REVIEW

# Clock genes and cancer development in particular in endocrine tissues

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## 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 tumourigenenesis, 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

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## 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

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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*), cryptochome 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.* 2018*a*,*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

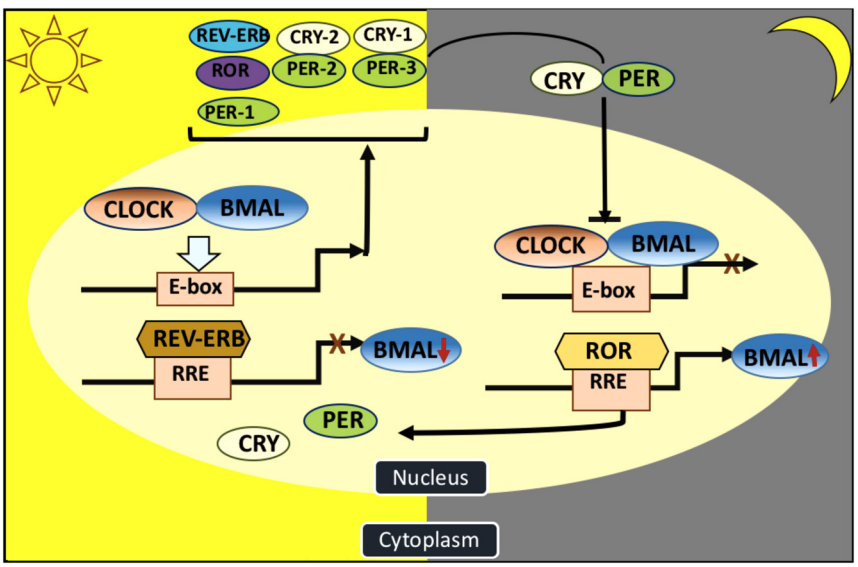


**Figure 1**
Transcription–translation feedback loop of clock genes. The cyclicity of circadian oscillation is generated by autoregulatory transcription–translation-based molecular oscillators, in which transcription at E-box sites occupies a pivotal position. The BMAL1 and CLOCK proteins form a complex 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 $Ca^{2+}$ and/or cAMP lead to the activation of ERK and CREB, and thereby activate the CRE-mediated PER1 expression.

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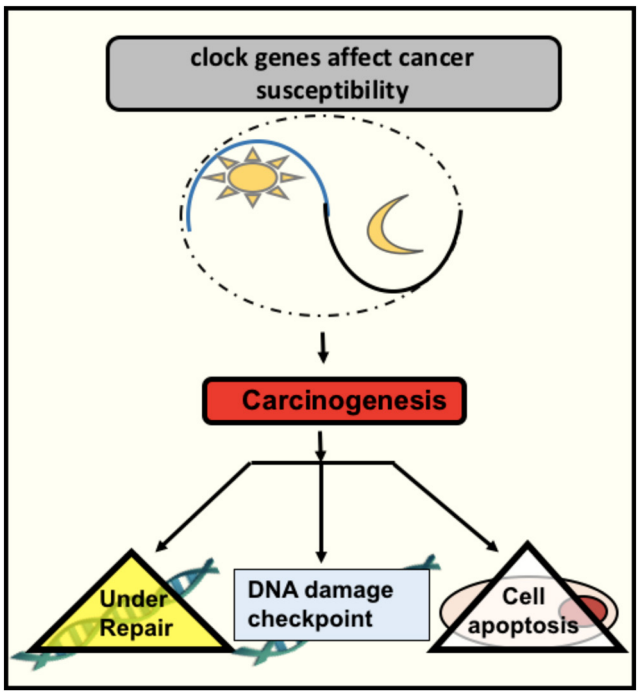


**Figure 2**
Circadian clock and carcinogenesis. Circadian clock disruption can lead to carcinogenesis through disruption of nucleotide excision repair and cell cycle checkpoints response as well as by influencing the intrinsic apoptosis, which is initiated by DNA-damaging agents, and the extrinsic apoptosis, which is initiated by cytokines, including death ligands such as tumour necrosis factor α (TNFα).

