REVIEW

Clock genes and cancer development in particular in endocrine tissues

Anna Angelousi1, Eva Kassi1,2, Narjes Ansari-Nasiri2, Harpal Randeva3, Gregory Kaltsas4,6* and George Chrousos5,6*

1Endocrine Unit, 1st Department of Internal Medicine, Laiko Hospital, National and Kapodistrian University of Athens, Athens, Greece
2Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, Athens, Greece
3Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM), University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK
4Endocrine Unit, 1st Department of Propaedeutic Medicine, Laiko University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
5First Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Correspondence should be addressed to A Angelousi: a.angelousi@gmail.com

*(G Kaltsas and G Chrousos contributed equally to this work)

Abstract

Circadian rhythms at a central and peripheral level are operated by transcriptional/translational feedback loops involving a set of genes called ‘clock genes’ that have been implicated in the development of several diseases, including malignancies. Dysregulation of the Clock system can influence cancer susceptibility by regulating DNA damage and repair mechanisms, as well as apoptosis. A number of oncogenic pathways can be dysregulated via clock genes’ epigenetic alterations, including hypermethylation of clock genes’ promoters or variants of clock genes. Clock gene disruption has been studied in breast, lung and prostate cancer, and haematological malignancies. However, it is still not entirely clear whether clock gene disruption is the cause or the consequence of tumourigenesis and data in endocrine neoplasms are scarce. Recent findings suggest that clock genes are implicated in benign and malignant adrenocortical neoplasias. They have been also associated with follicular and papillary thyroid carcinomas and parathyroid adenomas, as well as pituitary adenomas and craniopharyngiomas. Dysregulation of clock genes is also encountered in ovarian and testicular tumours and may also be related with their susceptibility to chemotherapeutic agents. The most common clock genes that are implicated in endocrine neoplasms are PER1, CRY1; in most cases their expression is downregulated in tumoural compared to normal tissues. Although there is still a lot to be done for the better understanding of the role of clock genes in endocrine tumourigenesis, existing evidence could guide research and help identify novel therapeutic targets aiming mainly at the peripheral components of the clock gene system.

Key Words
- clock genes
- circadian rhythm
- adrenal tumour
- thyroid cancer
- pituitary adenomas
- parathyroid adenomas
- testis tumour
- ovarian tumour

Introduction

Circadian rhythms are biological rhythms that exhibit a periodicity close to 24 h. In mammals, these rhythms are generated by a master biological clock, located centrally in the suprachiasmatic nucleus (SCN) of the hypothalamus and by clocks localized in peripheral tissues (Lowrey & Takahashi 2011, Kassi et al. 2013). Both clocks communicate with each other and generate circadian rhythmicity by the coordinated activation/inactivation
of self-oscillating transcription factors (Charmandari et al. 2011). In particular, external daytime information (darkness vs. light) is transferred from the retina of the eye to the SCN synchronizing the SCN clock with the external light-darkness cycle. The SCN passes information about the time of day to all tissues of the body through as yet unknown possibly neural or neuroendocrine mechanisms (Nicolaides et al. 2017). Loss of the SCN pacemaker, for example by surgical lesioning, in mice causes a gradual desynchronization of rhythmicity in peripheral tissues. In rats with ablated SCN, the normal diurnal adrenal corticosterone rhythm is no longer detectable (Kolbe et al. 2018).

At the molecular level, circadian clocks are operated by transcriptional/translational feedback loops involving a set of genes called ‘clock genes’ (Buhr et al. 2013, Partch et al. 2014) (Fig. 1). At least 20% of all mammalian genes are considered to be clock-controlled (Storch et al. 2002, Koike et al. 2012). To date at least 12 mammalian clock-related genes have been identified: Period-1 (PER-1), Period-2 (PER-2), Period-3 (PER-3), circadian locomotor output cycles kaput (CLOCK), cryptochrome 1 (CRY1), cryptochrome 2 (CRY2), the transcription factors aryl hydrocarbon receptor nuclear translocator-like (ARNTL or BMAL1), the Timeless (TIM), the retinoic acid-related orphan nuclear receptor (ROR) (Hsu et al. 1996, Shearman et al. 1997, Liu et al. 2017), the neuronal PAS domain protein 2 (NPAS2) (Reick et al. 2001), the nuclear receptor subfamily 1 group D member 1 and 2 (NR1D1 and NR1D2, also known as REV-ERB alpha and beta) (Preitner et al. 2002) and the casein kinase I epsilon (CSNK1E) (Bugge et al. 2012) (Fig. 1).

