

**Manuscript version: Author's Accepted Manuscript**

The version presented in WRAP is the author's accepted manuscript and may differ from the published version or Version of Record.

**Persistent WRAP URL:**

<http://wrap.warwick.ac.uk/149208>

**How to cite:**

Please refer to published version for the most recent bibliographic citation information. If a published version is known of, the repository item page linked to above, will contain details on accessing it.

**Copyright and reuse:**

The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions.

Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

**Publisher's statement:**

Please refer to the repository item page, publisher's statement section, for further information.

For more information, please contact the WRAP Team at: [wrap@warwick.ac.uk](mailto:wrap@warwick.ac.uk).

# 1 **Cancer clocks in tumorigenesis: The p53 pathway and beyond**

2 Ewan M Stephenson<sup>1,2,3</sup>, Laura E J Usselman<sup>2</sup>, Vinay Tergaonkar<sup>1</sup>, David M Virshup<sup>3</sup>, Robert  
3 Dallmann<sup>2</sup>

4 1. Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology  
5 and Research, Singapore

6 2. Division of Biomedical Sciences, Warwick Medical School, University of Warwick,  
7 Coventry, United Kingdom

8 3. Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore

9

## 10 **DECLARATION OF INTEREST**

11 The authors declare no conflict of interest that could undermine the impartiality of this  
12 review.

13

## 14 **FUNDING**

15 E.M.S. is supported financially by a doctoral scholarship from the Warwick-A\*STAR  
16 Research Attachment Programme (ARAP), the A\*STAR Graduate Academy and the Warwick  
17 University Doctoral College. L.E.J.U. is supported by the UK Medical Research Council  
18 Doctoral Training Partnership (MR/N014294/1) and Warwick University Doctoral College.  
19 V.T. lab is supported by IMCB core funds and Singapore National Research Foundation's  
20 Competitive Research Programme (NRF-CRP17-2017-02). D.M.V. lab is supported by the  
21 Singapore National Medical Research Council (NMRC/CIRG/1465/2017). R.D. lab is  
22 supported by the Warwick Innovation Fund and Cancer Research UK (C53720/A29468).

## 23 **Abstract**

24 Circadian rhythms regulate a vast array of physiological and cellular processes, as well  
25 as the hormonal milieu, to keep our cells synchronised to the light-dark cycle. Epidemiologic  
26 studies have implicated circadian disruption in the development of breast and other cancers,  
27 and numerous clock genes are dysregulated in human tumours. Here we review the evidence  
28 that circadian rhythms, when altered at the molecular level, influence cancer growth. We also  
29 note some common pitfalls in circadian-cancer research and how they might be avoided to  
30 maximise comparable results and minimise misleading data.

31 Studies of circadian gene mutant mice, and human cancer models *in vitro* and *in vivo*,  
32 demonstrate that clock genes can impact tumourigenesis. Clock genes influence important  
33 cancer related pathways, ranging from p53-mediated apoptosis to cell cycle progression.  
34 Confusingly, clock dysfunction can be both pro- or anti- tumourigenic in a model and cell type  
35 specific manner. Due to this duality, there is no canonical mechanism for clock interaction with  
36 tumourigenic pathways.

37 To understand the role of the circadian clock in patients' tumours requires analysis of  
38 the molecular clock status compared to healthy tissue. Novel mathematical approaches are  
39 under development, but this remains largely aspirational, and is hampered by a lack of temporal  
40 information in publicly available datasets.

41 Current evidence broadly supports the notion that the circadian clock is important for  
42 cancer biology. More work is necessary to develop an overarching model of this connection.  
43 Future studies would do well to analyse the clock network in addition to alterations in single  
44 clock genes.

45

46 **Alternative Gene Abbreviations:** BMAL1 (ARNTL, MOP3), DEC1/2  
47 (BHLHE40/BHLHE41), REV-ERB $\alpha/\beta$  (NR1D1/NR1D2), CK1 $\delta/\epsilon$  (CSNK1D, CSNK1E),  
48 NFIL3 (E4BP4)

## 49 **Introduction**

50 In 1729, French astronomer Jean-Jaques d'Ortous de Mairan observed the *Mimosa*  
51 *pudica* plant opening and closing its leaves in accordance with the geophysical day, even when  
52 deprived of a light source (de Mairan, 1729). In the centuries since, oscillations that coordinate  
53 normal physiology with the 24-hour light/dark cycle have been discovered across the kingdoms  
54 of life (Rosbash, 2009). Disrupted circadian rhythms contribute to many human diseases  
55 including diabetes, obesity and depression. An epidemiological link between circadian rhythms  
56 and cancer was established as early as 1996, and multiple papers on this topic have been  
57 subsequently published (Tynes et al., 1996). The WHO classifies circadian disruption as a  
58 probable human carcinogen (IARC, 2019), though there is debate as to the validity or strength  
59 of this association (Zhang and Papantoniou, 2019, Rivera et al., 2020, Dun et al., 2020,  
60 Wegrzyn et al., 2017).

61 Unsurprisingly, circadian influence over the endocrine system has been implicated in  
62 this proposed cancer-circadian link. In 1987, Stevens suggested that melatonin suppression  
63 from light at night may play a role in breast cancer biology (Stevens, 1987). This 'melatonin  
64 hypothesis' is out-of-vogue currently owing to contradictory data, these discrepancies and  
65 potential explanations for them are well reviewed by Hunter and Figueiro (Hunter and Figueiro,  
66 2017). There is established circadian variation in many cancer relevant hormones including  
67 melatonin, sex hormones, thyroid hormones and corticosteroids (Prasai et al., 2011). In fact,  
68 the link between hormones, circadian rhythms, and cancer is not one way. Pheochromocytomas  
69 have been observed disrupting circadian rhythms via aberrant hormone release (Tabebi et al.,  
70 2018).

71 At the cellular level, animal models deficient for one or more core circadian genes are  
72 cancer prone (Fu et al., 2002). Cancers are known for aberrant gene expression and therefore,  
73 it is possible that the circadian system is dysregulated within tumours, irrespective of the  
74 rhythm of the animal as a whole. Indeed, an association between cancer severity and circadian  
75 status of the tumour has been documented, and escape from circadian regulation is suggested  
76 to be an emerging hallmark of cancer (Papagiannakopoulos et al., 2016, El-Athman and  
77 Relógio, 2018). Despite this, our understanding of the role of the circadian system and the  
78 influence of specific circadian genes, in tumourigenesis is still developing.

