early stages in order to prevent and improve survival, particularly in hormone-dependent cancer cases.

Circadian regulation

Biological rhythms are perfectly orchestrated by environmental cues (e.g. photoperiod) to enhance survival, especially during environmental challenges [6, 7]. They also play a significant role in body homeostasis and in the temporal organization of biological responses with the changing environmental conditions [8, 9]. Photoentrainment is a nonvisual photo-response mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain the photopigment melanopsin [10]. The photoperiod signal is detected by ipRGCs and is propagated to the circadian clock in the hypothalamic suprachiasmatic nucleus (SCN) through the retino hypothalamic tract. Consequently, the SCN invigorates the synthesis and release of melatonin (MLT) from the pineal gland during the night, to the systemic circulation primarily under dark conditions. Hence, MLT is expected to be a major component of the mechanism of action, by which the circadian clocks regulate daily and seasonal rhythms in a wide array of biological functions, including energy homeostasis [6, 11, 12].

The circadian output of the clock is defined by a network of feedback loop interactions (Figure 1) between

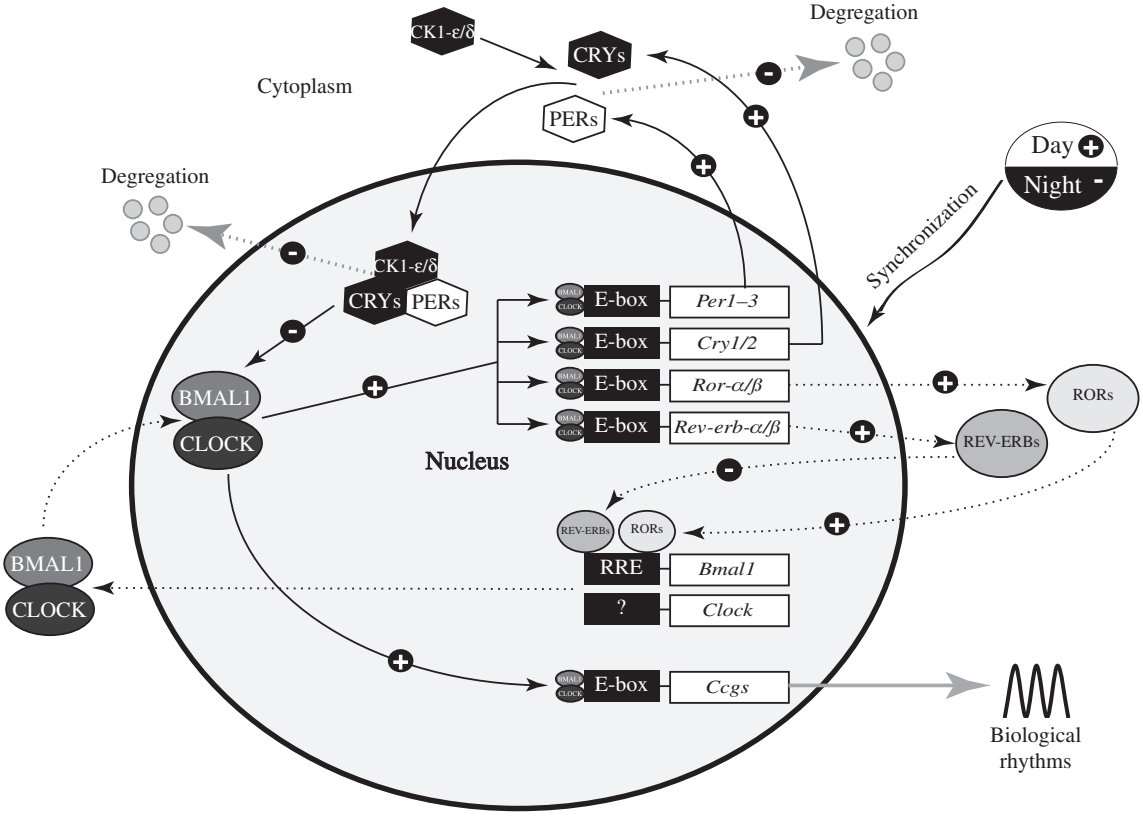

Figure 1: Simplified molecular model of the mammalian circadian clock.
Negative (–) and positive (+) transcription-translation feedback loops set the circadian rhythmicity. At the core clock (solid arrows), the transcription factors BMAL1 and CLOCK form the heterodimer to regulate their own transcription and those of other clock genes (e.g. Per and Cry families) by binding to clock E-box elements. The nuclear receptors, Rev-erb-α/β and Ror-α/β, form auxiliary (dashed arrows) feedback loops to inhibit and activate the transcription of transcription of Bmal1 and clock, respectively. Generally, these positive and negative feedback loops are synchronized by the light–dark cycle and are directly involved in the regulation of clock-controlled genes (Ccgs) that generate a wide array of circadian biological rhythms. Casein kinase 1 epsilon and delta (CK1-ε/δ) phosphorylates CRYs and PERs and finally leads to their degradation.

clock genes and their protein products [13, 14]. Overall, the core of this network consists of three *period* genes in the mouse (*Per1*, *Per2*, and *Per3*) and two *cyrptochrome Cry1* and *Cry2*, which are activated at the onset of the circadian day by a positive transcriptional feedback loop. The loop, which originates from heterodimer complexes, contains the following proteins: Circadian Locomotor Output Kaput (CLOCK), the Neuronal PAS Domain Protein 2 (NPAS2), and Brain and Muscle Arnt-like Protein 1 (BMAL1). BMAL1 hetero-dimerizes with either CLOCK or NPAS2 and acts by enhancing E-box sequences to activate the transcription of *Per1*, *Per2*, *Per3, Cry1*, and *Cry2* [3, 15]. The day activation of *Per* and *Cry* families is terminated at the onset of the circadian night by a negative translation feedback loop comprised of their protein products PER and CRY, which conjugate heteromeric complexes to block the CLOCK/NPAS2:BMAL1-induced transcription activation at the nucleus level. Subsequently, the available PER and CRY are gradually phosphorylated by casein kinase 1-epsilon and delta, disintegrated, and completely removed from the nucleus at the end of the circadian night, thus allowing a new cycle to be regenerated [16, 17].

These primary feedback loops are supported by secondary feedback arrays involving other protein complexes, which interact with the core feedback loops at different sites of action [18, 19]. The most common secondary feedback loops involve the action of the nuclear receptors, *Rev-erbα* and *Rorα/β*, which induce the activation and inhibition of the transcription of *Clock* and *Bmal1*, respectively, by their crosstalk with the retinoid-related orphan receptors [20, 21]. The post-interactions are suggested to play an important fine-tuning role in mediating reciprocated signals between the circadian clock components and physiological responses, particularly metabolism and energy balance at the cellular levels [22]. Although the SCN is the master circadian clock, the circadian feedback loop interactions are omnipresent in eventually all cells in peripheral tissues, including the heart, spleen, lung, liver, endocrine glands, and others [23, 24]. The peripheral oscillators present a very similar molecular machinery like that of the SCN, although this is regulated in a different manner compared with the central oscillator. Peripheral oscillators are not directly entrained by light signals like the SCN and present delayed circadian responses compared with those detected in the SCN, indicating that the peripheral oscillators are modulated by central signals originating from the SCN [25–27]. Together, the central and peripheral oscillators crosstalk and eventually regulate the circadian expression of genes involved in a broad spectrum of physiological responses, such as metabolic, endocrine, and immune functions [28–30].

Circadian disruption

A significant disruption of the normal entrained circadian rhythms, also called circadian disruption, is a direct consequence of our modern lifestyle, such as prolonged nocturnal activity with excessive ALAN exposure, long-term and frequent shift work, sleep deprivation (activities known as 24/7 days), and flights across different time zones [31]. Circadian disruption is persistently associated with several pathological consequences, including sleep-wake disorders [32], psychiatric disorders [33], cardiovascular diseases [34], immunological disorders [35], metabolic disorders [36], obesity [37], and cancer progression [38]. Nearly a decade ago, the International Agency for Research on Cancer of the World Health Organization (WHO) stated that shift work, which involves circadian disruption is "probably carcinogenic to human" [39]. More recently, the adverse potential of circadian disruption on human health has been anchored in a resolution passed by the American Medical Association (AMA), which states that ALAN is a source of pollution that interrupts both normal sleep patterns and daily rhythms in humans [40]. ALAN, as the most prominent circadian disruptor, vigorously ceases pineal MLT synthesis and secretion and consequently disrupts circadian rhythms [31, 41]. The melanopsin-containing ipRGCs, which regulate the pineal MLT synthesis via the SCN, are extremely sensitive to short wavelength light exposure even at low irradiance [42] and for a short period [43] as they can suppress the synthesis of the pineal hormone MLT. The spectral sensitivity of MLT synthesis suppression demonstrates wavelength-dependent sensitivity with robust effects in response to short wavelength (450–500 nm) exposure [44, 45]. Accordingly, a comprehensive study on the effect of various artificial lights on MLT suppression revealed that 4000 K and 5000 K LED lights were the most effective in the suppression of the hormone compared with counterpart technologies, such as incandescent, halogen, and florescent lightening systems [46]. Furthermore, a different study demonstrated an inverse association between MLT synthesis and the irradiances of narrowband blue LED exposure (peak $\lambda = 469$ nm; ½ peak bandwidth = 26 nm), and the impact of this spectrum was evidently larger than that of 4000 K of white florescent at twice the energy of the former [44, 47]. Finally, the negative effects of circadian disruption on public health are expected to be exacerbated by the increasing exposure to ALAN of LED illumination emitted from variant sources, including electronic screens used by people of all ages, particularly adolescents [48]. ALAN-induced circadian disruption has been related to several health risks, including overweight, obesity, and cancer [49, 50].

Epigenetic

Epigenetic modifications regulate heritable changes without any alteration in the sequence of the DNA nucleic acids. In differentiated cells, different mechanisms of epigenetic regulation exist, including histone remodeling and DNA methylation [51]. One of the most described histone modifications in terms of post-transcriptional promotion of genes expression is the acetylation of lysine of H3 and H4 N-terminal tail domains [52]. H3 acetylation at lysine 4 activates gene expression by chromatin opening, consequently making the gene more accessible to DNA-binding transcriptional factors [53]. On the contrary, H3 histone heterochromatin protein 1 methylation at lysine 9 silences gene expression by tightening the DNA warp around the histone, thereby blocking the accessibility of the gene to transcriptional factors [54]. In mammalian cells, DNA methylation is the major epigenetic modification for regulating DNA transcription to RNA, thus controlling gene expression [55]. DNA methylation involves the conversion of cytosine nucleotide to 5-methylcytosine by the attachment of a methyl group to the C5 position of cytosine bases that occur next to guanine bases, referred to as CpG sites [56]. Typically, DNA methylation within the promotor CpG sites involves transcriptional gene silencing and consequently decreased RNA synthesis by blocking both gene accessibility to both transcriptional factors and RNA polymerase [57, 58]. DNA methylation is regulated by DNA methyltransferases (DNMTs), which are a family of enzymes that regulate all types of methylation. The mammalian cells express three types of DNMTs that differ in their activity with regards to methylation status during the cell lifespan [59]. The methyltransferases DNMT3a and DNMT3b are generally responsible for setting the patterns of methylation in the cells by regulating de novo DNA methylation, whereas the activity of DNMT1 is generally related to the maintenance of the de novo methylation patterns during DNA replication [60].

DNA methylation can occur in mammalian cells in response to a wide array of environmental factors and may represent an adaptive significance to the organism in relation to environmental challenges [61]. Conversely, epigenetic modifications, such as DNA methylation and histone protein remodeling, have been reported to be involved in the generation of several pathological alterations, including obesity [62] and cancer morbidity [63].

