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Mini-review

Circadian molecular clocks and cancer

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ABSTRACT

Physiological processes such as the sleep-wake cycle, metabolism and hormone secretion are controlled by a circadian rhythm adapted to 24 h day-night periodicity. This circadian synchronisation is in part controlled by ambient light decreasing melatonin secretion by the pineal gland and co-ordinated by the suprachiasmatic nucleus of the hypothalamus. Peripheral cell autonomous circadian clocks controlled by the suprachiasmatic nucleus, the master regulator, exist within every cell of the body and are comprised of at least twelve genes. These include the basic helix-loop-helix/PAS domain containing transcription factors; Clock, BMal1 and Npas2 which activate transcription of the periodic genes (*Per1* and *Per2*) and cryptochrome genes (*Cry1* and *Cry2*). Points of coupling exist between the cellular clock and the cell cycle. Cell cycle genes which are affected by the molecular circadian clock include *c-Myc*, *Wee1*, *cyclin D* and *p21*. Therefore the rhythm of the circadian clock and cancer are interlinked. Molecular examples exist including activation of *Per2* leads to c-myc overexpression and an increased tumor incidence. Mice with mutations in Cryptochrome 1 and 2 are arrhythmic (lack a circadian rhythm) and arrhythmic mice have a faster rate of growth of implanted tumors. Epidemiological finding of relevance include '*The Nurses' Health Study*' where it was established that women working rotational night shifts have an increased incidence of breast cancer. Compounds that affect circadian rhythm exist with attendant future therapeutic possibilities. These include casein kinase I inhibitors and a candidate small molecule KL001 that affects the degradation of cryptochrome. Theoretically the cell cycle and malignant disease may be targeted vicariously by selective alteration of the cellular molecular clock.

1. Introduction to circadian physiology

There is circadian synchronisation of numerous molecular, physiological, biochemical and behavioural processes [1–4]. The diurnal variation in cortisol is one such circumstance. The rate of production of melatonin (N-acetyl-5-methoxytrptamine) an indoleamine hormone which is mainly produced by the pineal gland is very low during the daytime, ascends in the evening and peaks between 02 00 h and 04 00 h. It then gradually declines. The circadian system is perturbed by exposure to light at night with commensurate suppression of melatonin production and dysregulation of circadian genes that have been implicated in cancer development [5]. Melatonin binding to its receptor MT-1 modulates pathways implicated in cancer. Night shift work is viewed as a vicarious indicator of exposure to light at night.

Molecularly cryptochromes are the circadian photoreceptors in mammals and have differing regional expression patterns in the retino-neuro-anatomical system. They absorb light and the electromagnetic signal is transmitted to the circadian molecular clock using a pterin and flavin adenine dinucleotide as chromophore-cofactors. They are structurally related to the DNA repair enzyme photolyase [6]. Cry1 and Cry2 are differentially expressed in the retina relative to the opsin based visual photoreceptors. Within the retina, the axons of some of the ganglion cells enter the retinohypothalamic tract, which leave the dorsal surface of the optic chiasm and synapse with the suprachiasmatic nucleus. The suprachiasmatic nucleus of the hypothalamus is considered to be the master regulator of cellular circadian rhythm. Its neurons are tightly interconnected and synchronized as an organ by crosstalk coupling mediated by vasoactive intestinal polypeptide and other neurotransmitters [7–9]. Within the suprachiasmatic nucleus, Cry1 has high expression with circadian periodicity. Transplantated pancreatic adenocarcinomas or osteosarcomas in mice in which the suprachiasmatic nucleus is ablated have an accelerated rate of tumor growth compared to mice with an intact suprachiasmatic nucleus [10].

Visual input leads to synchronization of the circadian neuronal firing rhythm of the suprachiasmatic neurones with the ambient lighting, so that they fire on a 24 h day-light cycle and the suprachiasmatic nucleus in turn synchronises the peripheral molecular

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clock in each cell [11,12]. This anatomically is responsible for the influence that varying levels of ambient light have on pituitary gonadotropin secretion, as well as pineal secretion of melatonin. Diurnal levels of pituitary gonadotrophins is of self-evident interest in the biology of endocrine related tumours such as breast and prostate cancer. Mice with suprachiasmatic lesions have an accelerated growth rate of implanted neoplasms [10]. Axons arising within the suprachiasmatic nucleus contact the orexin neurons within the lateral hypothalamus and the dorsomedial hypothalamic nucleus that projects caudally to the locus coeruleus.

As well as environmental cycles, circadian rhythms are also created in a cell autonomous manner by a transcriptional regulatory network of circadian clock genes. This circadian clock controls approximately 10% of all genes [1,2,4,13,14]. A cellular transcription-translation feedback loop termed the peripheral clock is present in each cell [15]. Physiologic processes in peripheral tissues are controlled by tissue specific clock controlled genes such as *p21*, *Wee-1* and thymidilate synthase [16–22]. Perturbations in the function of the circadian clock can occur by environmental disturbances (e.g. shift work, jet lag) or else by genetic mutations. Shift work that involves circadian disruption is deemed to be a Group2A ('probable') carcinogen by the International Agency for Research on Cancer and epidemiologic studies have demonstrated that circadian rhythm disruption increases the risk of breast, colon, prostate, lung, ovarian and hepatocellular carcinoma [23–25]. Pathologically, many types of cancer cells have cell-autonomous clocks. The relative contributions of disrupted circadian rhythm and disrupted circadian genes to cancer risk may be informative as to the pathogenesis of differing cancers and new treatment interventions. In one potentially confounding consideration sleep deprivation leads to immunodeficiency [26,27]. Natural killer cell activity is supressed and alteration in the T-helper 1/T-helper 2 cytokine balance decreases cellular immunity and tumour immune surveillance [28,29].