79

80

## 81 **Molecular Clockwork**

82 In most mammals, light is a strong ‘Zeitgeber’, i.e., an external cue that can entrain  
83 circadian rhythms (Duffy and Czeisler, 2009). Light triggers a signal which is transmitted to  
84 the suprachiasmatic nuclei (SCN), an area of the ventral hypothalamus referred to as the  
85 ‘central pacemaker’ (Gooley et al., 2001, Hattar et al., 2002). These signals reset the clock in  
86 these neurons by activating light inducible elements in core clock gene promoters (Astiz et al.,  
87 2019). The SCN then goes on to synchronise peripheral clocks in cells throughout the body.  
88 Notably, melatonin and glucocorticoid secretion are under SCN regulation and are thought to  
89 have key roles in synchronising peripheral clocks. Glucocorticoids are such strong circadian  
90 synchronisers that they are often used to synchronise cells *in vitro* for circadian experiments  
91 (Balsalobre et al., 2000, Prasai et al., 2011).

92 The ultimate units of circadian timekeeping are cell-autonomous  
93 transcriptional/translational/post-translational feedback loops (Fig 1). In the core circadian  
94 loop, the circadian locomotor output cycles kaput (CLOCK) protein forms a heterodimer with  
95 brain and muscle ARNT-like protein 1 (BMAL1). At cycle start, this CLOCK/BMAL1  
96 heterodimer can bind to E-box promoter elements (CANNTG) and promote transcription.  
97 Amongst those genes activated in this way are the period (*PER1*, *PER2* & *PER3*) and  
98 cryptochrome (*CRY1* & *CRY2*) genes. PER and CRY proteins then accumulate in the cytoplasm  
99 and eventually multimerise forming a ~1 megadalton complex along with Casein Kinase 1  $\delta$   
100 and  $\epsilon$  (CK1 $\delta$ /CK1 $\epsilon$ ) and Rab5-activating protein 6 (GAPVD1) (Aryal et al., 2017, Brown et  
101 al., 2005).

102 In the cytoplasm, post-translational modifications fine tune the system. PER2 is post-  
103 translationally regulated via a phosphoswitch mechanism (Zhou et al., 2015). CK1 can  
104 phosphorylate two distinct regions of PER2. Phosphorylation on the FASP (Familial Advanced  
105 Sleep Phase) site leads to PER2 stabilisation, whereas phosphorylation of the degron leads to  
106 degradation via  $\beta$ -TrCP. The ‘decision making’ region appears to be a loop of CK1, the  
107 conformation of which can influence site specificity (Philpott et al., 2020). If PER2 is not  
108 degraded it can be translocated into the nucleus along with CRY proteins and CK1.

109 In the nucleus, PERs and CRYs repress activity of the CLOCK/BMAL1 heterodimer  
110 by preventing its transcriptional activity or dissociating the complex from DNA (Ye et al.,

111 2014). Therefore, PERs and CRYs inhibit their own transcription and the transcription of the  
112 numerous genes controlled by CLOCK/BMAL1, which closes the loop. It is worth noting that  
113 CLOCK/BMAL1 is not the only activating complex that can bind to E-boxes. NPAS2 can  
114 substitute for CLOCK at least in some cells (DeBruyne et al., 2007). Moreover, for example,  
115 MYC is also capable of binding to E-boxes in direct competition to the CLOCK/BMAL1  
116 complex and, therefore, MYC can dampen or stop the clock (Altman et al., 2015).

117 A secondary loop adds to the stability and complexity of the system. The *REV-ERB $\alpha$*   
118 and *REV-ERB $\beta$*  promoters contain an E-box, and are transcriptionally activated by the  
119 CLOCK/BMAL1 heterodimer. REV-ERB $\alpha$  and REV-ERB $\beta$  compete with retinoic acid-related  
120 orphan receptors (ROR $\alpha$ , ROR $\beta$  & ROR $\gamma$ ) for ROR binding sites in the BMAL1 promoter.  
121 RORs activate *BMAL1* transcription and REV-ERBs repress it. These primary and secondary  
122 feedback loops constitute the canonical circadian system and have analogues across a variety  
123 of species (Brown et al., 2012). In mammals, however, there are a number of sub-loops which  
124 interlock with either the primary or secondary core loop, proteins involved in these include  
125 DBP, DEC1 and DEC2 (Takahashi, 2016, Nakashima et al., 2008).

126 Manipulation of key components of these interlocking feedback loops has dramatic  
127 effects on the clock. This is well studied in circadian gene knockout and mutation models in  
128 *Drosophila* and mice. These modifications to the clock elements range from absolute  
129 arrhythmicity to changes in circadian period, as measured by activity monitoring in whole  
130 animals (Bunger et al., 2000, Lee et al., 2004). Notably, *BMAL1* knockout mice are completely  
131 arrhythmic in their behaviour, with nocturnal activity replaced by more random patterns of  
132 activity irrespective of time of day (Bunger et al., 2000).

## 133 **Clocks in Tumourigenesis**

134 This intricate system, and disruptions therein, can have profound effects on human  
135 health (Roenneberg and Merrow, 2016). Circadian rhythms influence homeostasis and regulate  
136 a variety of integral hormones including insulin, glucagon, oestrogen and progesterone, as well  
137 as controlling a host of other processes ranging from immunity to metabolism (Petrenko and  
138 Dibner, 2017, Rahman et al., 2019). It is, therefore, unsurprising that circadian biology has  
139 been implicated in a variety of prevalent conditions, including metabolic syndromes, obesity  
140 and cardiovascular disease. This review focuses on the interplay between circadian rhythms  
141 and cancer (Bae et al., 2019, Buurma et al., 2019, Noh, 2018).

142 Expression levels of circadian genes, the amplitude of oscillation, and clock output  
143 genes differ between cell types and tissues (Zhang et al., 2014, Mure et al., 2018). Due to this,  
144 there may be distinct pro- or anti-tumourigenic roles for each core clock gene in various tumour  
145 types. A number of studies have attempted to elucidate the roles of each of the clock genes in  
146 various *in vitro* and *in vivo* models (Fig 2).