Circadian clock and metabolism

Currently, it is becoming clear that energy homeostasis and distinct metabolic responses are the outcomes of several integrated circadian signals [49, 64, 65]. Indeed, chronobiological studies have shown that several metabolic enzymes regulating variant metabolic processes are under circadian control [66, 67]. Peripheral circadian oscillators have been demonstrated in both white and brown adipose tissues that play a prominent role in energy conservation and adaptive metabolic responses [68–70]. Additionally, normal satiety hormone levels, such as leptin [71], ghrelin [72], insulin [73], and glucagon [74], tend to exhibit circadian oscillation. Finally, functional connections between core clock genes and metabolic processes are increasingly being characterized using mutant mice. The *Per2* mutant mice exhibited severe obesity when offered a high fat diet [75]. The *Clock* knockout mice show significant metabolic abnormalities, such as mitigated feeding rhythms, increased appetite, obesity, hyperglycemia, and others [66]. Additionally, in *Clock*-deficient mice, significant increases in food intake, body mass, and body fat percentage was measured compared with counterpart normal mice [76]. Loss function of the clock gene *Bmal1* affects daily rhythms of glucose and triglyceride levels in mice [77]. Nonetheless, other clock gene mutant mice (e.g. *Bmal1* and *mPer2*) were reported to not becoming obese [78].

Meanwhile, transcriptional regulation of rate limiting-steps in energy homeostasis is a prominent factor controlling oscillated metabolic responses [79]. The conspicuous metabolic enzyme hepatic 3-hydroxy-3-methylglutarul CoA reductase (rate controlling-factor for cholesterol biosynthesis), exhibit robust diurnal variations with higher levels during the night [80]. The expression of glycogen synthase 2 (rate limiting-enzyme for glycogenesis) in the liver is under circadian control [81]. The transcriptional activation of glycogen synthase 2 is regulated by CLOCK through binding to E-box elements [82]. Moreover, the rhythmic expression of nicotinamide phosphoribosyltranferase, which regulates a significant limiting-step in the nicotinamide adenine dinucleotide (NAD+) salvage pathway (involved in oxidation-reduction reactions), is controlled by the transcriptional factors CLOCK:BMAl1 [83]. Consistently, *Clock*- and *Bmal1*-mutant mice exhibit no obvious rhythmic oscillation in the expression of Nicotinamide phosphoribosyltransferase [84]. The clock transcription components are closely involved in the regulation of several nuclear receptors, such as Retinoic acid-related orphan receptor α and Peroxisome proliferator-activated receptor [85]. These receptors are directly involved in a wide array of metabolic pathways, such as lipid, glucagon, and cholesterol metabolism [86]. Finally, the transcriptional circadian factors can interact with certain nutrient sensors that reliably reflect the

cellular energetic status, such as Sirtuin 1 and Adenosine monophosphate-activated protein kinase [86, 87]. These sensors can interact jointly or independently with key clock transcriptional factors, such as CLOCK, BMAL1, and CRY1, to modulate distinct metabolic processes, including gluconeogenesis, mitochondrial biogenesis, glycogen and fatty acid metabolism, steroid synthesis, and others [86].

LAN and circadian disruption of metabolic responses

Urbanization has resulted in immense increase in the abundance of both indoor and outdoor ALAN, causing a serious light pollution that leads to adverse consequences for human health and the environment [88]. In laboratory and non-laboratory rodents, ALAN treatment has been consistently demonstrated to be associated with metabolic disorders. ALAN treatments result in a significant reduction in daily energy expenditure and body mass of social voles (*Microtus socialis*) and laboratory rats [89, 90]. In another mice experiment, circadian disruption by dim florescent ALAN has been reported to change feeding behavior and increased mass gain [91, 92]. This contrasting effect of ALAN on body mass could be at least partially explained by the different light intensity levels used in the comparative studies; where the direction of body mass change can be directly related to light intensity levels. Furthermore, ALAN exposure disrupts circadian clock function in the pancreatic islet, causing glucose-unresponsive insulin secretion in rats [93]. Human studies also support the association between circadian disruption and metabolic diseases. Circadian disruption by shift work is strongly associated with risk of obesity, type 2 diabetes, high blood levels of glucose and triglycerides, high blood pressure, and coronary artery disease [66, 78].

Generally, the organization of energy homeostasis expresses the relations between energy intake and energy expenditure [94]. Several human and non-human studies have demonstrated that energy intake and energy expenditure are modulated at the molecular, physiological, and behavioral levels by the SCN [78, 95, 96]. The dysregulation of the circadian transcriptional factors by environment cues, such as ALAN, could compromise daily rhythms in energy intake and energy expenditure, thus causing metabolic disorders. Indeed, a direct link between core molecular clock and energy expenditure has been established. *Per2*, *Cry1*, and *Cry2* mutant mice fed with a high fat diet showed higher energy expenditure compared with wild-type mice [69]. Consistently, blocking the nuclear receptors REV-ERB-α and REV-ERB-β by synthetic agonists increases energy expenditure in mice [97].

The metabolic effects of ALAN exposure, including night shift work, are mediated by the suppression of nocturnal pineal MLT production [98, 99]. ALAN exposure prompts circadian disruption and resulted in metabolic disorders and tumor development in mice and rats, and oral MLT administration rectified the ALAN-induced adverse effects [100; Figure 2]. MLT regulates multiple clock gene expression in peripheral oscillators, such as the mouse pituitary pars tuberalis via its *mt1* receptor [101, 102]. These studies demonstrated that the transcriptional factors PER1, CRY1, CLOCK, and BMAL1 are downregulated in *mt1* knockout mice. Similar MLT-dependent expression sensitivity of clock genes in the SCN and pars tuberalis has also been suggested in the diurnal Soya sheep, *Ovis aries* [103, 104]. In animal models, MLT modifies circulating levels of insulin [105], leptin [106], ghrelin, and peptide YY [107]. In patients with chronic nonalcoholic liver disease, a significant increase in plasma levels of adiponectin, leptin, and ghrelin have been detected, in response to MLT treatment [108]. MLT can also exert its effects on metabolic processes throughout epigenetic modifications [109].

Epigenetic and metabolic disorder

The functional and molecular link between epigenetic modification and energy homeostasis has been established in the literature [110]. DNA methylation and histone modification are expected to play an influential regulatory role in diabetes and obesity development by silencing the transcription of genes associated with these metabolic disorders [111]. Obesity-related epigenetic modifications occurring throughout the adult lifespan could follow encoded blueprints in the chromatin during the embryonic early stages of development [112]. Accordingly, maternal genotype and maternal environmental exposures (e.g. smoking, alcohol, drugs, ALAN and others) can affect birth weight and set the foundation for epigenetic-related obesity risk and other metabolic disorders. Furthermore, silencing genes in obesity development can be predetermined by maternal or paternal [113] epigenetic-induced genomic imprinting. For example, birth mass can be affected by paternal imprinting of gene on chromosome 11 and chromosome 6q [114]. Likewise, the development of obesity can be associated with maternal imprinting of the Gsα gene, which encodes α subunit of the Gs-protein [115].

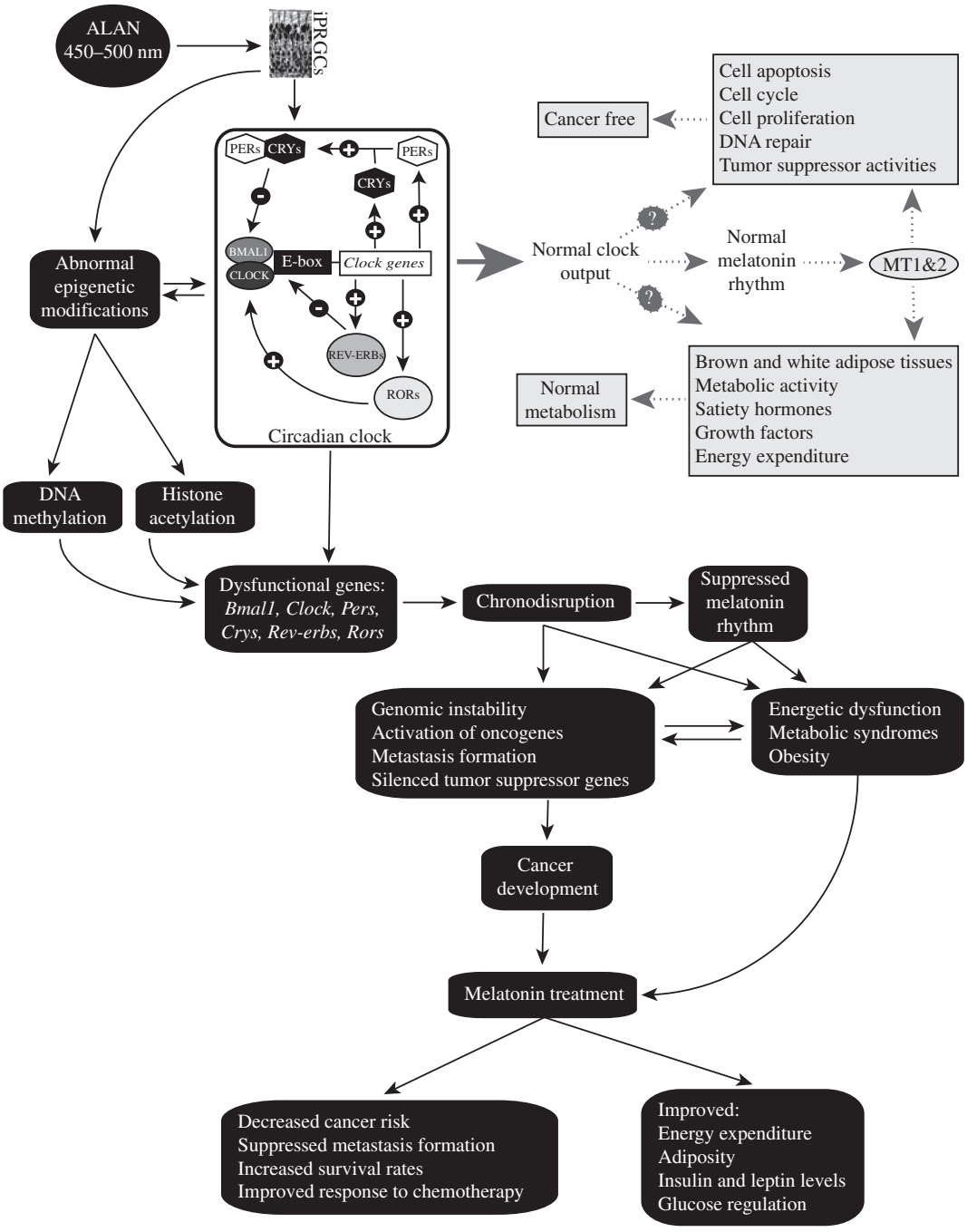

Figure 2: Schematic representation of the connections between the circadian clock and both metabolism and cancer development in mammals under normal (gray boxes) and ALAN exposure (black boxes).
ALAN short wavelength exposure is conveyed to the circadian clock at the SCN by intrinsically photosensitive retinal ganglion cells (ipRGCs). Abnormal epigenetic modifications are suggested to mediate the ALAN-induced metabolic and carcinogenic adverse effects, which might be rectified by melatonin treatment.