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* and *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, PER3, 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.*

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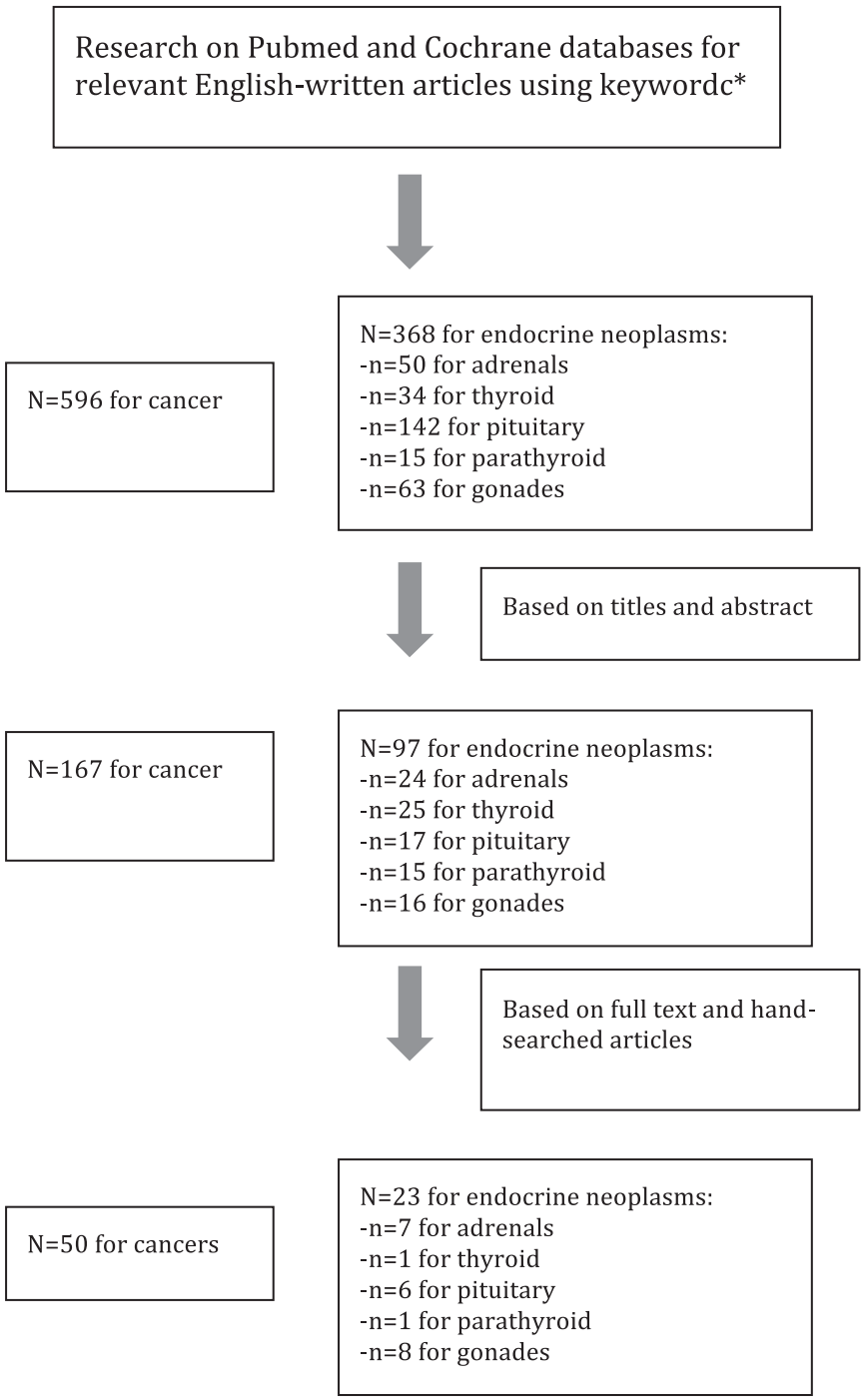


**Figure 3**
Flow diagram. *Keywords (clock genes) AND (adrenals), (clock genes) AND (thyroid), (clock genes) AND (pituitary adenomas), (clock genes) AND (parathyroid), (clock genes) AND (ovarian OR testes) COMBINED with circadian and tumour.

2005, Yeh *et al.* 2005, Gery *et al.* 2007, Hernández-Rosas *et al.* 2018), colorectal cancer (Štorcelová *et al.* 2013) and pancreatic ductal adenocarcinoma (Relles *et al.* 2013), and has been associated with a poor prognosis in these cancers (Zhou *et al.* 2016).