## 147 **ISSUES WITH MOLECULAR CLOCK RESEARCH IN TUMOURS**

148 Based on animal as well as human studies, more than 20% of all transcripts are clock  
149 regulated and can vary many-fold over the day (Zhang et al., 2014). A lack of timing  
150 information for human tissue samples plagues many of the studies to be discussed. If timing  
151 information is unavailable for bio-samples it is impossible to know if a difference in expression  
152 is due to true differential expression or sampling at different phases of the circadian cycle.  
153 Therefore, care must be taken in interpreting claims of up or downregulated oscillating genes.  
154 Some studies address this by comparing tumour clock gene expression to that of the adjacent  
155 tumour margin (Ye et al., 2018). This approach should still be applied with caution, as tumour  
156 margin cells are not equivalent to healthy cells and it is only assumed that the tumour margin  
157 and the tumour are in phase with each other (Aran et al., 2017).

158 Even studies in experimental *in vitro* and *in vivo* models can suffer from a lack of  
159 information. Overexpression, knockdown or knockout of circadian genes will result in  
160 modified rhythmic gene expression. For example, overexpression of PER2 reduces  
161 transcription of *CRY1* & *CRY2* and will, therefore, have profound effects on circadian  
162 oscillation (Chen et al., 2009). Therefore, circadian gene manipulations should be proceeded  
163 and succeeded by an assessment of the cell's overall circadian function. These knock-on effects  
164 may underlie observed phenotypes. Moreover, to compare the consequence of a clock gene  
165 modification across cell lines it would be pertinent to have time-of-sampling information even  
166 for *in vitro* experiments as this, combined with knowledge of the cells modified rhythmic  
167 behaviour, could shed light on the mechanisms behind cell specific tumourigenic effects of  
168 clock genes. Additionally, clock genes can have pleiotropic effects independent of their core  
169 clock function. For example, CLOCK/BMAL1 can bind to E-box sequences regardless of  
170 whether it is regulated in an oscillatory manner. CLOCK/BMAL1 regulated by the clock,  
171 CLOCK/BMAL1 unregulated by the clock, and complete loss of CLOCK/BMAL1 proteins  
172 each would have different consequences for cell function. These distinctions are likely to apply  
173 to most all circadian proteins. To understand clock gene modifications in the context of the

174 overall circadian clock, i.e., functional analysis via reporter assays, multiple timed samples or  
175 the use of one sample combined with the use of a multi-dimensional mathematical model is  
176 required.

## 177 **BMAL1**

178 In humans, *BMAL1* has been associated with a number of cancers including breast,  
179 pancreatic and thyroid tumours, though its role in these endocrine cancers is incongruous.  
180 BMAL1 is often depicted as the centre of the core circadian system, owing to the  
181 BMAL1/CLOCK heterodimer being the canonical positive arm and driver of circadian gene  
182 transcription (Fig 1), and *BMAL1* is the only known single gene knockout that is considered to  
183 completely stop the clock (Baggs et al., 2009). Unsurprisingly, this has led it to be amongst the  
184 best studied circadian regulators in regard to tumourigenesis. In breast cancer, reduced *BMAL1*  
185 is consistently associated with increased risk of metastasis (Ramos et al., 2020). Similarly,  
186 pancreatic cancer cells are reported to have less *BMAL1* than non-cancerous controls (Jiang et  
187 al., 2016). However, thyroid carcinoma and a subset of malignant plural mesothelioma (MPM)  
188 had upregulated *BMAL1* (Ye et al., 2018, Elshazley et al., 2012). Despite its links to endocrine  
189 related cancers in humans, little evidence thus far has been unearthed linking BMAL1 to  
190 endocrine pathways in tumourigenesis.

191 In xenograft models *BMAL1* appears to be anti-tumourigenic. BMAL1 knockdown  
192 (k/d) in a pancreatic cancer cell line with high endogenous BMAL1 expression (BxPC-3)  
193 increased subcutaneous tumour size and matrix metalloproteases (MMPs) 9 and 2. BMAL1  
194 overexpression in a pancreatic cell line with low endogenous BMAL1 expression (AsPC-1)  
195 showed converse effects (Jiang et al., 2016). This may be a p53 pathway effect as BMAL1 was  
196 shown to be bound to the p53 promoter and overexpression of BMAL1 induced an upregulation  
197 of phospho-p53 whereas BMAL1 k/d reduced it. It is not clear how transcriptional regulation  
198 via BMAL1 could upregulate phospho-p53, but not overall p53 (Jiang et al., 2016). Similarly,  
199 in hepatocellular carcinoma (HCC), BMAL1 also appears to inhibit tumour growth.  
200 Canonically, hepatocyte nuclear factor 4 alpha (HNF4 $\alpha$ ) is a tumour suppressing transcriptional  
201 repressor. However, a subset of liver tumours expresses an isoform named P2-HNF4 $\alpha$ . Both  
202 isoforms of HNF4 $\alpha$  can repress BMAL1 transcription, however P2-HNF4 $\alpha$  has a much  
203 stronger effect. Forcing P2-HNF4 $\alpha$  expressing HCC cells to re-express BMAL1 caused a  
204 reduction in tumour growth subcutaneously in mice and an induction of both p53 and cleaved  
205 caspase 3 (Fekry et al., 2018).