In mammals the circadian clock regulation is made up of two main interlocking, regulatory feedback loops. The BMAL1/CLOCK heterodimer activates the PER-1, -3 and the CRY1-2 genes. After accumulation in the cytoplasm PER/CRY complexes relocate into the nucleus to inactivate CLOCK/BMAL1 transactivation, thereby downregulating their own expression (Hunt et al. 2001, Machicao et al. 2016). RORα is a core part of the clock machinery that positively regulates the expression of BMAL1 (Hunt et al. 2001, Machicao et al. 2016) (Fig. 1).

Dysregulation of clock genes’ expression is associated with various diseases such as diabetes type 1, rheumatoid arthritis, inflammatory bowel diseases and polyglandular autoimmune disease (Angelousi et al. 2018a,b). In vitro and in vivo studies have shown that clock genes are also implicated in carcinogenesis through a number of different pathways. In particular, clock genes may affect cancer susceptibility regulating nucleotide excision repair, DNA damage checkpoints and apoptosis (Sahar et al. 2009, Sancar et al. 2015) (Fig. 2).

Clock genes affect also critical pathways of autophagy such as the mechanistic target of rapamycin (mTOR), the AMP-activated protein kinase and the silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1), as well as proliferative mechanisms that involve the Wnt/β-catenin pathway (Maiese 2017), regulators and checkpoints of the cell cycle as well as growth factors (i.e. vascular epithelial growth factor (VEGF)) (Sahar et al. 2009).

The Clock system may also impact on the efficacy of cancer treatment by modulating the pharmacokinetics and pharmacodynamics of chemotherapeutic drugs as well as the

---

**Figure 1**

Transcription–translation feedback loop of clock genes. The cyclicity of circadian oscillation is generated by autoregulatory transcription–translation–based molecular oscillators, in which transcription at E-box sites occupies a pivotal position. The BMAL1 and CLOCK proteins form a complex and bind to E-box (CACGT[G/T]) in the promoter regions of PER genes (PER1 and PER2). PERs translocate to the nucleus, heterodimerize with CRY and inhibit CLOCK–BMAL1-mediated E-box transcription. After a period of time, the PER–CRY repressor complex is degraded and CLOCK–BMAL1 can then activate a new cycle of transcription. Assisting this core oscillatory loop, accessory loops (REV/ERB, ROR) modulate the amplitude and the phase of the genes they regulate. The extracellular signals that increase the content of Ca²⁺ and/or cAMP lead to the activation of ERK and CREB, and thereby activate the CRE-mediated PER1 expression.
activity of the DNA repair enzymes regulated by anticancer drugs (Sancar et al. 2015). Some studies suggest that loss of mammalian circadian clock proteins such as PER2 can lead to enhanced mTOR activity and chemotherapy drug resistance (Chen et al. 2017), whereas in other studies BMAL1 overexpression increases the sensitivity of colorectal cancer to chemotherapy (Zeng et al. 2014).

Over the last few years, increasingly more studies have been published on the association of clock genes with cancer. Most of the data concern breast cancer, lung, prostate or haematological malignancies (Chen et al. 2005, Taniguchi et al. 2009, Sephton et al. 2013). On the contrary, only few data exist on the role of clock genes in the tumourigenesis of endocrine organs and concern mainly case reports and few original articles. This is the first review to present currently available data looking at the association of clock genes and endocrine neoplasms.

Methods

To identify studies and determine eligibility a systematic search was conducted in the PubMed, MEDLINE and Cochrane databases. The search terms included the following: ‘clock genes’, ‘circadian rhythms’, ‘adrenal tumours’, ‘thyroid cancer’, ‘pituitary adenomas’, ‘parathyroid adenomas’, ‘ovarian tumours’, ‘testicular tumours’. The above keywords were also combined with the Boolean operators AND and OR. Two of the authors (AA and EK) independently examined all potentially eligible titles and abstracts. Full manuscripts were obtained as necessary to finalize eligibility. Reference lists of eligibility studies were also searched thoroughly to identify additional studies. Only English language papers were selected (Fig. 3).