The clock machinery comprises distinct circadian histone remodeling at the promoters of active clock genes that are regulated via clock transcriptional factors, such as CLOCK and BMAL1. The putative epigenetic control of these transcriptional factors has been suggested to interact with certain metabolic indicators, particularly Sirtuin 1, which is a key regulator of energy homeostasis [83, 116]. Sirtuin 1 is a mammalian enzyme that deacetylates histone in response to NAD+/NADH ratios, which reflect the cell metabolic status [117]. Sirtuin 1 interacts

with CLOCK and BMAL1 to regulate rhythmic acetylation at the promoters of clock-associated genes in mouse liver [118] as well as to deacetylate and degrade PER2 [119]. Furthermore, Significant association between methylation levels at the CpG sites of *CLOCK*, *BMAL1*, and *PER2* clock genes and obesity risk has been reported in women during the mass reduction program [120].

Circadian clock and breast cancer

The circadian clock plays a pivotal role in homeostasis maintenance, and the association between circadian-disruption with homeostasis pathologies, such as cancer, is well-established [2, 121]. The core clock genes are intimately involved in controlling diverse carcinogenic activities in cancer cells, including proliferation, apoptosis, metastatic capability, and regulation of tumor-related genes [122, 123]. Accelerated tumor development in response to circadian disruption and clock gene deregulation has been recorded. Transcription upregulation of *Per1* and *Per2* blocks breast-cancer cell proliferation [124] probably by induced cell apoptosis [125]. *Per2* has been demonstrated to be a tumor suppressor gene in MCF-7 breast cancer epithelial cells [126] by linking the Estrogen-receptor-α (ER-α) to the circadian system regulating its degradation [127]. Single nucleotide polymorphism in the core clock genes *BMAL1* and *CLOCK* has been suggested to be associated with high risk of breast cancer among night work-shift Norwegian nurses [128].

In vitro knocking down the gene *CLOCK* increases the expression of a network of cancer-related genes and decreases the activity of several tumor suppressor genes related to breast cancer [129]. In the same study, researchers also noted lower expression levels of the *CLOCK* gene in women free from breast cancer. Conversely, *CRY2* shows lower expression levels in breast cancer cells compared with the counterpart normal cells [130]. A positive correlation between expression level of core clock genes and breast cancer risk has been reported for the gene *TIMLESS* in peripheral blood lymphocytes of 80 breast cancer women compared with age-matched tumor-free equal cases [131]. Researchers have also discovered a significant association between number of successive night shifts and risk of breast cancer in women who have worked night shifts with single nucleotide polymorphism in the gene *BMAL1* and related orphan receptor β (*ROR-β*), r [128]. The association between high risk of breast cancer and single nucleotide polymorphism in other core clock genes, such as *BMAL2* and *PER3* has been reported [128]. The overexpression of most core clock-genes, such as *PER1*, *PER2, PER3, CLOCK*, and *CRY2*, has been associated with significant lower metastasis formation and longer survival rates [132]. An in vitro inhibition of the circadian transcription product cryptochrome (CRY) by synthetic product, referred to as KS15, inhibited cell proliferation and increased the sensitivity to anti-cancer drug in breast cancer cells [133]. This result suggests the important role of the clock gene *CRY* in breast cancer development, whereas the overexpression of this gene inhibits the growth of breast cancer cells. In Chinese women, different mutations at the core clock genes contribute differently to the level of breast cancer risk [134].

Circadian disruption and breast cancer

Breast cancer is the most prevalent malignancy among western women and has been increasingly being associated with circadian disruption provoked by shift work, jetlag, sleep deprivation, and ALAN exposures [48, 135–137]. ALAN exposure as a prevailing feature of our modern 24/7 (h/days) active society is recognized as the most powerful factor, behind circadian disruption [138–140; Figure 2]. The evidence for a causal and direct association between ALAN-induced circadian disruption and breast cancer development comes primarily from both epidemiological observations [141–143] and experimental studies [144–146].

A comprehensive meta-analysis, based on several chronobiological studies evaluating the effects of different types of circadian disruptions (e.g. shift work, sleep deprivation, and ALAN exposure) demonstrated a direct association between circadian disruption and breast cancer risk. This risk showed a dose-dependent increase with the number of years devoted to shift work, in which 16% risk increase is estimated for every 10 years of shift work [147]. Additionally, covering night shift work for 20 years or more has been shown to be notably associated with increased risk of ER-α-related breast cancer [148]. Positive correlation between ALAN intensity (using satellite images) with breast cancer incidences has been clearly demonstrated among Israeli women across 147 communities [149] and also worldwide with approximately 70% and 50% higher risk, respectively, of breast cancer incidences at the highest ALAN intensity compared with the lowest exposure [150]. Furthermore, several observational studies have demonstrated a plausible association between bedroom chronic exposure to moderate levels of

ALAN and increased breast cancer prevalence [151–153]. A high risk for developing breast cancer relates not only to indoor levels of ALAN, but also to outdoor levels [142]. In a recent case-control study of 93 cases and 185 controls comprising Israeli women matched by age and residential area, high risk of breast cancer has been found to be linked with substantial exposure to ALAN from outdoor illumination, whereas reduced risk has been detected among women exposed to moderate bedroom ALAN without noteworthy external exposures [154]. Finally, several studies that have assessed the relation between breast cancer risk and the degree of visual impairment suggest an inverse dose-dependent relation between the two variables, with significant decrease in breast cancer risk among completely blind women [155–157].

The experimental evidence on the effect of ALAN exposure on cancer development in animal models suggest a significant association between the light exposure and breast cancer development. In nude rats, accelerated growth of MCF-7 human breast cancer xenografts has been noted in response to constant ALAN exposure compared with the counterpart rats kept under normal alternating light–dark cycle [144, 158]. In support of this finding, ALAN exposure as low as 0.2 lux has been demonstrated to be sufficient to induce tumor growth and increase tumor lipid uptake and metabolism [159]. In female Balb/c mice 4T1 mammary carcinoma model, tumor growth clearly accelerated in response to 1X30 min/day ALAN exposure (white fluorescent, 450 Lux, 469 nm) for 21 days compared with mice maintained under the same conditions, but without ALAN [145]. ALAN affected 4T1 tumor growth in a wavelength-dependent manner. Mice exposed to blue LED ($\lambda_{Dominant} = 460$ nm, 350 Lux) ALAN exposure showed higher tumor growth rates and promoted lung metastasis formation compared with incandescent ($\lambda_{Dominant} = 580$ nm) ALAN-exposed mice at the same intensity level. Furthermore, the tumor-free interval duration, from cell inoculation to the appearance of measurable tumors, was only 3 days for LED ALAN-treated mice, compared with the 5 days in ALAN incandescent counterpart group [160]. Most of the epidemiological and experimental data provide a well-established association between circadian disruption by shift work, ALAN, and breast cancer risk. However, some other studies failed to establish a clear association [161]. This case-control study evaluated the association between variant night shift factor and breast cancer incidences in Western Australia using a self-administered questionnaire and telephone interview to obtain details about demography, lifestyle, occupational history, and shift work factors, including ALAN, phase shift, and sleep duration [161]. In this study, night shift has been found to be associated with a small but statistically significant increase risk for breast cancer, but the association with other shift work factors, such as duration of exposure to ALAN and sleep disturbance, showed inconsistent results.

Epigenetic and breast cancer

In recent years, the connection between epigenetic modifications with cancer progression has been extensively studied, particularly in breast cancer. In normal cells, the promoter region of tumor-suppressive genes generally show un-methylated CpG sites, whereas the transcriptional silencing of these genes by abnormal hyper-methylation is a common feature in cancer cells [162]. Hyper-methylation has been reported in several genes in breast cancer, including *BRC1*, the most commonly investigated human gene in relation to breast cancer progression [163, 164]. *BRC1* promoter methylation in free peripheral blood DNA has been associated with a 3.5-fold increased risk of early developing breast cancer [165]. Hyper-methylation has also been reported at CpG promoter sites in other tumor suppressor genes (e.g. *CDKN2A* and *PTEN*) that are associated with breast cancer development [166]. Generally, these tumor suppressor genes are involved in different carcinogenic-related processes, such as cell cycle regulation, DNA repair, hormone/receptor-mediated signaling pathways (ER-α), cell apoptosis, and metastasis formation [167].

In long-term shift work, DNA samples extracted from whole blood of 117 Danish women showed an extinctive GDM modification in several genes including onco-related genes, at more than 5000 CpG sites [168]. The genome of breast cancer cells is typically characterized by global hypo-methylation, and this assumption is supported by results of a meta-analysis study, in which breast cancer risk has been found to be significantly higher for women with the lowest GDM compared with the counterpart group with the highest GDM levels [169]. Global hypo-methylation in white blood cells has also been reported to be related with increased risk of breast cancer [170, 171]. Global DNA hypo-methylation can promote breast cancer development via several signaling pathways, including triggering genomic instability, activation of oncogenes, and inducing metastasis formations [172, 173]. Furthermore, GDM levels revealed positive correlation with carcinogenic clinical characteristics including, stage of the cancer, tumor growth rates, and high grade [160, 174, 175].

Epigenetic modifications in core clock genes are linked with carcinogenic activity [176]. The hypo-methylation of

promoters and hyper-methylation of the core clock genes, *CLOCK* and *CRY2*, are expected to be associated with several breast cancer types [129, 130]. The transcriptional deregulation of the clock genes *PER1*, *PER2*, and *PER3*, by CpG site hyper-methylation at the promoter region has been suggested to be related to breast cancer development [177, 178]. Recently, wide genome hypo-methylation in night shift workers has been demonstrated at CpG sites of several genes, including circadian core clock genes, such as *PER3* [179].

ALAN, obesity, and cancer – the epigenetic nexus to melatonin

Melatonin and obesity

ALAN exposure, particularly of short wavelength (i.e. blue-white LED illumination) is a highly effective inhibitor of N-acetyltransferase, the rate-limiting enzyme in MLT synthesis, thus resulting in markedly lower MLT levels and increased human health risk [180; Figure 2]. In humans, ALAN-induced suppression of MLT is suggested to be associated with obesity development intermediated by reduced metabolic activity in brown adipose tissue (BAT) [181, 182]. BAT specializes in heat production, particularly in small rodents and infants, by uncoupling oxidative phosphorylation in mitochondria, mainly during cold exposure [183]. Until recently, BAT has been thought to fulfil marginal role in adult humans, but a new line of evidence support an important role for BAT in body mass regulation [184, 185]. MLT increases BAT activity, thus boosting energy expenditure and consequently decreasing body mass [186]. Both MLT receptors MT1 and MT2 are expressed in human brown and white adipose tissues, suggesting a regulatory metabolic role for the hormone in these tissues [187]. In rats, MLT treatment in the drinking water resulted in a significant decrease in body mass [188, 189]. A past study reported that abolishing the nocturnal MLT levels by pinealectomy tends to inhibit the short-day induced body mass loss in Siberian hamsters [190]. MLT treatment consistently reduced adiposity and body mass in several rodent species [182]. Several studies have demonstrated that chronic exposure to dim light at night increases body mas gain in mice, and this effect is suggested to be mediated by the ALAN-induced MLT suppression [91, 92, 191, 192]. Finally, MLT has been reported to influence several metabolic factors, such as insulin, insulin-like growth factor, growth hormone, glucose metabolism, and others, which could directly control body mass homeostasis [193].

MLT and cancer

The relationship between MLT and malignancy has been extensively studied in human and non-human models, showing an extraordinarily consistent oncostatic property of the pineal hormone. In premenopausal and postmenopausal women, urinary 6-sulfatoxymelatonin (6-SMT) levels, the major urinary metabolite of MLT [194], show an inverse relation with risk of breast cancer [195, 196]. Additionally, the inverse relationship between 6-SMT and breast cancer risk has been confirmed by other case-control studies in women [197]. In breast cancer patients, nocturnal 6-SMT levels were 48% lower compared with counterpart controls, and the metabolite levels significantly correlated negatively with tumor size [198]. An oral dose of MLT at 20 mg/day has been demonstrated to stabilize the disease progression and improve 1-year survival compared with patients treated with supportive care alone. Furthermore, MLT treatment improved the tumor response to chemotherapy and ameliorated the clinical side effect of the chemotherapy treatment [199]. In metastatic colorectal cancer, the efficiency of the chemotherapeutic drug irinotecan, enhanced by a concomitant nocturnal administration of MLT [200].