206 *BMAL1*'s role in tumours appears cell type specific in *in vivo* models utilising mouse  
207 cancer cells and *in vitro* models. Murine colon adenocarcinoma (C26) cells with *BMAL1* k/d  
208 formed significantly larger tumours than their wildtype (WT) counterparts (Zeng et al., 2010).  
209 In a genetically engineered mouse model of lung adenocarcinoma, tumour specific loss of  
210 *BMAL1* increased tumour burden, tumour weight, proliferation and *MYC* production. Notably,  
211 this effect was absent if performed in a *p53* deficient background, adding credence to the  
212 proposed *p53*-*BMAL1* interplay in tumourigenesis (Papagiannakopoulos et al., 2016).  
213 Conversely, in an acute myeloid leukaemia (AML) model in mice it was demonstrated that  
214 *BMAL1* k/d cells are depleted *in vivo* compared to their WT counterparts. This suggests that,  
215 in this instance, *BMAL1* confers a survival advantage to the cancer cells (Puram et al., 2016).  
216 Consistent with these findings, HCT116 (human colorectal carcinoma) cells with *BMAL1* k/d  
217 formed significantly smaller tumours when grafted onto zebrafish embryos than HCT116 WT.  
218 This model system is further removed from mammalian biology and it is unclear if this  
219 observation is a result of the cell type or the model. Interestingly, in HCT116 *BMAL1* k/d cells,  
220 *p53* was shown convincingly to oscillate at the mRNA level despite no oscillation at the  
221 *BMAL1* promoter (Basti et al., 2020). Studies *in vitro* have demonstrated *BMAL1* acting as a  
222 suppressor of proliferation in BxPC-3, AsPC-1, HCT116, C26 and U87MG (glioblastoma) cell  
223 lines and in patient derived glioblastoma stem cells, but acting as a proliferation enhancer in  
224 the human MPM cell lines ACC-MESO-1 and NCI-H290 (Jiang et al., 2016, Basti et al., 2020,  
225 Zeng et al., 2010, Gwon et al., 2020, Dong et al., 2019, Elshazley et al., 2012).

226 *BMAL1* is suggested to play a role in multiple endocrine and non-endocrine cancers,  
227 though its role does not seem consistent across various models, even within the same cancer  
228 type. Interestingly, the tumour types where *BMAL1* is suggested to be pro-tumourigenic,  
229 thyroid carcinoma, mesothelioma and AML are cancer types with low incidence of *p53*  
230 mutations in humans with ~1%, 15% and 5%, respectively (Donehower et al., 2019). As *p53*  
231 is prevalent in downstream analysis of *BMAL1* k/d effects it is plausible that some *p53*  
232 mutations in the tumour may influence tumourigenic effects of *BMAL1*. Discoveries such as  
233 *p53* oscillation upon *BMAL1* k/d in HCT116 hint at a link to rhythmicity. *MMP9* has also been  
234 reported to be suppressed by *BMAL1* in glioblastoma, pancreatic and breast cancer suggesting  
235 that *BMAL1* may be tied to metastatic and angiogenic potential (Gwon et al., 2020, Jiang et  
236 al., 2016, Wang et al., 2019). It would also be pertinent to have better models in these studies  
237 to more effectively demonstrate clinical relevance. Orthotopic xenografts are largely absent in  
238 *BMAL1* manipulation studies and should be considered for their greater clinical relevance in

239 BMAL1 and other clock gene studies. Alternatively, as the clock is tied to inflammation and  
240 immunity, the use of humanised mouse models or simply immunocompetent mouse models  
241 may unearth new pathways by which abrogation of the molecular clock impacts tumourigenesis  
242 (Comas et al., 2017).

## 243 **CLOCK**

244 In humans, CLOCK has been linked to breast cancer and oestrogen signalling (Rossetti  
245 et al., 2012, Xiao et al., 2014). Though it is as yet unclear if there are links between CLOCK  
246 and other tumour relevant hormones. CLOCK, together with its binding partner BMAL1, is a  
247 transcriptional activator. CLOCK is not completely indispensable for circadian function,  
248 though CLOCK has other unique functions such as acting as an acetyltransferase (Doi et al.,  
249 2006). Cadenas et al. (2014) found an association between clock genes and tumours in humans,  
250 combining 766 microarray transcriptomes from several cohorts of breast tumour patients.  
251 Prolonged metastasis free survival was associated with higher expression of a number of  
252 circadian genes, including *CLOCK* (Cadenas et al., 2014). Fittingly, the six most frequent  
253 single nucleotide polymorphisms (SNPs) that occur in *CLOCK* are associated with significant  
254 changes in breast cancer risk (Hoffman et al., 2010). Furthermore, in higher grade gliomas and  
255 ER $\alpha$ -positive breast tumours *CLOCK* have been reported to be upregulated (Chen et al., 2013,  
256 Xiao et al., 2014).

257 *In vitro* and *in vivo* models show CLOCK as a tumour enhancer. Though it is  
258 significantly less well studied than BMAL1. In a murine AML model cells lacking CLOCK  
259 were depleted compared to WT, much like BMAL1 k/d, and both knockdowns prevented cell  
260 cycle progression (Puram et al., 2016). Similarly, in a subcutaneous flank model of human  
261 colon cancer SW480 cells endogenously expressing low levels of CLOCK, CLOCK  
262 overexpression led to larger tumours after two weeks. Mechanistic interrogation found that  
263 overexpressing CLOCK caused a decrease in apoptosis related proteins BAX and BID and an  
264 increase in p-AKT (Wang et al., 2015b). CLOCK k/d cells also had reduced metastatic potential  
265 in mice and reduced expression of HIF1 $\alpha$ , ARNT and VEGF, all known to be key players in  
266 angiogenesis (Wang et al., 2017). *In vitro*, SW620 cells endogenously expressing high levels  
267 of CLOCK showed decreased proliferation and decreased cell migration after CLOCK k/d,  
268 while CLOCK overexpression in an endogenously low expressing cell line, SW480, resulted  
269 in the opposite effect (Wang et al., 2015b, Wang et al., 2017). CLOCK k/d in U87MG cells  
270 (human glioblastoma) increased apoptosis, along with decreased MYC and CCNB1 (Cyclin

271 B1) (Wang et al., 2016). It is at present unclear if the apoptosis mechanism here is the same as  
272 seen in Wang et al. (2015b). Fittingly, CLOCK k/d has also been demonstrated to reduce  
273 viability in glioblastoma stem cells and induce cleaved caspase 3 (Dong et al., 2019). In  
274 addition to apoptosis and angiogenic related pathways, CLOCK may be integrated closely with  
275 oestrogen (E2) and oestrogen receptor  $\alpha$  (ER $\alpha$ ) signalling in cancer. In ER $\alpha$  positive breast  
276 cancer cell lines, E2 has been demonstrated to upregulate CLOCK protein and mRNA by  
277 increasing ER $\alpha$  binding to the CLOCK promoter. A CLOCK k/d in these cells reduced  
278 proliferation (Xiao et al., 2014).