2. The molecular circadian clock

Despite the ubiquitous expression of circadian oscillators, they regulate the expression of genes in a tissue specific way [30]. The molecular clock involves transcription-translation feedback loops in a small number of core circadian genes in which there is the rhythmic production followed by protracted degradation of protein complexes that negatively impact on their own production [31,32].

(i) In the primary feedback loop (illustrated in Fig. 1), there are 3 basic helix-loop-helix/PAS domain containing transcription factors, *Clock*, *BMal1* (Brain-muscle Arnt-like) and *Npas2* (the name for the PAS protein domain is an acrynom of the first letter of three different proteins in which it occurs; Per-period circadian protein, Arnt-aryl hydrocarbon receptor nuclear translocation protei, and Sim-single minded protein). Basic helix–loop–helix (bHLH) proteins are transcription factors that can be transcriptional activators or repressors. They possess highly basic regions and helix–loop–helix motifs. bHLH proteins form homo dimers or heterodimers with other bHLH proteins through their helix–loop–helix domains, which enable their basic regions to form DNA-binding motifs that recognise E-box sequences.

They activate the transcription of the period genes (*Per1* and *Per2*) and cryptochrome genes (*Cry1* and *Cry2*) by binding to *cis* elements of the E-box within the genes' promoters. The PER/CRY complex subsequently inhibits transcription of its own gene(s) by blocking BMAL1/CLOCK activity [33]. Most circadian output which is mediated by the CLOCK-BMAL1 complex induces the expression of other transcription factors [34–36]. Once the Per and Cry proteins translocate to the cytoplasm they form heterodimeric complexes which translocate to the nucleus and bind to the Bmal1 promoter to inhibit Bmal1 expression and thereby inhibit their own transcription.

(ii) The core clock proteins are modulated by proteins including CK1 (epsilon) and Rev-Erbα leading to greater precision of the molecular oscillator with approximately 24 h periodicity. A second feedback loop involves the transcription of the orphan nuclear receptors RORα and REV-erbα. This transcription is activated during daytime by the CLOCK/BMAL1 complex subsequent to which they exert positive and negative transcriptional effects on the *BMAL1* gene through Rev-erbα/RORα response elements in the *Bmal1* promoter [12,31,37].

(iii) The circadian system affects other physiologic processes by clock-controlled nuclear receptor genes such as *PPARα* and *PPARγ* and cell cycle genes including *c-MYC*, *WEE1*, *cyclin D* and *p21* [38–41]. Importantly the rhythmic expression of some transcripts involved in the cell cycle including several cyclins is regulated by the circadian clock [42].

As an aside, it is of interest that the *NPAS2* gene encodes a protein that is an analogue of clock which functionally is operative in the forebrain circadian rhythm [43].

3. Epidemiologic evidence

Approximately 15–20% of workers in Europe and the US are involved in shift work which involves night work. The prevalence exceeds 30% in manufacturing, mining, transport, health care, communications and the hospitality sector. Two independent cohort studies of nurses involved in night-time shift work have observed a modestly increased incidence of breast cancer [24,44,45]. A small increase in the incidence of breast cancer has also been observed in female flight attendants but potential confounders include exposure to cosmic rays, detection bias, and proxy measures of exposure [46]. Lack of sleep may also be associated with a more aggressive breast cancer phenotype [47]. In one study, OncotypeDX disease recurrence scores in ER positive patients, were correlated with the average number of hours slept each night in the 2 years preceding diagnosis of breast cancer. Fewer hours of sleep was associated with greater recurrence scores ($R = -0.30$, $p = 0.0031$). This correlation was restricted to post-menopausal patients (post-menopausal patients $R = -0.41$, $p = 0.0011$; premenopausal patients $R = -0.05$, $p = 0.80$). Consistent with this, it has been found that patients with an altered circadian rhythm have a worse survival [48]. Again considering increased risk, in a nested case control study of 49,402 Norwegian nurses, a significantly increased risk of breast cancer was associated with working $\geqslant 5$ years and for $\geqslant 6$ consecutive nights (odds ratio 1.8, 95% confidence interval 1.1, 2.8) [49]. The investigators inferred that risk may be related to the number of consecutive night shifts. It is to be recalled that light at night can disrupt genes in the circadian clock in addition to affecting melatonin levels. In an epigenetic consideration, night shift workers have altered methylation of numerous genes including *Cry2* and *Clock* [50].

A separate combined analsysis of three National Health and Nutrition Examination Surveys in the United States correlated shift work with the risk of developing prostate cancer in men aged 40–65 years [51]. An odds ratio of 2.48 was found for an age-adjusted association between current shift work and a PSA equal or greater than 4.0 ng/mL (95% confidence interval, 1.08–5.70; $P = 0.03$). The confounder adjusted odds-ratio for individuals with