Clock genes and cancer

The mechanisms of dysregulation of the core circadian genes in human cancers discovered to date include epigenetic silencing by promoter methylation, deregulation at the transcriptional and post-transcriptional levels, and structural variations of clock proteins due to gene polymorphisms. Clock proteins also modify intracellular pathways in peripheral target tissues leading to either proliferation or suppression of carcinogenesis.

In 2007, the International Agency for Research on Cancer listed ‘shift work leading to a disruption in circadian rhythm’ as a probable human carcinogen (Straif et al. 2007). On a genome-wide scale, 5409 cytosine-guanosine (CpG) sites were found to be differentially methylated during day versus night-time shift workers, and a 66% of these loci were hypermethylated (Zhu et al. 2011). Similarly, CpG methylation of the PER1, PER2, PER3 promoters was found to correlate with changes in protein expression in 50% of breast tumours compared to normal tissue originating from the same patients (Chen et al. 2005). PER1, PER2 and PER3 promoters was found to correlate with changes in protein expression in 50% of breast tumours compared to the non-tumour samples. CRY2 expression was found to be significantly downregulated, while CLOCK expression was upregulated in breast tumour samples compared to the non-tumour samples. CRY2 and NPAS2 were also downregulated in the poorly differentiated breast cancer in comparison with well and moderately differentiated ones suggesting a possible prognostic role in breast cancer (Lesicka et al. 2018).

Moreover, BMAL1 silencing due to CpG promoter methylation was found in 19.7% of diffuse large B-cell lymphomas, 33.3% of acute lymphocytic leukaemias and 19.2% of acute myeloid leukaemias (Taniguchi et al. 2009). Decreased expression of PER1 or PER2 has been also associated with promoter hypermethylation in endometrial, non-small lung cancer cells (Chen et al. 2005).
Research on Pubmed and Cochrane databases for relevant English-written articles using keywords*

N=368 for endocrine neoplasms:
- n=50 for adrenals
- n=34 for thyroid
- n=142 for pituitary
- n=15 for parathyroid
- n=63 for gonades

N=596 for cancer

Based on titles and abstract

N=97 for endocrine neoplasms:
- n=24 for adrenals
- n=25 for thyroid
- n=17 for pituitary
- n=15 for parathyroid
- n=16 for gonades

N=167 for cancer

Based on full text and hand-searched articles

N=23 for endocrine neoplasms:
- n=7 for adrenals
- n=1 for thyroid
- n=6 for pituitary
- n=1 for parathyroid
- n=8 for gonades

N=50 for cancers

In a recent systematic review and meta-analysis out of 366 clock gene variants which were investigated, 10 polymorphisms in 7 genes were associated with susceptibility to several cancers including breast cancer, prostate cancer, non-Hodgkin’s lymphoma, glioma, chronic lymphocytic leukaemia, colorectal cancer, non-small cell lung cancer and ovarian cancer (Benna et al. 2017).

In addition, CRY2, PER1, PER2, PER3, BMAL1 and CLOCK were found to be upregulated at mRNA levels in osteosarcoma cells (Yu et al. 2013), whereas CRY1 knockdown enhanced proliferation and migration of osteosarcoma cells through activation of Akt/P53/P21 signalling pathway. In chronic lymphocytic leukaemia...
CRY1 was upregulated whereas its silencing was associated with an indolent clinical course (Habashy et al. 2018, Peng et al. 2019). Overexpression of BMAL1 suppressed nasopharyngeal carcinoma cell proliferation in vitro and in vivo (Neilsen et al. 2019). Although the role of BMAL1 in cancer is not fully understood, studies suggested that BMAL1 may be a regulator of the p53 tumour suppressor pathway (Mullenders et al. 2009). More recently, it was found that BMAL1 can suppress cancer invasion by inhibiting the AKT signalling pathway (Jung et al. 2013). TIME was also overexpressed in colon cancer cells at least in part due to ERK signalling activation depletion, which slowed colon cancer cell proliferation by inducing G2/M arrest as a result of DNA damage (Neilsen et al. 2019).