279 The two pathways suggested to give rise to a CLOCK mediated effect on  
280 tumorigenesis are apoptosis and oestrogen signalling. Oestrogen signalling is an attractive  
281 avenue as it has been previously demonstrated that BMAL1 k/d can modify ER signalling and  
282 lead to aberrant breast acinar morphogenesis (Rossetti et al., 2012). It may be pertinent to  
283 investigate if other circadian genes have similar links to oestrogen. Apoptosis-related proteins  
284 have been implicated in this system in multiple studies, therefore it would be useful for future  
285 studies to probe for those proteins that have been implicated before. This would demonstrate if  
286 apoptosis instigated by CLOCK k/d is via the same mechanism in all cell types. Additionally,  
287 some caution must be taken in assuming that CLOCK acts as a tumorigenic enhancer in all  
288 cancers. It is conceivable that its effects are just as cell-type specific as those of its  
289 heterodimeric partner and the research is simply sparser. Notably, in human patients, an anti-  
290 tumorigenic role for CLOCK was suggested that is not seen in xenograft models. NPAS2 is a  
291 paralogue of CLOCK that can compensate somewhat for CLOCK loss in the brain, moreover  
292 some evidence suggests that it may also be able to do this in the periphery (DeBruyne et al.,  
293 2007, Landgraf et al., 2016). In future it would be interesting for studies to take this into account  
294 and perform double knockdowns.

## 295 **PERIOD GENES**

296 Of all clock genes, the PER proteins have perhaps the most variable effects on  
297 tumorigenesis, with evidence for a tumour suppressive role and a tumour enhancing role in  
298 both human data and in experimental models. PER1 and PER2 are core components of the  
299 negative arm of the circadian clock as they suppress the function of the CLOCK/BMAL1  
300 heterodimer. PER2 specifically is a very well researched clock protein with a complex  
301 circadian function (Konopka and Benzer, 1971, Philpott et al., 2020). As its expression  
302 oscillates distinctly over the circadian cycle, PER2 is a good rhythm marker. Overexpression

303 of PER2 can stop the circadian clock similar to BMAL1 k/o (Chen et al., 2009). Unlike PER1  
304 and PER2, which have similar and well-defined roles in the clock, PER3 remains elusive. Its  
305 role, if any, in cell autonomous clocks appears tissue specific (Pendergast et al., 2012).  
306 However, a number of PER3 polymorphisms have been described with a variety of sleep  
307 phenotypes (Hida et al., 2014).

308 Patient sample data thus far suggests a tumour suppressive function for *PER1&3*, with  
309 *PER2*'s role being less clear. A large study of datasets in The Cancer Genome Atlas suggested  
310 an overall tumour suppressive role for the *PERs*, however, *PER1,2&3* expression were  
311 associated with pro-tumourigenic inhibition of apoptosis and activation of RAS/MAPK or  
312 receptor tyrosine kinase (RTK) signalling in a sizeable minority of the cancer types analysed  
313 (Ye et al., 2018). Expression of all three *PERs* differed in 95% of breast tumours relative to  
314 margins, though no statistical analysis was presented for this data. Notably, the *PER* expression  
315 differed between individuals and between cell populations within tumour slices (Chen et al.,  
316 2005). Other groups have found similar results with *PER1&2* being under-expressed in breast  
317 tumours and gliomas, and low expression of *PER1,2&3* correlating with poorer overall survival  
318 in cases of pancreatic ductal adenocarcinoma (PDA) (Winter et al., 2007, Xia et al., 2010,  
319 Relles et al., 2013). In head and neck squamous cell carcinoma, low expression of both *PER1*  
320 and *PER3* was predictive of poorer 2-year survival. Low *PER3* expression was associated with  
321 larger tumour size and increased invasiveness, which are potentially better metrics than  
322 survival as this specific study had a low sample size and high overall 2-year survival (Hsu et  
323 al., 2012). *PER1* is reported to be down-regulated in breast tumours relative to healthy controls  
324 and in colorectal cancer low expression was associated with increased metastasis (Gery et al.,  
325 2006, Oshima et al., 2011). In gastric cancer *PER2* was actually found to be upregulated  
326 compared to paired margin biopsies, complicating its potential role (Hu et al., 2014). Finally,  
327 hetero- or homozygosity for a particular variant of *PER3* resulted in 1.7-fold elevated risk of  
328 breast cancer (Zhu et al., 2005).

329 Experimentally, PER2 appears anti-tumourigenic *in vivo* and *in vitro*. K562, human  
330 chronic myelogenous leukaemia (CML), cells have demonstrated reduced growth capacity in  
331 bone marrow upon PER2 overexpression. Induction of p53 as well as reduced CCNB1 and  
332 MYC were suggested as potential mechanisms (Sun et al., 2010). Similarly, mutation of PER2  
333 increased proliferation of lung cancer cells in mice, this was conserved in a p53 deficient  
334 background (Papagiannakopoulos et al., 2016). In contrast, HCT116 xenografts in zebrafish  
335 embryos showed no change upon PER2 k/d (Basti et al., 2020). Overexpression of *PER2* in

336 murine Lewis Lung Carcinoma or EMT6 (mouse mammary carcinoma) cells *in vitro* results in  
337 reduced proliferation and increased apoptosis (Hua et al., 2006). Delivery of PER2 ectopically  
338 to murine lung tumours can reduce their size and growth (Hua et al., 2007). In human pancreatic  
339 cancer cell lines *PER2* increases BAX and reduces BCL-X in a dose dependant manner, as well  
340 as in human osteosarcoma where a reduction in *PER2* increased phospho-AKT and BCL-2 and  
341 reduced p27 and p21, implicating both cell cycle and apoptosis pathways (Oda et al., 2009, Qin  
342 et al., 2018). Conversely, Basti et al. found, despite the lack of effect *in vivo*, their PER2 k/d in  
343 HCT116 conferred the highest rate of proliferation *in vitro* until day 5, similar to PER2's  
344 tumour suppressive effects in CML and lung cancer cells *in vitro* (Basti et al., 2020, Sun et al.,  
345 2010, Papagiannakopoulos et al., 2016).