In mice inoculated with 4T1 breast cancer cells, ALAN exposure has been associated with the promotion of tumor growth compared with unexposed mice [145, 160]. In these recent studies, the addition of MLT to the drinking water blocked the accelerated tumor growth effects of ALAN. In rodent models of tumorigeneses, the metastasis formation of solid tumors increased in response to pinealectomy and induced-metastatic surgery effect, whereas pharmacological MLT administration reversed the formation [201]. In MCF-7 human breast-cancer cell lines, MLT administration reduced the tumor invasion potential [202], and in MCF-7 tumor-bearing mice, MLT lessened metastasis formation compared with counterpart untreated mice [203]. In rats bearing the estrogen receptor α positive (ERα+) human breast cancer, a concomitant administration of MLT and adriamycin has been shown to reduce tumor mass and increase both survival rates and life quality [204]. In Fibrosarcoma ascites and Ehrlich solid tumors, the effect of MLT administration on tumor growth rates, has been related to the time of the hormone administration, as stimulating and inhibiting effects have been detected in response to MLT injection in the morning and in the late afternoon, respectively [205]. Finally, MLT treatment suppressed the proliferation of ERα+ and ERα– human

breast-cancer cell lines [206]. Two membrane G-protein associated coupled receptors, MT1 and MT2, are expressed across many tissues and cells in the body and have been found to mediate most of the oncostatic responses to MLT [207]. The MLT-induced oncostatic activities in ERα+ MCF-7 human breast-cancer cells are mediated mainly by the MT1 receptor, which is coupled with several G-proteins, including $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{q}$, and $G\alpha_{11}$ [201].

At the molecular level, the involvement of MLT in tumorigenesis is mediated throughout several genes that are responsible for regulating variant cellular cancer-related activity, including apoptosis, cell cycle, cell proliferation, DNA repair, and tumor suppressor activity [208; Figure 2]. MLT can affect breast cancer cell development by blocking the linoleic acid uptake via MT1-induced cyclic adenosine monophosphate (cAMP) suppression; this promotes the downregulation of the mitogenic signaling molecule 13-hydroxyoctadecadienoic that is responsible for the activation of growth factor pathways that are essential for cells growth and survival [201]. MLT can modulate the expression patterns of different core clock genes and other circadian-related genes involved in cellular carcinogenic activities [209]. Finally, epigenetic modification is a promising molecular mechanism for metabolic and oncostatic action of MLT, in response to environmental exposure to polluting sources, such as ALAN. These reversible modifications are profoundly associated with the expression of several genes related with obesity development [112] and carcinogenic activity [210].

MLT and epigenetic modifications

Currently, there is converging evidence supporting the notion that MLT modulates energy metabolism breast cancer- and prostate cancer-related responses throug various epigenetic modifications, particularly protein histone acetylation and DNA methylation [109, 211]. MLT can modulate histone acetylation and DNA methylation [212] to regulate a wide range of several metabolic process, including energy expenditure, adiposity, insulin, and leptin levels as well as pancreatic β-cells glucose regulation [105, 213–215]. Recently, it has been demonstrated that MLT can stimulate epigenetic modification in the rat brain via histone hyper-acetylation [216], and that this effect is mediated by its *mt1* receptor [217]. MLT can also regulate epigenetic modification by inhibiting DNA methyltransferase, thereby causing global DNA hypo-methylation [212].

MLT can influence carcinogenic activity through various mechanisms, including anti-proliferative activity, inhibiting synthesis of steroids, interacting with ERα to affect cellular actions of estrogen, modulating cancer-related gene expression, mediating apoptosis, blocking tumor metabolic processes, and anti-metastatic activity [206]. In vitro MLT-treated C17.2 neural stem cells, noticeably showed both increased mRNA expression of several histone deacetylase and histone h3 acetylation [217]. In MLT-treated MCF-7 cell lines, the induced increase in DNA methylation levels has been associated with the downregulation of the oncogenes EGR3 (early growth responsive gene 3) and POU4F2/Brn-3b; in comparison, the transcription levels of the tumor and metastasis suppressor gene glypican-3 (GPC-3) increased [218]. Furthermore, in estrogen-receptor-related breast cancer, MLT has been found to decrease expression levels of the aromatase gene (CYP19), which are responsible for the activity status of estrogens plausibly via methylation of the CYP19 gene or deacetylation of CYP19 histone at the promotor level [109]. Under both blue-LED and florescent ALAN exposures, Blab/c 4T1 breast-cancer cell tumor-inoculated female mice clearly demonstrated decreased GDM levels and increased tumor growth rates, and both were reversed by the addition of MLT to the drinking water during nighttime [145, 160]. Overall, these findings suggest that MLT can induce epigenetic modifications of several metabolic- and carcinogenic-related genes, subsequently rendering them active or inactive by histone acetylation or DNA methylation.

Figure 3 proposes a signaling pathway for ALAN-induced metabolic and carcinogenic syndromes. ALAN exposure, particularly short-wavelength exposure, has been detected by iPRGCs and thereafter the exposure signal has been conveyed to the SCN. As a result, pineal MLT production is suppressed and global DNA hypomethylation developed in several genes in response to decreased DNMTs activity. The aberrant methylation in these genes, including core clock genes, can advance their silencing or activation, and thus prompt metabolic and carcinogenic syndromes. Supportively, a recent review of the signaling pathway linking between the ALAN-induced MLT suppression and breast cancer progression in human and non-human models, has implied the occurrence of epigenetic modifications, particularly GDM as a potential molecular mechanism linking environmental exposures (e.g. ALAN) and adverse health outcomes [219]. According to this review, MLT levels are regulated by ipRGCs, which control the inhibition of the pineal hormone in response to short wavelength ALAN and consequently advance tumorigenesis mediated by epigenetic modification, such as DNA methylation. Considering the suggested association between obesity and the development of variant cancers

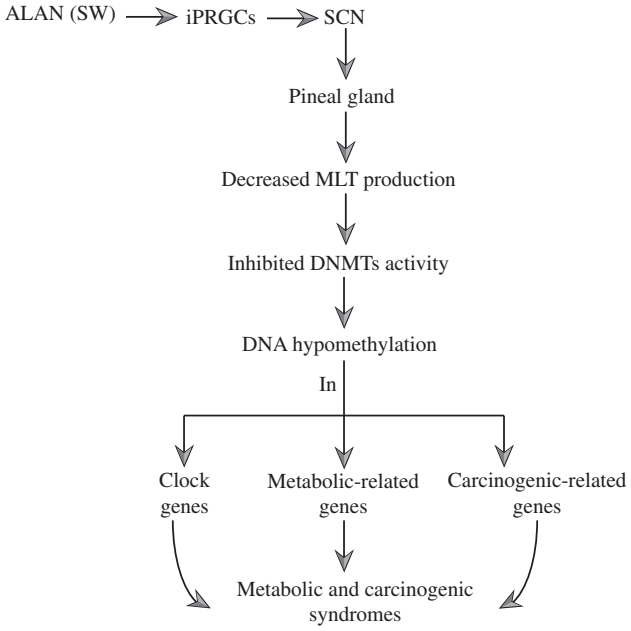

Figure 3: ALAN signaling pathway.
ALAN exposure of short wavelength (SW) are detected by intrinsically photosensitive retinal ganglion cells that transfer the signal to the SCN and the pineal MLT levels decrease rapidly. Consequently, the activity of DNA methyltransferases (DNMTs) is inhibited, and global DNA hypomethylation occurs in several genes that control metabolic and carcinogenic activities, including core clock genes. A direct signaling of MLT to the cells is also plausible.

[220], the suggested nexus between ALAN, MLT suppression, epigenetic modifications, and adverse health consequences [219] is of significant clinical importance.

Circadian disruption biomarkers in obesity and cancer research

The most important step in the diagnosis of circadian disruption is to establish the daily rhythm of MLT levels. Generally, the indole hormone rhythm is highly sensitive to every type of circadian disruption, including sleep disorder, jetlag, shift work, and most important ALAN [48, 137]. MLT levels, measured as 6-SMT, have been found to be significantly reduced in night shift workers compared with counterpart day workers, and the effect correlates positively with the intensity of ALAN and the number of nights worked [221]. Changing the sleep-wake cycle and ALAN resulted in phase advance of the MLT rhythm, which also reduced the circadian amplitude of the hormone in healthy men [222]. MLT suppression by ALAN has a dose-dependent response to both irradiance level and duration of the exposure [223]. Furthermore, the phase advance and suppression in MLT rhythms are intimately wavelength-dependent with more manifested effect at short wavelengths compared with long wavelengths [44, 45, 224]. Finally, the ALAN-induced MLT suppression is not age-related [225]. MLT levels can be directly determined virtually in all body fluids, including blood, saliva, and urine, using straightforward non-invasive methods. The most pervasive protocols for determination of MLT in blood, urine, and saliva are radioimmunoassay and enzyme-linked immunoassay [194]. The major MLT metabolite in urine, 6-SMT, reliable reflects pineal MLT secretion in human and the metabolite circadian rhythm can faithfully constitute a clinical diagnosis of circadian disruption-related disorders [226].

In the last few years, epigenetic modifications have been the focus of intensive research effort due to their potential application as biomarkers for various health disorders, including obesity and cancer [227]. DNA methylation is a significant candidate for biomarker development, because the methylated CpG sites present high stability compared with high mutation variability in any given type of cancer [228]. DNA hyper-methylation can silence genes that are profoundly involved in the secretion of insulin and glucagon [229]. Patients with type 2 diabetes express altered GDM in skeletal muscles, adipose tissue, and pancreatic islets [230]. DNA methylation of one CpG site at the promotor glocagone-like-peptide-1 (GLP1R) receptor gene decreases the transcription levels of GLP1R and correlated positively with the body mass index [231]. Increased methylation at the promoter within the tumor necrosis factor-α (TNF-α) cytokine shows direct correlation with expression level and body mass lose [232]. Additionally, higher DNA methylation at the promoter of the adrenoreceptor-β-3 gene, which encodes protein belonging to the β-adrenergic receptor family and expressed mainly in adipose tissues, are associated with obesity and other related disorders [233]. In obese children, DNA methylation levels at two CpG sites within the promoter of the Fas apoptotic inhibitory molecule 2 gene are clearly associated with obesity [234].