All these data suggest the involvement of clock genes altered expression in human carcinogenesis and in some cases to prognosis. Loss of circadian homeostasis not only promotes cancer development, but is also associated with poor response to anticancer treatments. Some studies suggest that loss of mammalian circadian clock proteins such as PER2 can lead to enhanced mTOR activity and chemotherapy drug resistance (Chen et al. 2017), whereas in other studies BMAL1 overexpression increases the sensitivity of colorectal cancer to chemotherapy (Zeng et al. 2014). They are also used as independent prognosis factors for survival and therapeutic response of patients with metastatic breast, lung and colorectal cancers (Innominato et al. 2012, Sephton et al. 2013).

However, their therapeutic role in clinical practice remains still under investigation. In vitro, two major pharmacological modulators of the circadian clock system have been developed; the small molecule inhibitors that mediate a shortening of the period of circadian oscillation are the inhibitors of the GSK-3β in osteosarcoma cells which is known to directly phosphorylate several core-clock proteins including PER2, CRY2, REV-ERBeta, CLOCK and BMAL1 (Yin et al. 2006, Sahar et al. 2009, Spengler et al. 2009), leading to either their degradation (in case of CRY2, CLOCK and BMAL1) or their increased nuclear translocation (PER2) or stabilization (REV-ERBeta). The second modulator is a small molecule (named longdaysin) that potently lengthened the circadian period in a variety of cultured cells and in explants of mouse SCN (Hirota et al. 2010).

Clock genes in endocrine neoplasms

Clock genes and adrenal neoplasms

Recent data in vitro and in animal studies have clearly demonstrated the circadian expression of clock genes in the adrenal cortex as well as in the adrenal medulla (Ishida et al. 2005, Guo et al. 2006, Oster et al. 2006). In mice, both the adrenal cortex and the medulla displayed day/night variation in PER1, CRY2 and BMAL1 protein expression. PER1 and CRY2 peaked in the middle of the light phase, whereas BMAL1 peaked in the darkness phase (Ishida et al. 2005, Guo et al. 2006, Oster et al. 2006, Torres-Farfan et al. 2006). Studies in rat pheochromocytoma showed the existence of circadian rhythm of BMAL1, PER1, REV-ERB and CRY1 expression, although in stress condition, circadian oscillation affected mainly the apoptosis and neural differentiation via the activation of the transcription factor S (Atf5) and much less the catecholamine synthesis via the enzyme of tyrosine hydroxylase (Lemos et al. 2007).

Although clock genes’ expression has been reported in the nonhuman primate and rodent adrenal gland, data on human studies are scarce. Only one study demonstrated expression of PER1, PER2, CRY2, CLOCK and BMAL1 genes in normal human adrenal tissues (Campino et al. 2011) with predominant expression of PER1 mRNA, whereas the remaining genes showed only a weak expression (Campino et al. 2011) (Table 1).

A recent study showed that in human aldosterone-producing adenomas, CRY1 was overexpressed, while CRY2 was downregulated in the tissues, when compared with the paired adjacent adrenal cortex (Tetti et al. 2018). In addition, type II 3-hydroxyl steroid dehydrogenase (HSD3B2), overexpressed 300-fold compared to HSD3B1, was the principal isoform in these adenomas (Kono-Fukaya et al. 2015). Both dehydrogenases were more expressed in aldosterone-producing adenomas compared to the adjacent cortex. Treatment with angiotensin II resulted in a significant upregulation of CRY1 and downregulation of CRY2 through activation of the angiotensin type 1 receptor (Tetti et al. 2018). A previous study indicated that downregulation of PER1 was associated with lower plasma aldosterone levels and reduced HSD3B expression in H295R human adrenal cell line as well as in mice (Richards et al. 2013). On the contrary, incubation with angiotensin II led to increased PER1 protein levels in H295R adrenal cell lines suggesting a downregulation of PER1 in hyperaldosteronism (Romero et al. 2007).