346 Experimental evidence for the role of PER1 and PER3 in tumourigenesis is scarce and  
347 that which is available is purely *in vitro*. PER1 overexpression is reported to increase DNA  
348 damage induced apoptosis in HCT116 cells, PER1 k/d having the opposite effect (Gery et al.,  
349 2006). In PaCa2 (human pancreatic cancer cells) PER1 k/d caused a decrease in proliferation,  
350 PER3 also caused a dip in proliferation but it was found to be statistically insignificant. These  
351 findings in PaCa2 are interesting as TNF $\alpha$  treatment of PaCa2 increases proliferation and  
352 downregulates PER1 and PER3 expression, suggesting PERs may be downstream of this TNF $\alpha$   
353 effect (Suzuki et al., 2008). PaCa-2 and PANC-1 cell lines both demonstrate increased  
354 apoptosis upon PER1 k/d and display an increase in BAX and cleaved PARP as well as a  
355 decrease in BCL-2 (Sato et al., 2009).

356 Apoptosis and the cell cycle have both been implicated in the role of the PERs in  
357 tumours. PER2 is fairly well studied however, PER1 lacks *in vivo* data and PER3 is generally  
358 understudied. The finding that p53 is dispensable for PER2's effect on tumourigenesis in  
359 murine lung cancer, contrary to BMAL1, clearly demonstrates that the mechanism of action of  
360 each clock gene in tumourigenesis can be distinct, despite phenotypic similarities  
361 (Papagiannakopoulos et al., 2016). From the data discussed here it seems likely that the anti-  
362 tumourigenic effect of PER2 is via a combination of apoptotic and cell cycle effects, though it  
363 remains unclear if the exact same parts of these diverse pathways are involved across multiple  
364 tumour types. PER1 is thought to have a similar role in the circadian system as PER2, however  
365 the minimal mechanistic information in cancer seems to show an opposite effect. PER1  
366 increases BCL-2 and decreases BAX and vice versa for PER2. A study interrogating the  
367 tumourigenic role of both PER1 and PER2 in the same cell line would be ideal. Sato et al.  
368 suggest PER1's effect on tumourigenesis may be due to PER1 dysregulation having a wider

369 impact on the circadian system as a whole, highlighting the aforementioned importance of  
370 assessing the whole molecular clock in models (Sato et al., 2009).

## 371 **CRY1 & CRY2**

372 In human cancer the role of the CRYs is very unclear, with studies suggesting they are  
373 tumour suppressive and others suggesting them to be tumour enhancing. The CRY proteins are  
374 important for circadian rhythms across the animal kingdom. In mammals they seem to have  
375 forgone their ancient light sensing ability, but still remain transcriptional repressors which,  
376 along with the PERs form the primary negative arm of the circadian clock (Michael et al.,  
377 2017). In humans, *CRY2* is associated with prolonged metastasis free survival in breast cancer  
378 and lower *CRY2* expression is associated with worsened overall survival in PDA, suggesting a  
379 link between oestrogen related cancer and *CRY* expression which sadly has not been addressed  
380 in models (Cadenas et al., 2014, Relles et al., 2013). In colorectal cancer, however, *CRY1*  
381 expression correlates with worse overall survival, and in gastric cancer high *CRY1* expression  
382 relative to paired tumour margin is associated with higher cancer stage (Yu et al., 2013, Hu et  
383 al., 2014).

384 Facilitated by the availability of knock-out mouse models, studies on *CRY1*- and  
385 *CRY2*-deficient mice are abundant (van der Horst et al., 1999). Tumour autonomous *CRY1*  
386 and *CRY2* data is much scarcer. *CRY1* k/d in HOS and U2OS cell lines (human osteosarcomas)  
387 led to larger tumours in mice (Zhou et al., 2018). This effect was also seen *in vitro* (Zhou et al.,  
388 2018). HCC cells with *CRY* double knockout in a p53 knockout (k/o) background have been  
389 shown to grow at the same rate as the same cells with just p53 k/o, however the *CRY* loss  
390 sensitised them to TNF $\alpha$  treatment, suggesting a link to apoptosis and the NF- $\kappa$ B pathway (Lee  
391 and Sancar, 2011). It should be noted that this HCC study was performed in immunodeficient  
392 mice; with a functional immune system these tumours would potentially die due to TNF $\alpha$   
393 produced by lymphocytes in the tumour microenvironment without the need for endogenous  
394 TNF $\alpha$ .

395 *In vitro*, *CRY2* appears to be anti-tumourigenic in osteosarcoma but has negligible  
396 effects in breast cancer. *CRY2* k/d in HOS cells enhanced cell cycle progression, proliferation  
397 and migration, reducing p53 expression but increasing MYC, CCND1, MMP-2,  $\beta$ -Catenin and  
398 ERK1/2 phosphorylation. The knockdown also modified the circadian landscape in the cells,  
399 increasing *CRY1*, *PER1*, *PER2*, *BMAL1*, and *CLOCK* expression (Yu et al., 2018). *CRY2* k/d  
400 in MCF7 cells showed no change in cell cycle distribution or apoptosis with or without methyl

401 methanesulfonate insult. The only difference observed was a difference in DNA damage  
402 accumulation and an upregulation of p21 and Cyclin D1 (Hoffman et al., 2010).

403 CRY2's inhibition of MYC seems important to its role in cancer. An increase in MYC  
404 upon CRY2 k/d is likely due to CRY2's known role in degrading MYC via cooperative action  
405 with the E3 ubiquitin ligase FBXL3 (Huber et al., 2016). Indeed, in humans, data concerning  
406 PDA, a cancer type inextricably linked to MYC, suggests an increase in CRY2 as beneficial  
407 for patient survival. However, the import of this in tumours is unclear as CRY2 k/o has proved  
408 insufficient for primary cell transformation; expression of MYC combined with CRY2 k/o was  
409 not sufficient to cause colony formation on soft agar (Huber et al., 2016). Therefore, CRY2  
410 may act as an inhibitor of tumour progression but be insufficient to contribute to tumour  
411 initiation. In mouse models CRY's role seems reversed when p53 is mutated, with the CRYs  
412 acting as tumour enhancers, as loss of CRY acts as a sensitisation factor to TNF $\alpha$  mediated  
413 apoptosis (Ozturk et al., 2009). This suggests a link between CRY2 and p53, which is also  
414 potentially on display *in vitro* with p21 accumulation upon CRY2 k/d demonstrated by  
415 Hoffman et al. (2010). In human studies it would be interesting to contextualise the role of  
416 CRY in terms of the MYC and p53 status of the tumour. More data is required to fully  
417 understand the role of CRYs in cancers. There is a wealth of data concerning whole animal  
418 knockouts, but they are limited for modelling the clinical situation in human cancers in a  
419 meaningful way, there are no known human conditions which completely remove circadian  
420 proteins in all body cells.