Likewise, abnormalities in epigenetic activity are expected to be evident long before the cancer becomes asymptomatic and impose fatal health risk [235]. Therefore, epigenetic modifications, particularly DNA methylation levels, are of great clinical importance as a non-invasive tool for early detection and staging of cancer. *BRC1* promoter methylation is closely associated with increased risk for early development of breast cancer [165]. In invasive ductal carcinoma, the gene *GHSR* (Growth hormone secretagogue receptor), a member of

the G-protein coupled receptor family, which binds to gherlin to regulate energy homeostasis and body mass, exhibited higher methylation levels compared with adjacent normal tissues [236]. Few years ago, a genome-wide methylated study demonstrated MCF-7 breast-cancer cell lines that are characterized by both extensive global DNA hypo-methylation and more clustered hyper-methylation at CpG-rich specific loci [237]. In breast cancer, aberrant global methylation is associated with genomic instability and increased activity of oncogenes [238], whereas hyper methylation at promoter CpG sites advance chromatin closing and further silencing of tumor suppressor genes, cell proliferation, and tumor development [167]. Furthermore, promoter hyper-methylation of breast cancer oncogenes tends to inhibit growth regulatory genes, resulting in irregular cell division, whereas global DNA hypo-methylation can stimulate the expression of metastatic genes that are required for cancer cells dissemination [239]. Recently, a whole genome methylation analysis on cell free circulating DNA detected seven novel markers presenting frequent and high levels of methylation in both ER-positive and ER-negative cancer samples compared with low levels in counterpart normal samples [240]. These markers included *AKR2B2*, *COL6A2*, *GPX7*, *HIST1H3C*, *HOXB4*, *RASGRF2*, and *TM6SF1*, for which the specificity and sensitivity of the screening analysis has been found to be ~96%. We can use these novel markers as a predictive and monitoring tool in order to assess the efficiency of treatment response. In another research that studied DNA methylation in circulating free DNA, clear hyper-methylation has been demonstrated in the promoter of the tumor suppressor genes *ITIH5*, *DKK3*, and *RASSF1A*, with high sensitivity (50%–100%) to discriminate cancer patients from healthy and benign controls [241]. The methylation levels at the promotor of the tumor suppressor genes *BCAP31* and *OGG1* have been found to be inversely associated with survival rates and prognosis in breast cancer patients [242]. Finally, the association between promoter hyper-methylation of tumor suppressor genes and breast cancer development has also been demonstrated in other studies [243, 244].

Concluding remarks

Light pollution has become a defining feature of human lifestyle mainly in urban areas. ALAN and rotating shift work are increasingly becoming major sources of circadian disruption that subsequently cause serious health threats as well as ecological impacts. Currently, metabolic syndrome, diabetes, obesity, and cancer have been experimentally and observationally associated with the misregulation of the transcriptional-translational feedback loops of the mammalian clock components. MLT is a leading candidate for mediating the putative ALAN-induced circadian disruption at the molecular, hormonal, and epigenetic levels. Therefore, MLT serves as an operational biomarker for circadian disruption and a promising chronobiotic drug target for circadian disruption-related diseases [245]. Enhancement of the circadian amplitude by pharmalogical agents, such as Nobiletin, has been found to ameliorate circadian disruption-related syndromes, such as obesity and metabolic disorders [246]. The Nobiletin signal transduction is suggested to be directly mediated by retinoid, which is an acid receptor related-orphan receptor that modulates core circadian clock genes, thus enhancing the circadian amplitude to moderate the pathological consequences of disruption of the circadian rhythms. Accordingly, MLT and other proven chronobiotic compounds can be utilized to rectify circadian disruption and other related diseases, particularly cancer, obesity, and metabolic disorders.

Aberrant epigenetic modifications are increasingly being associated with both obesity and cancer development. These reversible modifications can be developed at early embryonic stages and can even continue to present during later life. Furthermore, epigenetic modifications can provide a simple and non-invasive monitoring tool for circadian disruption, which can lead to the development of public health risks. In breast cancer, improving survival rates by diagnosis at early stages seems possible, as the rates are not encouraging after metastasis formation [247]. Consequently, the early detection of metabolic disorders and breast cancer development induced by circadian disruption, particularly by ALAN, provide a promising opportunity for individuals of a risk group to control the disease development by simply changing their habitual lifestyles. Further studies are warranted to address other intervention measures to counteract circadian disruption-related health risks, such as developing environmentally friendly illumination, improving circadian adaptation in rotating shift workers, and developing chronobiotic drugs that can modulate the circadian clock and prevent circadian disruption-related diseases.

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References

1. Stevens RG, Zhu Y. Electric light, particularly at night, disrupts human circadian rhythmicity: is that a problem? Philos Trans R Soc Lond B Biol Sci 2015;370:1–9.
2. Sollars PJ, Pickard GE. The neurobiology of circadian rhythms. Psychiatr Clin North Am 2015;38:645–65.
3. Fuhr L, Abreu M, Pett P, Relógio A. Circadian systems biology: when time matters. Comput Struct Biotechnol J 2015;13:417–26.
4. Haim A, Portnov BA. Light pollution as a new risk factor for human breast and prostate cancers. Dordrecht, The Netherlands: Springer Netherlands, 2013.
5. Wright KP Jr, McHill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED. Entrainment of the human circadian clock to the natural light–dark cycle. Curr Biol 2013;23:1554–8.
6. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. Annu Rev Neurosci 2012;35:445–62.
7. Frank E, Sidor MM, Gamble KL, Cirelli C, Sharkey KM, Hoyle N, et al. Circadian clocks, brain function, and development. Ann N Y Acad Sci 2013;1306:43–67.
8. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature 2002;418:935–41.
9. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet 2005;6:544–56.
10. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, et al. Measuring and using light in the melanopsin age. Trends Neurosci 2014;37:1–9.
11. Pevet P, Challet E. Melatonin: both master clock output and internal time–giver in the circadian clocks network. J Physiol Paris 2011;105:170–82.
12. Kitazawa M. Circadian rhythms, metabolism, and insulin sensitivity: transcriptional networks in animal models. Curr Diab Rep 2013;13:223–8.
13. Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006;15:R271–7.
14. Debruyne JP. Oscillating perceptions: the ups and downs of the CLOCK protein in the mouse circadian system. J Genet 2008;87:437–46.
15. Parekh PK, McClung CA. Circadian mechanisms underlying reward–related neurophysiology and synaptic plasticity. Front Psychiatry 2016;6:187.
16. Eide EJ, Woolf MF, Kang H, Woolf P, Hurst W, Camacho F, et al. Control of mammalian circadian rhythm by CKIepsilon-regulated proteasome-mediated PER2 degradation. Mol Cell Biol 2005;25:2795–807.
17. Virshup DM, Eide EJ, Forger DB, Gallego M, Harnish EV. Reversible protein phosphorylation regulates circadian rhythms. Cold Spring Harb Symp Quant Biol 2007;72:413–20.
18. Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, et al. Dec1 and Dec2 are regulators of the mammalian molecular clock. Nature 2002;419:841–4.
19. Ueda HR, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, et al. Systemlevel identification of transcriptional circuits underlying mammalian circadian clocks. Nat Genet 2005;37:187–92.
20. Ripperger JA, Albrecht U. REV-ERB-erating nuclear receptor functions in circadian metabolism and physiology. Cell Res 2012;22:1319–21.
21. Zhao X, Cho H, Yu RT, Atkins AR, Downes M, Evans RM, et al. Nuclear receptors rock around the clock. EMBO Rep 2014;15:518–28.
22. Duguay D, Cermakian N. The crosstalk between physiology and circadian clock proteins. Chronobiol Int 209;26:1479–513.
23. Fukuhara C, Tosini G. Peripheral circadian oscillators and their rhythmic regulation. Front Biosci 2003;8:d642–51.
24. Cuninkova L, Brown SA. Peripheral circadian oscillators: interesting mechanisms and powerful tools. Ann N Y Acad Sci 2008;1129:358–70.
25. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 2010;72:517–49.
26. Brown SA, Azzi A. Peripheral circadian oscillators in mammals. Handb Exp Pharmacol 2013;217:45–66.
27. Schibler U, Gotic I, Saini C, Gos P, Curie T, Emmenegger Y, et al. Clock-talk: interactions between central and peripheral circadian oscillators in mammals. Cold Spring Harb Symp Quant Biol 2015 80:223–32.
28. Hastings M, O'Neill J S, Maywood ES. Circadian clocks: regulators of endocrine and metabolic rhythms. J Endocrinol 2007;195:187–98.
29. Zanquetta MM, Corrêa-Giannella ML, Monteiro MB, Villares SM. Body weight, metabolism and clock genes. Diabetol Metab Syndr 2010;2:53.
30. Cermakian N, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, et al. Crosstalk between the circadian clock circuitry and the immune system. Chronobiol Int 2013;30:870–88.
31. Reiter RJ, Rosales-Corral S, Coto-Montes A, Boga JA, Tan DX, Davis JM, et al. The photoperiod, circadian regulation and chronodisruption: the requisite interplay between the suprachiasmatic nuclei and the pineal and gut melatonin. J Physiol Pharmacol 2011;62:269–74.
32. Abbott SM, Reid KJ, Zee PC. Circadian rhythm sleepwake disorders. Psychiatr Clin North Am 2015;38:805–23.
33. Jones SG, Benca RM. Circadian disruption in psychiatric disorders. Sleep Med Clin 2015;10:481–93.
34. Alibhai FJ, Tsimakouridze EV, Reitz CJ, Pyle WG, Martino TA. Consequences of circadian and sleep disturbances for the cardiovascular system. Can J Cardiol 2015;31:860–72.
35. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol 2010;185:5796–805.
36. Reinke H, Asher G. Circadian clock control of liver metabolic functions. Gastroenterology 2016;150:574–80.
37. Laermans J, Depoortere I. Chronobesity: role of the circadian system in the obesity epidemic. Obes Rev 2016;17:108–25.
38. Sigurdardottir LG, Valdimarsdottir UA, Fall K, Rider JR, Lockley SW, Schernhammer E, et al. Circadian disruption, sleep loss, and prostate cancer risk: a systematic review of epidemiologic studies. Cancer Epidemiol Biomarkers Prev 2012;21:1002–11.
39. Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol 2007;8:1065–6.