Recent data have shown that cortisol-producing adenomas, non-functional adenomas and adrenal hyperplasias showed downregulation of mRNA and protein levels in six clock-related genes (CLOCK, BMAL1, PER1, CRY1, REV-ERB and ROR) compared to their peritumoural normal tissues (Angelousi et al. 2018a,b). Conn adenomas showed upregulation in
Table 1  Clock genes disruption in endocrine tumours.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Studied samples</th>
<th>CLOCK</th>
<th>BMAL1</th>
<th>CRY1</th>
<th>CRY 2</th>
<th>PER1</th>
<th>PER 2</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetti et al. (2018)</td>
<td>- Human APA vs normal adrenal tissues - Mice silenced for PER1</td>
<td>Nd</td>
<td>Nd</td>
<td>- (mRNA)</td>
<td>(mRNA) after treatment with Ang II</td>
<td>(mRNA)</td>
<td>(mRNA)</td>
<td>aldosterone levels in PER1 heterozygous mice</td>
</tr>
<tr>
<td>Romero et al. (2007)</td>
<td>H295R adrenocortical cells</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Angelousi et al. (2018)</td>
<td>Human CPA vs normal tissues</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Angelousi et al. (2018)</td>
<td>Human NFA vs normal tissues</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Angelousi et al. (2018)</td>
<td>Human Conn adenomas vs normal tissues (mRNA)</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Angelousi et al. (2018)</td>
<td>ACC vs human normal tissues</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Mannic et al. (2013)</td>
<td>Human FTC and PTC tissues vs normal thyroid tissue</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Becquet et al. (2014)</td>
<td>Human pituitary tissues</td>
<td>No alteration in protein level</td>
<td>Nd</td>
<td>No alteration in protein level</td>
<td>Nd</td>
<td>(mRNA) but not in the protein level</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Joustra et al. (2014)</td>
<td>Patients with NFA (blood)</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Altered melatonin secretion</td>
</tr>
<tr>
<td>Lissoni et al. (1992)</td>
<td>Patients with prolactinoma and GH-secreting adenomas</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Increased daytime secretion of melatonin and low increase in night-time</td>
</tr>
<tr>
<td>Terzolo et al. (1995)</td>
<td>Patients with GH-secreting adenomas (blood)</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Increased daytime secretion of melatonin with no correlation however with GH and IGF 1 levels</td>
</tr>
<tr>
<td>Lipton et al. (2009)</td>
<td>Patients with craniopharyngioma (blood)</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Decreased night-time and increased daytime values of melatonin</td>
</tr>
<tr>
<td>Sadowski et al. (2018)</td>
<td>Human parathyroid adenoma or hyperplasia vs normal parathyroid tissues</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>
mRNA levels of all six genes compared to peritumoural normal tissue as well as compared to cortisol-producing adenomas and non-functional adenomas. Interestingly, in adrenocortical carcinomas (ACCs), although BMAL1 mRNA exhibited a similar expression, CRY1 and PER1 mRNA levels were decreased and CLOCK mRNA levels were increased compared to the peritumoural tissues (Angelousi et al. 2018a,b).

Clock genes and thyroid cancer

Thyroid cancer represents the most common endocrine malignancy and accounts for 1% of all human malignancies (Philippe & Dibner 2015). The expression of the BMAL1 was upregulated in tissue samples of follicular thyroid carcinoma (FTC), and of papillary thyroid carcinoma (PTC), as compared with normal thyroid tissue and benign nodules, whereas CRY2 was downregulated in FTC and PTC (Mannic et al. 2013). Human thyrocytes derived from normal thyroid tissue exhibited high-amplitude circadian oscillations of BMAL1-luciferase reporter expression and endogenous clock transcripts. Similarly, thyrocytes derived from FTC and PTC exhibited clock transcript oscillations similar to those of normal thyroid tissue and benign nodules except for PER2 that was altered only in PTC, whereas cells obtained from poorly differentiated thyroid carcinoma exhibited altered circadian oscillations (Mannic et al. 2013) (Table 1).

Hence, while the transition from benign to malignant thyroid nodules does not completely abolish the functional thyroid clock, it is certainly associated with clock alterations, probably restricted to the first cycle kinetics. Moreover, changes of BMAL1 levels in PTC nodules further underline the link between cancerous transformation and changes in the circadian clock or in the individual core-clock genes in the periphery (Mannic et al. 2013).