## 421 **REV-ERB $\alpha$ & REV-ERB $\beta$**

422 There is evidence to suggest that the REV-ERBs play a role in oestrogen related  
423 tumours in humans, and in *in vivo* and *in vitro* cell based studies, although the suggested effect  
424 on tumourigenesis is occasionally inconsistent. The REV-ERBs are part of the secondary  
425 circadian loop as they can bind to elements in the *BMAL1* promoter and suppress its  
426 transcription, they have ties to inflammatory pathways and are therefore, attractive targets for  
427 anti-cancer compounds. A number of agonists and antagonists of REV-ERBs have been  
428 developed (Wang et al., 2020). High expression of REV-ERB $\alpha$  is associated with better  
429 prognosis in triple negative breast cancer when the patients were treated with chemotherapy,  
430 leading to better overall and disease-free survival (Na et al., 2019).

431 REV-ERBs seem to be anti-tumourigenic in most, but not all, cell lines tested thus far.  
432 There are two available REV-ERB agonists which are used in many of the studies discussed

433 here, named SR9009 and SR9011. These have proved effective in killing astrocytoma (Becker),  
434 leukaemia (Jurkat), breast (MCF7), colon (HCT116) and melanoma (A375) cell lines *in vitro*  
435 (Sulli et al., 2018). SR9009 reduced autophagy and increased apoptosis in skin naevi,  
436 glioblastoma and subcutaneous small cell lung cancer in mice (Sulli et al., 2018, Shen et al.,  
437 2020). In HCT116, REV-ERB $\alpha$  k/d is reported to reduce proliferation and micrometastasis  
438 formation *in vivo* in a zebrafish embryo model. This is in contrast to the *in vitro* information  
439 from the same study which found enhanced proliferation on REV-ERB $\alpha$  k/d (Basti et al., 2020).  
440 In T98G glioblastoma and HepG2 cells introduction of SR9009 is cytotoxic, but in other  
441 hepatocellular carcinoma cells (Huh7 and HCCLM3) REV-ERB $\beta$  appears to increase cell  
442 viability and increase epithelial-mesenchymal transition (Wagner et al., 2019, Tong et al.,  
443 2020). Undifferentiated and partially differentiated gastric cancer (BGC-823 & SGC-7901),  
444 demonstrated increased proliferation upon REV-ERB $\alpha$  k/d and the glycolysis and pentose  
445 phosphate pathway were suggested as the potential mechanism (Tao et al., 2019). It must be  
446 noted, however, that these cell lines are known to have misattributed identity, so this data is  
447 potentially more related to HeLa cells than gastric cancer (Ye et al., 2015).

448 REV-ERB $\alpha$  is tied to breast cancer as it and HER-2 lie on the same amplicon. REV-  
449 ERB $\alpha$  k/d reduces viability of HER-2 positive breast cancer cells (BT474), potentially due to  
450 a link between REV-ERB $\alpha$  and fatty acid synthesis (Kourtidis et al., 2010). Conversely, in  
451 other breast cancer cell lines (MDA-MB-231, MCF7, BT474 and MDA-MB-361) SR9011  
452 reduced cell viability. A REV-ERB $\beta$  k/d stopped the SR9011 effect therefore, even though  
453 SR9011 acts via both REV-ERBs, in these breast cancer cells SR9011 acted via REV-ERB $\beta$   
454 (Wang et al., 2015a). Both SR9009 and SR9011 were tested on glioblastoma stem cells and  
455 were found to reduce proliferation compared to non-cancerous controls, and they reduced  
456 expression of glioblastoma stem cell markers. SR9011 combined with a CRY stabilisation  
457 agent reduced glioblastoma stem cell viability synergistically, both agents targeting different  
458 molecular feedback loops of the clock (Dong et al., 2019).

459 REV-ERB research is in an odd position, as there is contradictory data within the same  
460 cancer type in both breast cancer and hepatocellular carcinoma. In a number of breast cancer  
461 cell lines REV-ERB $\beta$  appears to be the important REV-ERB, though this may not be true in all  
462 types of breast cancer. From the human data it would be expected that REV-ERB agonists  
463 would be anti-tumourigenic which was true in many, but not all, cell lines studied. The  
464 comparison is not perfect as the human data was specifically in triple negative breast cancer  
465 and involved chemotherapy and the model data was *in vitro*. An experiment involving REV-

466 ERB knockdowns and agonists in a triple negative breast cancer line (e.g., MDA-MB-231) in  
467 a clinically relevant *in vivo* model (e.g., orthotopic) would give greater insight into the role  
468 these proteins play in human tumour biology. As there are already small molecules which show  
469 good efficacy and high specificity at targeting the clock via REV-ERBs, these genes are a  
470 promising route towards circadian based cancer therapies, though it should be noted that the  
471 agonists currently available have been suggested to have anti-proliferative effects independent  
472 of their REV-ERB agonism (Dierickx et al., 2019, Dong et al., 2019).

## 473 **TIMELESS**

474 TIMELESS was one of the first circadian proteins to be cloned in flies where it is an  
475 integral part to the circadian system (Sehgal et al., 1994). In mammals, a TIMELESS homolog  
476 exists, but its function in mammalian circadian biology is poorly understood, presumably in  
477 part due to the embryonic lethality of TIMELESS k/o (Gotter et al., 2000, Kurien et al., 2019).  
478 In humans, high expression of *TIMELESS* is associated with shorter metastasis free survival in  
479 breast cancer, meaning its only link to cancer in humans is in oestrogen related cancer (Cadenas  
480 et al., 2014).