40. AMA. AMA adopts new policies at annual meeting. 2012. Available at: http://www.ama-assn.org/resources/doc/csaph/x-pub/a12-csaph4-lightpollution-summary.pdf. Accessed 20 February 2016.
41. Davis S, Mirick DK. Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. Cancer Causes Control 2006;17:539–45.
42. Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab 2015;90:1311–6.
43. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol 2001;535:261–7.
44. Erren TC, Pape HG, Reiter RJ, Piekarski C. Chronodisruption and cancer. Naturwissenschaften 2008;95:367–82.
45. Falchi F, Cinzano P, Elvidge CD, Keith DM, Haim A. Limiting the impact of light pollution on human health, environment and stellar visibility. J Environ Manage 2011;92:2714–22.
46. Aubé M, Roby J, Kocifaj M. Evaluating potential spectral impacts of various artificial lights on melatonin suppression, photosynthesis, and star visibility. PLoS One 2013;8:e67798.
47. West KE, Jablonski MR, Warfield B, Cecil KS, James M, Ayers MA, et al. Blue light from light-emitting diodes elicits a dose-dependent suppression of melatonin in humans. J Appl Physiol 2011;110:619–26.
48. Smolensky MH, Sackett-Lundeen LL, Portaluppi F. Nocturnal light pollution and underexposure to daytime sunlight: complementary mechanisms of circadian disruption and related diseases. Chronobiol Int 2015;32:1029–48.
49. Froy O. Circadian rhythms and obesity in mammals. ISRN Obes 2012;2012:437198.
50. Savvidis C, Koutsilieris M. Circadian rhythm disruption in cancer biology. Mol Med 2012;18:1249–60.
51. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev 2009;23:781–3.
52. An W. Histone acetylation and methylation: combinatorial players for transcriptional regulation. Subcell Biochem 2007;41:351–69.
53. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, et al. Active genes are tri–methylated at K4 of histone H3. Nature 2002;419:407–11.
54. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 2001;410:120–4.
55. Mazzio EA, Soliman KF. Basic concepts of epigenetics: impact of environmental signals on gene expression. Epigenetics 2012;7:119–30.
56. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev 2011;25:1010–22.
57. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484–92.
58. Long HK, Blackledge NP, Klose RJ. ZF-CxxC domain–containing proteins, CpG islands and the chromatin connection. Biochem Soc Trans 2013;41:727–40.
59. Du J, Johnson LM, Jacobsen SE, Patel DJ. DNA methylation pathways and their crosstalk with histone methylation. Nat Rev Mol Cell Biol 2015;16:519–32.
60. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology 2013;38:23–38.
61. Reik W, Dean W. DNA methylation and mammalian epigenetics. Electrophoresis 2001;22:2838–43.
62. Smith CJ, Ryckman KK. Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome. Diabetes Metab Syndr Obes 2015;8:295–302.
63. Martinez-Cardús A, Vizoso M, Moran S, Manzano JL. Epigenetic mechanisms involved in melanoma pathogenesis and chemoresistance. Ann Transl Med 2015;3:209.
64. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. Science 2010;330:1349–54.
65. Garaulet M, Madrid JA. Chronobiological aspects of nutrition, metabolic syndrome and obesity. Adv Drug Deliv Rev 2010;62:967–78.
66. Eckel-Mahan K, Sassone-Corsi P. Metabolism and the circadian clock converge. Physiol Rev 2013;93:107–35.
67. Lee Y, Kim EK. AMP-activated protein kinase as a key molecular link between metabolism and clockwork. Exp Mol Med 2013;45:e33.
68. Johnston JD. Adipose circadian rhythms: translating cellular and animal studies to human physiology. Mol Cell Endocrinol 2012;349:45–50.
69. Shostak A, Husse J, Oster H. Circadian regulation of adipose function. Adipocyte 2013;2:201–6.
70. Yoshino J, Klein S. A novel link between circadian clocks and adipose tissue energy metabolism. Diabetes 2013;62:2175–7.
71. Gómez-Abellán P, Gómez-Santos C, Madrid JA, Milagro FI, Campion J, Martínez JA, et al. Site-specific circadian expression of leptin and its receptor in human adipose tissue. Nutr Hosp 2011;26:1394–401.
72. Bodosi B, Gardi J, Hajdu I, Szentirmai E, Obal F Jr, Krueger JM. Rhythms of ghrelin, leptin, and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. Am J Physiol Regul Integr Comp Physiol 2004;287:R1071–9.
73. Coomans CP, van den Berg SA, Lucassen EA, Houben T, Pronk AC, van der Spek RD, et al. The suprachiasmatic nucleus controls circadian energy metabolism and hepatic insulin sensitivity. Diabetes 2013;62:1102–8.
74. Vieira E, Marroquí L, Figueroa AL, Merino B, Fernandez-Ruiz R, Nadal A, et al. Involvement of the clock gene Rev-erb alpha in the regulation of glucagon secretion in pancreatic alpha-cells. PLoS One 2013;8:e69939.
75. Yang S, Liu A, Weidenhammer A, Cooksey RC, McClain D, Kim MK, et al. The role of mPer2 clock gene in glucocorticoid and feeding rhythms. Endocrinology 2009;150:2153–60.
76. Shostak A, Meyer-Kovac J, Oster H. Circadian regulation of lipid mobilization in white adipose tissues. Diabetes 2013;62:2195–203.
77. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2004;2:e377.
78. Williams DL, Schwartz MW. Out of synch: clock mutation causes obesity in mice. Cell Metab 2005;1:355–6.
79. Holzberg D, Albrecht U. The circadian clock: a manager of biochemical processes within the organism. J Neuroendocrinol 2003;15:339–43.
80. Brown MS, Goldstein JL, Dietschy JM. Active and inactive forms of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver of the rat. Comparison with the rate of cholesterol synthesis in different physiological states. J Biol Chem 1979;254:5144–9.

81. Ferrer JC, Favre C, Gomis RR, Fernández-Novell JM, García-Rocha M, de la Iglesia N, et al. Control of glycogen deposition. FEBS Lett 2003;546:127–32.
82. Doi R, Oishi K, Ishida N. CLOCK regulates circadian rhythms of hepatic glycogen synthesis through transcriptional activation of Gys2. J Biol Chem 2010;285:22114–21.
83. Bellet MM, Orozco-Solis R, Sahar S, Eckel-Mahan K, Sassone-Corsi P. The time of metabolism: NAD+, SIRT1, and the circadian clock. Cold Spring Harb Symp Quant Biol 2011;76: 31–8.
84. Imai S. "Clocks" in the NAD World: NAD as a metabolic oscillator for the regulation of metabolism and aging. Biochim Biophys Acta 2010;1804:1584–90.
85. Sahar S, Sassone-Corsi P. Regulation of metabolism: the circadian clock dictates the time. Trends Endocrinol Metab 2012;23:1–8.
86. Fan W, Downes M, Atkins A, Yu R, Evans RM. Nuclear receptors and AMPK: resetting metabolism. Cold Spring Harb Symp Quant Biol 2011;76:17–22.
87. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 2010;5: 253–95.
88. Bedrosian TA, Nelson RJ. Influence of the modern light environment on mood. Mol Psychiatry 2013;18:751–7.
89. Cos S, Mediavilla D, Martínez-Campa C, González A, Alonso-González C, Sánchez-Barceló EJ. Exposure to light-at-night increases the growth of DMBA–induced mammary adenocarcinomas in rats. Cancer Lett 2006;235:266–71.
90. Zubidat AE, Nelson RJ, Haim A. Spectral and duration sensitivity to light-at-night in 'blind' and sighted rodent species. J Exp Biol 2011;214:3206–17.
91. Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, et al. Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci USA 2010;107:18664–9.
92. Fonken LK, Aubrecht TG, Meléndez-Fernández OH, Weil ZM, Nelson RJ. Dim light at night disrupts molecular circadian rhythms and increases body weight. J Biol Rhythms 2013;28:262–71.
93. Qian J, Block GD, Colwell CS, Matveyenko AV. Consequences of exposure to light at night on the pancreatic islet circadian clock and function in rats. Diabetes 2013;62:3469–78.
94. Flier JS, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. Cell 1998;92:437–40.
95. Jéquier E, Tappy L. Regulation of body weight in humans. Physiol Rev 1999;79:451–80.
96. Morgan PJ, Ross AW, Mercer JG, Barrett P. Photoperiodic programming of body weight through the neuroendocrine hypothalamus. J Endocrinol 2003;177:27–34.
97. Solt LA, Wang Y, Banerjee S, Hughes T, Kojetin DJ, Lundasen T, et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 2012;485:62–8.
98. Navara KJ, Nelson RJ. The dark side of light at night: physiological, epidemiological, and ecological consequences. J Pineal Res 2007;43:215–24.
99. Dumont M, Lanctôt V, Cadieux-Viau R, Paquet J. Melatonin production and light exposure of rotating night workers. Chronobiol Int 2012;29:203–10.
100. Anisimov VN, Vinogradova IA, Panchenko AV, Popovich IG, Zabezhinski MA. Light-at-night-induced circadian disruption, cancer and aging. Curr Aging Sci 2012;5:170–7.
101. Jilg A, Moek J, Weaver DR, Korf HW, Stehle JH, von Gall C. Rhythms in clock proteins in the mouse pars tuberalis depend on MT1 melatonin receptor signalling. Eur J Neurosci 2005;22:2845–54.
102. von Gall C, Weaver DR, Moek J, Jilg A, Stehle JH, Korf HW. Melatonin plays a crucial role in the regulation of rhythmic clock gene expression in the mouse pars tuberalis. Ann N Y Acad Sci 2005;1040:508–11.
103. Lincoln G, Messager S, Andersson H, Hazlerigg D. Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. Proc Natl Acad Sci USA 2002;99:13890–5.
104. West A, Dupré SM, Yu L, Paton IR, Miedzinska K, McNeilly AS, et al. Npas4 is activated by melatonin, and drives the clock gene Cry1 in the ovine pars tuberalis. Mol Endocrinol 2013;27:979–89.
105. Peschke E, Schucht H, Mühlbauer E. Long-term enteral administration of melatonin reduces plasma insulin and increases expression of pineal insulin receptors in both Wistar and type 2-diabetic Goto-Kakizaki rats. J Pineal Res 2010;49:373–81.
106. Kus I, Sarsilmaz M, Colakoglu N, Kukne A, Ozen OA, Yilmaz B, et al. Pinealectomy increases and exogenous melatonin decreases leptin production in rat anterior pituitary cells: an immunohistochemical study. Physiol Res 2004;53:403–8.
107. Aydin M, Canpolat S, Kuloğlu T, Yasar A, Colakoglu N, Kelestimur H. Effects of pinealectomy and exogenous melatonin on ghrelin and peptide YY in gastrointestinal system and neuropeptide Y in hypothalamic arcuate nucleus: immunohistochemical studies in male rats. Regul Pept 2008;146: 197–203.
108. Gonciarz M, Bielański W, Partyka R, Brzozowski T, Konturek PC, Eszyk J, et al. Plasma insulin, leptin, adiponectin, resistin, ghrelin, and melatonin in nonalcoholic steatohepatitis patients treated with melatonin. J Pineal Res 2013;54:154–61.
109. Korkmaz A, Topal T, Tan DX, Reiter RJ. Role of melatonin in metabolic regulation. Rev Endocr Metb Disord 2009;10: 261–70.
110. Bellet MM, Sassone-Corsi P. Mammalian circadian clock and metabolism – the epigenetic link. J Cell Sci 2010;123:3837–48.
111. Drong AW, Lindgren CM, McCarthy MI. The genetic and epigenetic basis of type 2 diabetes and obesity. Clin Pharmacol Ther 2012;92:707–15.
112. Herrera BM, Keildson S, Lindgren CM. Genetics and epigenetics of obesity. Maturitas 2011;69:41–9.
113. Sullivan EL, Grove KL. Metabolic imprinting in obesity. Forum Nutr 2009;63:186–94.
114. Rao KR, Lal N, Giridharan NV. Genetic and epigenetic approach to human obesity. Indian J Med Res 2014;140:589–603.
115. Weinstein LS, Xie T, Qasem A, Wang J, Chen M. The role of GNAS and other imprinted genes in the development of obesity. Int J Obes 2010;34:6–17.
116. Orozco-Solis R, Sassone-Corsi P. Epigenetic control and the circadian clock: linking metabolism to neuronal responses. Neuroscience 2014;264:76–87.
117. Nogueiras R, Habegger KM, Chaudhary N, Finan B, Banks AS, Dietrich MO, et al. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. Physiol Rev 2012;92:1479–514.
118. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, et al. The NAD+-dependent deacetylase

SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 2008;134:329–40.
119. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 2008;134:317–28.
120. Milagro FI, Gómez-Abellán P, Campión J, Martínez JA, Ordovás JM, Garaulet M. CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. Chronobiol Int 2012;29:1180–94.
121. Dudek M, Gossan N, Yang N, Im HJ, Ruckshanthi JP, Yoshitane H, et al. The chondrocyte clock gene Bmal1 controls cartilage homeostasis and integrity. J Clin Invest 2016;126:365–76.
122. Uth K, Sleigh R. Deregulation of the circadian clock constitutes a significantfactor in tumorigenesis: a clockwork cancer. Part II. In vivo studies. Biotechnol Biotechnol Equip 2014;28: 379–86.
123. Soták M, Sumová A, Pácha J. Cross-talk between the circadian clock and the cell cycle in cancer. Ann Med 2014;46:221–32.
124. Yang X, Wood PA, Ansell C. Circadian time-dependent tumor suppressor function of period genes. Integr Cancer Ther 2009;8:309–16.
125. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. Mol Cell 2006;22:375–82.
126. Xiang S, Coffelt SB, Mao L, Yuan L, Cheng Q, Hill SM. Period-2: a tumor suppressor gene in breast cancer. J Circadian Rhythms 2008;6:4.
127. Gery S, Virk RK, Chumakov K, Yu A, Koeffler HP. The clock gene Per2 links the circadian system to the estrogen receptor. Oncogene 2007;26:7916–20.
128. Zienolddiny S, Haugen A, Lie JA, Kjuus H, Anmarkrud KH, Kjærheim K. Analysis of polymorphisms in the circadian-related genes and breast cancer risk in Norwegian nurses working night shifts. Breast Cancer Res 2013;15:R53.
129. Hoffman AE, Yi CH, Zheng T, Stevens RG, Leaderer D, Zhang Y, et al. CLOCK in breast tumorigenesis: genetic, epigenetic, and transcriptional profiling analyses. Cancer Res 2010;70: 1459–68.
130. Hoffman AE, Zheng T, Yi CH, Stevens RG, Ba Y, Zhang Y. The core circadian gene Cryptochrome 2 influences breast cancer risk, possibly by mediating hormone signaling. Cancer Prev Res (Phila) 2010;3:539–48.
131. Fu A, Leaderer D, Zheng T, Hoffman AE, Stevens RG, Zhu Y. Genetic and epigenetic associations of circadian gene TIMELESS and breast cancer risk. Mol Carcinog 2012;51:923–9.
132. Cadenas C, van de Sandt L, Edlund K, Lohr M, Hellwig B, Marchan R, et al. Loss of circadian clock gene expression is associated with tumor progression in breast cancer. Cell Cycle 2014;13:3282–91.
133. Chun SK, Chung S, Kim HD, Lee JH, Jang J, Kim J, et al. A synthetic cryptochrome inhibitor induces anti-proliferative effects and increases chemosensitivity in human breast cancer cells. Biochem Biophys Res Commun 2015;467:441–6.
134. Dai H, Zhang L, Cao M, Song F, Zheng H, Zhu X, et al. The role of polymorphisms in circadian pathway genes in breast tumorigenesis. Breast Cancer Res Treat 2011;127:531–40.
135. Harrington M. Location, location, location: important for jet-lagged circadian loops. J Clin Invest 2010;120:2265–7.
136. Haus EL, Smolensky MH. Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. Sleep Med Rev 2012;17:273–84.
137. Wang P, Ren FM, Lin Y, Su FX, Jia WH, Su XF, et al. Night-shift work, sleep duration, daytime napping, and breast cancer risk. Sleep Med 2015;16:462–8.
138. Fonken LK, Nelson RJ. The effects of light at night on circadian clocks and metabolism. Endocr Rev 2014;35:648–70.
139. Cho Y, Ryu SH, Lee BR, Kim KH, Lee E, Choi J. Effects of artificial light at night on human health: a literature review of observational and experimental studies applied to exposure assessment. Chronobiol Int 2015;32:1294–310.
140. Dominoni DM, Borniger JC, Nelson RJ. Light at night, clocks and health: from humans to wild organisms. Biol Lett 2016:12: pii: 20160015.
141. Bauer SE, Wagner SE, Burch J, Bayakly R, Vena JE. A case–referent study: light at night and breast cancer risk in Georgia. Int J Health Geogr 2013;12:23.
142. Hurley S, Goldberg D, Nelson D, Hertz A, Horn-Ross PL, Bernstein L, et al. Light at night and breast cancer risk among California teachers. Epidemiology 2014;25:697–706.
143. Kim YJ, Park MS, Lee E, Choi JW. High incidence of breast cancer in light-polluted areas with spatial effects in Korea. Asian Pac J Cancer Prev 2016;17:361–7.
144. Wu J, Dauchy RT, Tirrell PC, Wu SS, Lynch DT, Jitawatanarat P, et al. Light at night activates IGF-1R/PDK1 signaling and accelerates tumor growth in human breast cancer xenografts. Cancer Res 2011;71:2622–31.
145. Schwimmer H, Metzer A, Pilosof Y, Szyf M, Machnes ZM, Fares F, et al. Light at night and melatonin have opposite effects on breast cancer tumors in mice assessed by growth rates and global DNA methylation. Chronobiol Int 2014;31: 144–50.
146. Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, et al. Chronically Alternating Light Cycles Increase Breast Cancer Risk in Mice. Curr Biol 2015;25:1932–7.
147. He C, Anand ST, Ebell MH, Vena JE, Robb SW. Circadian disrupting exposures and breast cancer risk: a meta-analysis. Int Arch Occup Environ Health 2015;88:533–47.
148. Rabstein S, Harth V, Pesch B, Pallapies D, Lotz A, Justenhoven C, et al. Night work and breast cancer estrogen receptor status-results from the German GENICA study. Scand J Work Environ Health 2013;39:448–55.
149. Kloog I, Haim A, Stevens RG, Barchana M, Portnov BA. Light at night co-distributes with incident breast but not lung cancer in the female population of Israel. Chronobiol Int 2008;25:65–81.
150. Kloog I, Stevens RG, Haim A, Portnov BA. Nighttime light level co-distributes with breast cancer incidence worldwide. Cancer Causes Control 2010;21:2059–68.
151. O'Leary ES, Schoenfeld ER, Stevens RG, Kabat GC, Henderson K, Grimson R, et al. Electromagnetic Fields and Breast Cancer on Long Island Study Group. Shift work, light at night, and breast cancer on Long Island, New York. Am J Epidemiol 2006;164:358–66.
152. Li Q, Zheng T, Holford TR, Boyle P, Zhang Y, Dai M. Light at night and breast cancer risk: results from a population-based case-control study in Connecticut, USA. Cancer Causes Control 2010;21:2281–5.

153. Kloog I, Portnov BA, Rennert HS, Haim A. Does the modern urbanized sleeping habitat pose a breast cancer risk? Chronobiol Int 2011;28:76–80.
154. Keshet-Sitton A, Or–Chen K, Yitzhak S, Tzabary I, Haim A. Can avoiding light at night reduce the risk of breast cancer? Integr Cancer Ther 2016;15:145–52.
155. 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 2006;17:573–6.
156. Flynn–Evans EE, Stevens RG, Tabandeh H, Schernhammer ES, Lockley SW. Total visual blindness is protective against breast cancer. Cancer Causes Control 2009;20:1753–6.
157. 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.
158. 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;79:313–20.
159. Dauchy RT, Sauer LA, Blask DE, Vaughan GM. Light contamination during the dark phase in "photoperiodically controlled" animal rooms: effect on tumor growth and metabolism in rats. Lab Anim Sci 1997;47:511–8.
160. Zubidat AE, Fares B, Faras F, Haim A. Melatonin functioning through DNA methylation to constrict breast cancer growth accelerated by blue LED light at night in 4T1 tumor bearing mice. Gratis J Cancer Biol Ther 2015;1:57–73.
161. Fritschi L, Erren TC, Glass DC, Girschik J, Thomson AK, Saunders C, et al. The association between different night shiftwork factors and breast cancer: a case-control study. Br J Cancer 2013;109:2472–80.
162. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042–54.
163. Radpour R, Kohler C, Haghighi MM, Fan AX, Holzgreve W, Zhong XY. Methylation profiles of 22 candidate genes in breast cancer using high-throughput MALDI-TOF mass array. Oncogene 2009;28:2969–78.
164. Bediaga NG, Acha-Sagredo A, Guerra I, Viguri A, Albaina C, Ruiz Diaz I, et al. DNA methylation epigenotypes in breast cancer molecular subtypes. Breast Cancer Res 2010;12:R77.
165. Wong EM, Southey MC, Fox SB, Brown MA, Dowty JG, Jenkins MA, et al. Constitutional methylation of the BRCA1 promoter is specifically associated with BRCA1 mutation-associated pathology in early-onset breast cancer. Cancer Prev Res (Phila) 2011;4:23–33.
166. Stefansson OA, Esteller M. Epigenetic modifications in breast cancer and their role in personalized medicine. Am J Pathol 2013;183:1052–63.
167. Xiang TX, Yuan Y, Li LL, Wang ZH, Dan LY, Chen Y, et al. Aberrant promoter CpG methylation and its ranslational applications in breast cancer. Chin J Cancer 2013;32:12–20.
168. Zhu Y, Stevens RG, Hoffman AE, Tjonneland A, Vogel UB, Zheng T, et al. Epigenetic impact of long-term shiftwork: pilot evidence from circadian genes and whole-genome methylation analysis. Chronobiol Int 2011;28:852–61.
169. Woo HD, Kim J. Global DNA hypomethylation in peripheral blood leukocytes as a biomarker for cancer risk: a meta-analysis. PLoS One 2012;7:e34615.
170. Delgado-Cruzata L, Wu HC, Perrin M, Liao Y, Kappil MA, Ferris JS, et al. Global DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. Epigenetics 2012;7:868–74.
171. Kuchiba A, Iwasaki M, Ono H, Kasuga Y, Yokoyama S, Onuma H, et al. Global methylation levels in peripheral blood leukocyte DNA by LUMA and breast cancer: a case-control study in Japanese women. Br J Cancer 2014;110:2765–71.
172. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature 1998;395:89–93.
173. Kaneda A, Feinberg AP. Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk. Cancer Res 2005;65:11236–40.
174. Soares J, Pinto AE, Cunha CV, André S, Barão I, Sousa JM, et al. Global DNA hypomethylation in breast carcinoma: correlation with prognostic factors and tumor progression. Cancer 1999;85:112–8.
175. Cao Y, Li Y, Zhang N, Hu J, Yin L, Pan Z, et al. Quantitative DNA hypomethylation of ligand Jagged1 and receptor Notch1 signifies occurrence and progression of breast carcinoma. Am J Cancer Res 2015;5:1897–910.
176. Joska TM, Zaman R, Belden WJ. Regulated DNA methylation and the circadian clock: implications in cancer. Biology (Basel) 2014;3:560–77.
177. 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;26:1241–6.
178. Shih MC, Yeh KT, Tang KP, Chen JC, Chang JG. Promoter methylation in circadian genes of endometrial cancers detected by methylation-specific PCR. Mol Carcinog 2006;45:732–40.
179. Bhatti P, Zhang Y, Song X, Makar KW, Sather CL, Kelsey KT, et al. Nightshift work and genome–wide DNA methylation. Chronobiol Int 2015;32:103–12.
180. Rebec KM, Klanjšek-Gunde M, Bizjak G, Kobav MB. White LED compared with other light sources: age-dependent photobiological effects and parameters for evaluation. Int J Occup Saf Ergon 2015;21:391–8.
181. Frühbeck G, Becerril S, Sáinz N, Garrastachu P, García-Velloso MJ. BAT: a new target for human obesity? Trends Pharmacol Sci 2009;30:387–96.
182. Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev 2011;12:167–88.
183. Griggio MA. Thermogenic mechanisms in cold-acclimated animals. Braz J Med Biol Res 1988;21:171–6.
184. Betz MJ, Enerbäck S. Human brown adipose tissue: what we have learned so far. Diabetes 2015;64:2352–60.
185. Saely CH, Geiger K, Drexel H. Brown versus white adipose tissue: a mini-review. Gerontology 2012;58:15–23.
186. Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ. Melatonin, energy metabolism, and obesity: a review. J Pineal Res 2014;56:371–81.
187. Brydon L, Petit L, Delagrange P, Strosberg AD, Jockers R. Functional expression of MT2 (Mel1b) melatonin receptors in human PAZ6 adipocytes. Endocrinology 2001;142:4264–71.
188. Terrón MP, Delgado-Adámez J, Pariente JA, Barriga C, Paredes SD, Rodríguez AB. Melatonin reduces body weight gain and