Clock genes and pituitary neoplasms

In vitro studies have shown in cultures of corticotroph cells that a significant correlation exists between the mRNA expression of proopiomelanocortin (POMC) and PER2 (Tsukamoto-Yamauchi et al. 2015). Knockdown of CLOCK gene significantly reduced prolactin mRNA levels in GH3 cell lines, whereas knockdown of BMAL1 did not induce any change (Tsukamoto-Yamauchi et al. 2015). In vivo, BMAL1-R91A mutant rat exhibited loss of the circadian pattern of endogenous clock-controlled genes and prolactin gene suggesting the presence of a local oscillator in the pituitary (Becquet et al. 2014).
Up-to-date data concerning clock gene expression in pituitary neoplasias are scarce. In human autopsied pituitaries, PER1 mRNA expression showed daytime-dependent differences according to time of death with decreased levels observed at evening, whereas the protein levels of PER1, CRY1 and CLOCK did not fluctuate with the time of day (Becquet et al. 2014). In other studies, circadian rhythmicity has been indirectly studied through melatonin secretion and sleep disturbances (Joustra et al. 2014). Altered melatonin secretion was found in 41% of patients with non-functional pituitary macroadenomas (NFPA) (Joustra et al. 2014); those patients had high daytime levels, no evening rise or severe irregularity of melatonin secretion. Abnormally high serum levels of melatonin during the period of maximum light and abnormally low increases during the night were found in patients with prolactinomas and GH-secreting adenomas without any relation to tumour histotype (melatonin was increased during daytime and decreased during nighttime in both tumour types) (Lissoni et al. 1992). Another study showed increased daytime secretion of melatonin in patients with acromegaly without any correlation with GH and IGF levels (Terzolo et al. 1995) (Table 1). However, no study on clock gene alterations in these patients has been conducted yet.

Studies in patients with craniopharyngiomas showed that circadian rhythm based on body temperature and sleep-wake cycles was disrupted (Foschi et al. 2017) along with decreased nocturnal and increased daytime values of melatonin (Lipton et al. 2009). Daytime sleepiness scores were increased in patients treated for craniopharyngioma compared to healthy controls (although not statistically significant) similarly to patients with NFPA. Neither the type of surgery, previous radiotherapy nor age at diagnosis influenced the sleepiness scores in these patients (van der Klaauw et al. 2008). However, these results cannot clearly indicate whether this disruption of sleep cycle is a consequence of large tumours and/or their treatment in the hypothalamic/pituitary region, or a specific feature of craniopharyngiomas per se.

**Clock genes and parathyroid tumours**

Previous studies have indicated that osteoblasts and osteoclasts as well as bone metabolism are regulated under circadian conditions (Iimura et al. 2012, Dudek & Meng 2014, Fujihara et al. 2014, Hirai et al. 2014). Some bone metabolic markers, such as osteocalcin, N-terminal peptide (NTX) or C-terminal peptide (CTX), have also shown day-night variations in human plasma or urine (Ivaska et al. 2005, Generali et al. 2007). PER or CRY were the main genes influencing the pathways involved in the regulation of bone volume (Fu et al. 2005, Maronde et al. 2010).

Diurnal variation of PTH levels exhibited a pronounced circadian rhythm, obtaining peak levels in the early morning. When comparing patients with primary hyperparathyroidism with healthy subjects, the circadian rhythm of serum PTH level seems to be disrupted in patients, while restoration to normal rhythm was achieved after surgical treatment (Logue et al. 1990).

The mRNA levels of clock genes were significantly altered in parathyroid gland adenomas in patients with sporadic primary hyperparathyroidism or in parathyroid hyperplasia in patients with secondary hyperparathyroidism. In parathyroid adenomas, NFIL3 mRNA levels, encoding for a protein that represses expression of the core-clock components PER1 and PER2 was found downregulated. Similarly, mRNA expression of core-clock genes CRY1, CRY2 and PER2 was also downregulated (Table 1). Furthermore, in parathyroid hyperplasia NFIL3 and CRY2 mRNA levels were also found downregulated. However, no statistical differences were found in core-clock gene expression between parathyroid adenomas and hyperplasia. Of note, PER1 was the only clock gene that was significantly downregulated in parathyroid adenomas compared to normal tissues (Sadowski et al. 2018).

**Clock genes and genital (ovarian/testicular) tumours**

Ovarian cancer is the fourth most common malignant tumour in women and is the leading cause of death from gynaecologic malignancies (Xu et al. 2018). The expression of PER1, PER2, CRY2 and CLOCK in ovarian cancer was significantly lower compared to normal ovaries (Tokunaga et al. 2008). In contrast, CRY1 expression was highest followed by PER3 and BMAL1 suggesting that antiphase expression of CRY1 and BMAL1 may be preserved in ovarian cancers (Tokunaga et al. 2008). CRY1 and BMAL1 expression was significantly reduced in mucinous and grade 3 ovarian tumours compared to serous and endometrioid histological subtypes (Tokunaga et al. 2008). The combination of low CRY1 and low BMAL1 expression was significantly associated with overall survival. Thus, the combination of low CRY1 and low BMAL1 expression was an independent prognostic factor, along with the stage and histological subtype (Tokunaga et al. 2008).