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

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*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-ERBα, 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-ERBα). 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 5 (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 (Konosu-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.* 2018*a*,*b*). Conn adenomas showed upregulation in

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**Table 1** Clock genes disruption in endocrine tumours.

| Ref. | Studied samples | *CLOCK* | *BMAL1* | *CRY1* | *CRY 2* | *PER1* | *PER2* | Others |
|---|---|---|---|---|---|---|---|---|
| Tetti *et al.* (2018) | - Human APA vs normal adrenal tissues<br>- Mice silenced for *PER1* | Nd | Nd | -(mRNA)<br>-(mRNA) after treatment with Ang II | -(mRNA)<br>↓(mRNA) after treatment with Ang II | ↓aldosterone levels in *PER1* heterozygous mice | Nd | Nd |
| Romero *et al.* (2007) | H295R adrenocortical cells | Nd | Nd | Nd | Nd | ↓when stimulation with Ang II (mRNA) | Nd | Nd |
| Angelousi *et al.* (2018) | Human CPA vs normal tissues | ↓(mRNA) | ↓(mRNA) | ↓(mRNA) | Nd | ↓(mRNA) | Nd | REV-ERB, ROR=>↓ (mRNA) |
| Angelousi *et al.* (2018) | Human NFA vs normal tissues | ↓(mRNA) | ↓(mRNA) | ↓(mRNA) | Nd | ↓(mRNA) | Nd | REV-ERB, ROR=>↓ (mRNA) |
| Angelousi *et al.* (2018) | Human Conn adenomas vs normal tissues (mRNA) | ↓(mRNA) | ↓(mRNA) | Nd | Nd | Nd | Nd | REV-ERB, ROR=>↓(mRNA) |
| Angelousi *et al.* (2018) | ACC vs human normal tissues | -(mRNA) | Nd | ↓(mRNA) | Nd | ↓(mRNA) | Nd | Nd |
| Mannic et al. (2013) | Human FTC and PTC tissues vs normal thyroid tissue | Nd | -(mRNA) | Nd | ↓(mRNA) | Nd | ↓(mRNA) | Nd |
| Becquet *et al.* (2014) | Human pituitary tissues | No alteration in protein level | Nd | No alteration in protein level | Nd | ↓(mRNA) but not in the protein level | Nd | Nd |
| Joustra et al. (2014) | Patients with NFA (blood) | Nd | Nd | Nd | Nd | Nd | Nd | Altered melatonin secretion |
| Lissoni et al. (1992) | Patients with prolactinoma and GH-secreting adenomas | Nd | Nd | Nd | Nd | Nd | Nd | Increased daytime secretion of melatonin and low increase in night-time |
| Terzolo *et al.* (1995) | Patients with GH-secreting adenomas (blood) | Nd | Nd | Nd | Nd | Nd | Nd | Increased daytime secretion of melatonin with no correlation however with GH and IGF 1 levels |
| Lipton *et al.* (2009) | Patients with craniopharyngioma (blood) | Nd | Nd | Nd | Nd | Nd | Nd | Decreased night-time and increased daytime values of melatonin |
| Sadowski *et al.* (2018) | Human parathyroid adenoma or hyperplasia vs normal parathyroid tissues | Nd | Nd | ↓(mRNA) in adenoma | ↓(mRNA) in adenoma | ↓(mRNA) in adenoma and hyperplasia vs. normal | Nd | Nd |

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|---|---|---|---|---|---|---|---|---|---|
| Xu *et al.* (2018) | Ovarian cancer cells (cisplatin-sensitive A2780 vs. cisplatin-resistant CP70 cells) | ↓(mRNA and protein) in cisplatin-sensitive cells compared vs cisplatin-resistant cells | Nd | Nd | Nd | Nd | Nd | Nd | |
| Tokunaga *et al.* (2008) | Epithelial ovarian cancer vs. normal tissues | -(mRNA) | -(mRNA) | -(mRNA) | ↓(mRNA) | ↓(mRNA) | ↓(mRNA) | | |
| Lu *et al.* (2017) | Mouse testes injected with PIWIL2 and cells cultures | ↓(mRNA, protein) by PIWIL2 | ↓(mRNA, protein) through PIWIL2 | Nd | Nd | Nd | ↓(mRNA, protein) by PIWIL2 | Rev-Erbα: ↓(mRNA, protein) by PIWIL2 | |
| Michael *et al.* (2015) | Human cancer cells (H1299 cell line, SW480 cell line) | ↓(mRNA) by PASD1 | ↓(mRNA) by PASD1 | Nd | Nd | Nd | Nd | Nd | |

-, upregulated; ↓, downregulated; APA, Aldosterone-Producing Adenomas; Nd, No data; Ang II, Angiotensin II; GH, growth hormone; CPA, Cortisol-Producing Adenomas; NFA, Non-Functional Adenomas; ACC, Adrenocortical carcinoma; FTC and PTC, Follicular and Papillary Thyroid Carcinoma; PIWIL2, Piwi-like protein 2; PASD1, Protein PAS domain containing 1.

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.* 2018*a*,*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).

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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 night-time 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

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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 both 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

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a tumour suppressor mechanism, genetic alterations of clock genes in mice could predispose to neoplasia.

However, the prevailing view regarding the antitumor 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.

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