increases nocturnal activity in male Wistar rats. Physiol Behav 2013;118:8–13.
189. Hidayat M, Maha Y, Wasim H. Effect of melatonin on serum glucose and body weight in streptozotocin induced diabetes in albino rats. J Ayub Med Coll Abbottabad 2015;27:274–6.
190. Bartness TJ, Wade GN. Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci Biobehav Rev 1985;9: 599–612.
191. Borniger JC, Maurya SK, Periasamy M, Nelson RJ. Acute dim light at night increases body mass, alters metabolism, and shifts core body temperature circadian rhythms. Chronobiol Int 2014;31:917–25.
192. Aubrecht TG, Jenkins R, Nelson RJ. Dim light at night increases body mass of female mice. Chronobiol Int 2015;32:557–60.
193. Jenwitheesuk A, Nopparat C, Mukda S, Wongchitrat P, Govitrapong P. Melatonin regulates aging and neurodegeneration through energy metabolism, epigenetics, autophagy and circadian rhythm pathways. Int J Mol Sci 2014;15:16848–84.
194. de Almeida EA, Di Mascio P, Harumi T, Spence DW, Moscovitch A, Hardeland R, et al. Measurement of melatonin in body fluids: standards, protocols and procedures. Childs Nerv Syst 2011;27:879–91.
195. Schernhammer ES, Hankinson SE. Urinary melatonin levels and postmenopausal breast cancer risk in the Nurses' Health Study cohort. Cancer Epidemiol Biomarkers Prev 2009;18: 74–9.
196. Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, et al. Urinary 6-Sulphatoxymelatonin levels and risk of breast cancer in premenopausal women: the ORDET cohort. Cancer Epidemiol Biomarkers Prev 2010;19:729–37.
197. Basler M, Jetter A, Fink D, Seifert B, Kullak-Ublick GA, Trojan A. Urinary excretion of melatonin and association with breast cancer: meta-analysis and review of the literature. Breast Care (Basel) 2014;9:182–7.
198. Bartsch C, Bartsch H, Karenovics A, Franz H, Peiker G, Mecke D. Nocturnal urinary 6-sulphatoxymelatonin excretion is decreased in primary breast cancer patients compared to age-matched controls and shows negative correlation with tumor-size. J Pineal Res 1997;23:53–8.
199. Lissoni P. Is there a role for melatonin in supportive care? Support Care Cancer 2002;10:110–6.
200. Cerea G, Vaghi M, Ardizzoia A, Villa S, Bucovec R, Mengo S, et al. Biomodulation of cancer chemotherapy for metastatic colorectal cancer: a randomized study of weekly low-dose irinotecan alone versus irinotecan plus the oncostatic pineal hormone melatonin in metastatic colorectal cancer patients progressing on 5-fluorouracil-containing combinations. Anticancer Res 2003;23:1951–4.
201. Blask DE, Hill SM, Dauchy RT, Xiang S, Yuan L, Duplessis T, et al. Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. J Pineal Res 2011;51:259–69.
202. Mao L, Yuan L, Slakey LM, Jones FE, Burow ME, Hill SM. Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Res 2010;12:R107.
203. Leon-Blanco MM, Guerrero JM, Reiter RJ, Calvo JR, Pozo D. Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both in vivo and in vitro. J Pineal Res 2003;35:204–11.
204. Ma C, Li LX, Zhang Y, Xiang C, Ma T, Ma ZQ, et al. Protective and sensitive effects of melatonin combined with adriamycin on ER+ (estrogen receptor) breast cancer. Eur J Gynaecol Oncol 2015;36:197–202.
205. Bartsch H, Bartsch C. Effect of melatonin on experimental tumors under different photoperiods and times of administration. J Neural Transm 1981;52:269–79.
206. Hill SM, Belancio VP, Dauchy RT, Xiang S, Brimer S, Mao L, et al. Melatonin: an inhibitor of breast cancer. Endocr Relat Cancer 2015;22:R183–204.
207. Doghramji K. Melatonin and its receptors: a new class of sleep-promoting agents. J Clin Sleep Med 2007;3:S17–23.
208. Hill SM, Frasch T, Xiang S, Yuan L, Duplessis T, Mao L. Molecular mechanisms of melatonin anticancer effects. Integr Cancer Ther 2009;8:337–46.
209. Jung-Hynes B, Huang W, Reiter RJ, Ahmad N. Melatonin resynchronizes dysregulated circadian rhythm circuitry in human prostate cancer cells. J Pineal Res 2010;49:60–8.
210. Kochan DZ, Kovalchuk O. Circadian disruption and breast cancer: an epigenetic link? Oncotarget 2015;6:16866–82.
211. Korkmaz A, Sanchez-Barcelo EJ, Tan DX, Reiter RJ. Role of melatonin in the epigenetic regulation of breast cancer. Breast Cancer Res Treat 2009;115:13–27.
212. Korkmaz A, Reiter RJ. Epigenetic regulation: a new research area for melatonin? J. Pineal Res 2008;44:41–4.
213. Rasmussen DD, Boldt BM, Wilkinson CW, Yellon SM, Matsumoto AM. Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology 1999;140:1009–12.
214. Wolden-Hanson T, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM, et al. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology 2000;141:487–97.
215. Peschke E, Mühlbauer E. New evidence for a role of melatonin in glucose regulation. Best Pract Res Clin Endocrinol Metab 2010;24:829–41.
216. Niles LP, Pan Y, Kang S, Lacoul A. Melatonin induces histone hyperacetylation in the rat brain. Neurosci Lett 2013;541: 49–53.
217. Sharma R, Ottenhof T, Rzeczkowska PA, Niles LP. Epigenetic targets for melatonin: induction of histone H3 hyperacetylation and gene expression in C17.2 neural stem cells. J Pineal Res 2008;45:277–84.
218. Lee SE, Kim SJ, Yoon HJ, Yu SY, Yang H, Jeong SI, et al. Genome-wide profiling in melatonin-exposed human breast cancer cell lines identifies differentially methylated genes involved in the anticancer effect of melatonin. J Pineal Res 2013;54:80–8.
219. Haim A, Zubidat AE. Artificial light at night: melatonin as a mediator between the environment and epigenome. Philos Trans R Soc Lond B Biol Sci 2015;370:1–7.
220. Osório-Costa F, Rocha GZ, Dias MM, Carvalheira JB. Epidemiological and molecular mechanisms aspects linking obesity and cancer. Arq Bras Endocrinol Metabol 2009;53:213–26.
221. Papantoniou K, Pozo OJ, Espinosa A, Marcos J, Castaño-Vinyals G, Basagaña X, et al. Circadian variation of melatonin, light exposure, and diurnal preference in day and night shift workers of both sexes. Cancer Epidemiol Biomarkers Prev 2014;23:1176–86.

222. Dijk DJ, Duffy JF, Silva EJ, Shanahan TL, Boivin DB, Czeisler CA. Amplitude reduction and phase shifts of melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. PLoS One 2012;7:e30037.
223. Chang AM, Santhi N, St Hilaire M, Gronfier C, Bradstreet DS, Duffy JF, et al. Human responses to bright light of different durations. J Physiol 2012;590:3103–12.
224. Wright HR, Lack LC, Kennaway DJ. Differential effects of light wavelength in phase advancing the melatonin rhythm. J Pineal Res 2004;36:140–4.
225. Kim SJ, Benloucif S, Reid KJ, Weintraub S, Kennedy N, Wolfe LF, et al. Phase-shifting response to light in older adults. J Physiol 2014;592:189–202.
226. Middleton B. Measurement of melatonin and 6-sulphatoxymelatonin. Methods Mol Biol 2013;1065:171–99.
227. Brazel AJ, Vernimmen D. The complexity of epigenetic diseases. J Pathol 2016;238:333–44.
228. Salvianti F, Orlando C, Massi D, De Giorgi V, Grazzini M, Pazzagli M, et al. Tumor-related methylated cell-free dna and circulating tumor cells in melanoma. Front Mol Biosci 2015;2:76.
229. Dayeh T, Volkov P, Salö S, Hall E, Nilsson E, Olsson AH, et al. Genome-wide DNA methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. PLoS Genet 2014;10:e1004160.
230. Rönn T, Ling C. DNA methylation as a diagnostic and therapeutic target in the battle against type 2 diabetes. Epigenomics 2015;7:451–60.
231. Wu L, Zhao X, Shen Y, Huang G, Zhang M, Yan Y, et al. Influence of lifestyle on the FAIM2 promoter methylation between obese and lean children: a cohort study. BMJ Open 2015;5:e007670.
232. Campión J, Milagro FI, Goyenechea E, Martínez JA. TNF-alpha promoter methylation as a predictive biomarker for weight-loss response. Obesity (Silver Spring) 2009;17:1293–7.
233. Guay SP, Brisson D, Lamarche B, Biron S, Lescelleur O, Biertho L, et al. ADRB3 gene promoter DNA methylation in blood and visceral adipose tissue is associated with metabolic disturbances in men. Epigenomics 2014;6:33–43.
234. Wu L, Zhao X, Shen Y, Huang G, Zhang M, Yan Y, et al. Promoter methylation of fas apoptotic inhibitory molecule 2 gene is associated with obesity and dyslipidaemia in Chinese children. Diab Vasc Dis Res 2015;12:217–20.
235. van Veldhoven K, Polidoro S, Baglietto L, Severi G, Sacerdote C, Panico S, et al. Epigenome-wide association study reveals decreased average methylation levels years before breast cancer diagnosis. Clin Epigenetics 2015;7:67.
236. Ordway JM, Budiman MA, Korshunova Y, Maloney RK, Bedell JA, Citek RW, et al. Identification of novel high-frequency DNA methylation changes in breast cancer. PLoS One 2007;2:e1314.
237. Ruike Y, Imanaka Y, Sato F, Shimizu K, Tsujimoto G. Genome-wide analysis of aberrant methylation in human breast cancer cells using methyl-DNA immunoprecipitation combined with high–throughput sequencing. BMC Genomics 2010;11:137.
238. Byler S, Goldgar S, Heerboth S, Leary M, Housman G, Moulton K, et al. Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer Res 2014;34:1071–7.
239. Szyf M. DNA methylation signatures for breast cancer classification and prognosis. Genome Med 2012;4:26.
240. Fackler MJ, Lopez Bujanda Z, Umbricht C, Teo WW, Cho S, Zhang Z, et al. Novel methylated biomarkers and a robust assay to detect circulating tumor DNA in metastatic breast cancer. Cancer Res 2014;74:2160–70.
241. Kloten V, Becker B, Winner K, Schrauder MG, Fasching PA, Anzeneder T, et al. Promoter hypermethylation of the tumor-suppressor genes ITIH5, DKK3, and RASSF1A as novel biomarkers for blood-based breast cancer screening. Breast Cancer Res2013;5:R4.
242. Fleischer T, Edvardsen H, Solvang HK, Daviaud C, Naume B, Børresen-Dale AL, et al. Integrated analysis of high-resolution DNA methylation profiles, gene expression, germline genotypes and clinical end points in breast cancer patients. Int J Cancer 2014;134:2615–25.
243. Radpour R, Barekati Z, Kohler C, Lv Q, Bürki N, Diesch C, et al. Hypermethylation of tumor suppressor genes involved in critical regulatory pathways for developing a blood-based test in breast cancer. PLoS One 2011;6:e16080.
244. Agostini M, Enzo MV, Bedin C, Belardinelli V, Goldin E, Del Bianco P, et al. Circulating cell-free DNA: a promising marker of regional lymphonode metastasis in breast cancer patients. Cancer Biomark 2012;11:89–98.
245. Mirick DK, Davis S. Melatonin as a biomarker of circadian dysregulation. Cancer Epidemiol Biomarkers Prev 2008;17:3306–13.
246. He B, Nohara K, Park N, Park YS, Guillory B, Zhao Z, et al. The small molecule nobiletin targets the molecular oscillator to enhance circadian rhythms and protect against metabolic syndrome. Cell Metab 2016;23:610–21.
247. Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, et al. The case for early detection. Nat Rev Cancer 2003;3:243–52.