In addition, CLOCK mRNA and protein expression was significantly lower in cisplatin-sensitive ovarian
cancer cells compared to cisplatin-resistant ovarian cancer cells, indicating that CLOCK gene expression was strongly associated with cisplatin resistance in ovarian cancer cells (Xu et al. 2018). The upregulation of CLOCK in ovarian cancer cells reduced their sensitivity to cisplatin treatment (Xu et al. 2018). Following the knockdown of CLOCK in cisplatin-resistant ovarian cancer cells, cisplatin treatment was able to significantly inhibit the proliferation of cells and induce apoptosis (Xu et al. 2018) (Table 1). These findings indicated that inhibiting the circadian CLOCK gene expression can reverse the cisplatin resistance of ovarian cancer cell lines by affecting the protein expression of drug resistance genes. Thus, CLOCK gene may be designated as a novel candidate for targeted gene therapy in drug-resistant ovarian cancer (Sun et al. 2017).

Overexpression of BMAL1 inhibited cell growth and enhanced chemosensitivity of cisplatin in ovarian cancer cells suggesting that BMAL1 may be a tumour suppressor and is epigenetically silenced in ovarian cancer (Yeh et al. 2014). BMAL1 is epigenetically silenced by promoter methylation and histone modifications in ovarian cancer cell lines.

CRY1 is necessary for normal testicular function: CRY1 deficiency increased testicular germ cell apoptosis and decreased sperm count (Li et al. 2018). BMAL1 protein is exclusively expressed in mouse Leydig cells. Leydig cells rhythmically express BMAL1 protein, suggesting peripheral control of testosterone production by this clock protein (Alvarez et al. 2008).

Circadian clock is not functioning during spermatogenesis and can be disrupted in tumours. PIWIL2 (Piwi-like protein) belongs to the category of cancer/testis antigens that are expressed in human tumours but not in normal adult tissues except in the testis. It has been shown that PIWIL2 interacts with BMAL1 and CLOCK and can repress circadian rhythms in the normal testis and cancer cells. In particular, PIWIL2 can bind with E-Box sequences associated with the BMAL1/CLOCK complex to negatively regulate the transcriptional activation of promoters of clock-controlled genes (Lu et al. 2017) (Table 1). Another cancer/testis antigen (Protein PAS domain containing 1 – PASD1) suppresses also circadian rhythms. PASD1 is related to CLOCK and interacts with the CLOCK:BMAL1 complex to repress transcriptional activation (Michael et al. 2015).

**Clock genes and therapy in cancer**

The questions that could reasonably be raised is: What is the therapeutic application of the existing knowledge on clock genes involvement in tumourigenesis? Chronotherapy is the principle of judiciously timed administration of therapeutic interventions. It has been shown that timed delivery of a chemotherapy for colorectal cancer patients significantly improved the treatment efficacy and prolonged the patient's survival (Innominato et al. 2012). Moreover, daytime rather than night-time administration of seliciclib, a cyclin-dependent kinase inhibitor, reduced osteosarcoma progression, and improved the amplitude of clock gene expression rhythms within the tumour tissue (Iurisci et al. 2006).

According to the literature, clock genes have been mainly studied in peripheral endocrine tumours. Thus, peripheral clock system could be a potential therapeutic target. Resetting the circadian clock in tumour cells might inhibit or slow down tumour growth since most cancer cell lines and tumour tissues exhibit a local circadian clock system disruption (Kiessling et al. 2017). Indeed, Kiessling et al. showed that exposition of B16 mouse melanoma cells in various treatments such as dexamethasone (DEX), forskolin or heat shock protein restored rhythmic clock gene expression. Each of these clock-regulated treatments significantly slowed down B16 cell proliferation. Thus, targeting on the tumour clock system at peripheral level might represent an innovative strategy to improve the outcome of the treatments and potentially the patient’s survival.

**Clock genes disruption in tumourigenesis: an association of cause-causality**

Taking into consideration currently existing data on the potential role of clock genes in tumourigenesis irrespective of tissue origin the main hypothesis states that the circadian clock is an important tumour suppressor, and that disrupted circadian rhythms promote tumour development (Fu et al. 2013). In vivo studies including tumour-prone mice, expressing a mutated allele of p53 in mammary glands, showed higher rates of spontaneous tumour formation, when exposed to weekly alternating light cycles, suggesting that internal desynchronization and sleep disturbances contribute to de novo tumourigenesis (Van Dycke et al. 2015). Numerous studies reported that individual molecular components of the circadian clock, such as BMAL1 (Jung et al. 2013, Zeng et al. 2014), PER2 (Miyazaki et al. 2010) or PER1 (Gery et al. 2006) suppress proliferation or increase the sensitivity to anticancer drugs in different cancer cell lines. Thus, these data may lead to the assumption that if an intact circadian clock acts as
a tumour suppressor mechanism, genetic alterations of clock genes in mice could predispose to neoplasia.

However, the prevailing view regarding the antitumour activity of the circadian clock has been questioned by reports that some clock genes support proliferation in normal and cancer cells. For instance, human colorectal cancers often show higher expression of CLOCK or BMAL1 genes compared to healthy tissue (Karantanos et al. 2013, Wang et al. 2013).

These findings indicate that under certain circumstances, clock genes may promote cancer development and therefore their role as tumour suppressors must be re-evaluated. It could be speculated that unique epigenetic signatures of various cancer cell types are likely to define distinct subsets of clock-controlled genes, modulating influence of the circadian clock on proliferation, apoptosis and cell cycle progression, an hypothesis that needs however to be extensively tested.

Conclusions

Recent preclinical and clinical data along with in vitro studies have revealed a connection between the disruption of central and/or peripheral clock system and various cancers through several pathways of tumourigenesis that are dysregulated by clock genes. Whereas the functionality of molecular clocks in normal tissues has been extensively studied and recently resulted in significant breakthroughs, our knowledge of the circadian pattern in tumour cells and neoplasms is still limited and its significance not clearly defined. Data on endocrine neoplasms are scarce due to either the rarity of these neoplasms or their relative good prognosis. In almost all studies clock system has been investigated in the peripheral target organ through analysis of mRNA and protein levels in peripheral tissues. Only 24 h oscillation of melatonin levels has been studied in blood especially in patients with pituitary adenomas. It seems that the PER1 and CRY1 gene expression is the most commonly disrupted in endocrine tumour tissues being mostly downregulated suggesting a suppression of the peripheral clock system. In contrast, in testicular tumours CLOCK/BMAL1 were the most studied and found downregulated, whereas in ovarian cancer data suggest a preserved clock system. However, the role of the central clock and its implication in endocrine tumourigenesis is still unknown. Further studies are required to delineate the exact mechanisms of the central and peripheral clock genes implication, particularly in advanced and/or aggressive endocrine tumours.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

Gregory Kaltatsas and George Chrousos contributed equally to this work.

References


Buhl ED & Takahashi JS 2013 Molecular components of the mammalian circadian clock. Handbook of Experimental Pharmacology 217 3–27. (https://doi.org/10.1007/978-3-642-25950-0_1)


Fujiyama H, Kondo H, Noguchi T & Togari A 2014 Glucocorticoids mediate circadian timing in peripheral osteoclasts resulting in the circadian expression rhythm of osteoclast-related genes. *Bone* **61** 1–9.


Maiese K 2017 Moving to the rhythm with clock (circadian) genes, autophagy, mTOR and SIRT1 in degenerative disease and cancer. Current Neurovascular Research 14 299–304. (https://doi.org/10.2174/15672026146617018092010)


Torres-Farfan C, Seron-Ferre M, Dinet V & Korf HW 2006 Immunocytochemical demonstration of day/night changes of clock gene protein levels in the murine adrenal gland: differences between melanin-proficient (C3H) and melanin-deficient (C57BL) mice. *Journal of Pineal Research* **40** 64–70. (https://doi.org/10.1002/jpe.111.1060.079X.2005.00279.x)


Received in final form 28 March 2019
Accepted 4 April 2019
Accepted Preprint published online 4 April 2019