481 This oestrogen related cancer link is also reiterated *in vivo*. In an orthotopic breast  
482 cancer model TIMELESS k/d was reported to reduce the viability of MCF7 cells (Chi et al.,  
483 2017). Similarly, *in vitro* TIMELESS k/d in MCF7 cells reduces proliferation, and in the  
484 hepatocellular carcinoma cell lines HepG2 and Hep3B TIMELESS k/d causes cell cycle arrest  
485 and slightly increased apoptosis as well as a drop in migration in Hep3B cells (Mao et al., 2013,  
486 Elgohary et al., 2015). Data on TIMELESS is understandably minimal given its unclear role in  
487 the circadian system. The orthotopic *in vivo* model used by Chi et al. is an improvement over  
488 subcutaneous flank models and could be emulated for breast cancer studies on circadian genes  
489 in future.

## 490 **DEC1 & DEC2**

491 DEC1 and DEC2 are part of a smaller clock sub-loop which interlocks the primary  
492 circadian feedback loop. *DEC1* & *DEC2* are E-box containing genes whose corresponding  
493 proteins are known to inhibit CLOCK/BMAL1 heterodimer mediated transcription  
494 (Nakashima et al., 2008). In AML with MLL-AF6 chromosomal rearrangement, *DEC2* is  
495 overexpressed. Downregulation of *DEC2* increases apoptosis of these cells and reduces their  
496 viability *in vivo*, though the knockdown does not change the expression of other clock genes

497 (Numata et al., 2018). DEC1, however, has been reported to prevent cell cycle progression by  
498 directly binding to, and hence stabilising, Cyclin E (Bi et al., 2015). Overexpression of *DEC1*  
499 in MCF7 cells reduced tumour size in mouse xenografts significantly (Bi et al., 2015). In MCF7  
500 cells, *in vitro*, knockdown of DEC2 increased apoptosis, whereas DEC1 did not. Apoptosis  
501 related factors, namely FAS and BAX, were upregulated and MYC expression was decreased  
502 (Wu et al., 2015).

503 Genes outside of the primary and secondary canonical circadian loops, but which  
504 nonetheless have a role in circadian oscillations, are interesting and much of their function  
505 remains elusive. DEC1 and DEC2 are transcriptional repressors but, as evidenced by the role  
506 of DEC1 in stabilisation of Cyclin E, have roles outside of their transcriptional activity. The  
507 effect that these proteins have on tumourigenesis, as well as their specific role in tumour  
508 rhythms should be studied more closely.

### 509 **MECHANISM OVERVIEW - p53**

510 The p53 axis is a recurring mechanism linking the clock with cancer biology. This could  
511 prove pivotal as p53 is the single most frequently mutated gene in human cancers. Its ability to  
512 orchestrate cell cycle arrest via p21 and apoptosis via BCL-2 in response to DNA damage  
513 makes removal of p53 function essential for much of malignant growth (Kastenhuber and  
514 Lowe, 2017). As explored in this review, p53 has been linked to *BMAL1*, *PER1*, *PER2* and  
515 *CRY2*. The role p53 plays in the cancer biology of each gene is convoluted, for example *PER2*  
516 status modified p53 abundance in K562 cells, though its tumour suppressive role in lung cancer  
517 was demonstrated to be p53 independent. Additionally, p53 appears to begin cycling when  
518 *BMAL1* expression is lost, antithetical to the canonical idea that no BMAL1 means no circadian  
519 oscillation. The ubiquitous role of p53 in cancer makes the cell specific nature of its  
520 involvement confusing, though there are some possible explanations. Firstly, some studies do  
521 not probe for p53 and therefore it may be playing an unseen role in these cells. Secondly,  
522 *BMAL1* exerts pro-tumourigenic effects in cell lines with relatively low p53 mutation rates in  
523 patients. Perhaps the natural dependency of the cancer on p53 pathways may give rise to these  
524 cell specific effects.

525 This mechanism is further complicated by circadian-p53 crosstalk. It has been shown  
526 previously that a reduction of p53 upon *PER2* k/d can occur due to PER2 preventing MDM2  
527 mediated ubiquitination of p53 (Gotoh et al., 2014). In turn p53 can block CLOCK/BMAL1  
528 binding to the E-box elements in the *PER2* promoter (Miki et al., 2013). If p53 has such strong

529 links to PER2 then other clock genes that have an effect on p53 may in fact just be modifying  
530 *PER2* expression. Nevertheless. p53 remains an interesting mechanism by which the clock and  
531 cancer may interact.

## 532 **MECHANISM OVERVIEW - APOPTOSIS**

533 Another potential mechanism by which these genes influence tumourigenesis is via  
534 apoptosis pathways independent of p53. A link between the circadian clock and p53  
535 independent cell death has been previously established, demonstrating a link between NF-κB  
536 signalling and CRY in p53 null cells (Lee and Sancar, 2011). Whilst this is a good indicator of  
537 a circadian-apoptosis link, the data presented here have shown that aberrations in different  
538 clock proteins can lead to differing effects. Across a number of the aforementioned studies,  
539 changes in apoptosis related proteins such as p21 and BAX are fairly common. However, it is  
540 difficult to know if these effects can be traced back to p53. Apoptosis pathways converge  
541 significantly, and the effects of p53 are widespread. Sadly, a number of studies that identify  
542 increased apoptosis as a potential mechanism of tumourigenic modifications by circadian genes  
543 do not probe for p53 specifically, so it is difficult to ascertain which effects are p53 independent  
544 and which are p53 dependant.

## 545 **MECHANISM OVERVIEW – CELL CYCLE**

546 Cross talk between circadian oscillators and the cell cycle is well established (Farshadi  
547 et al., 2020). Dysregulation of the cell cycle is a key driver of increased proliferative capacity.  
548 In the majority of studies mentioned here there is a change in proliferation upon clock gene  
549 modification. Cell cycle arrest is repeatedly implicated in circadian rhythms and cancer.  
550 Primarily cells were found arrested in G2 (Sun et al., 2010, Elgohary et al., 2015, Wang et al.,  
551 2015a). It would be presumptuous to assume that this G2 arrest across various genes and cell  
552 types is caused by the same mechanism, though the consistency is interesting. There are a  
553 number of genes that are important for circadian gene effects on tumourigenesis that also have  
554 some function in the cell cycle (*WEE1, MYC, AKT, CYCLINB1, P21, P27, K167*) although  
555 many of these are also involved in apoptosis. Splitting up circadian genes' role in apoptosis  
556 and proliferation may prove folly as the scope of circadian control is so vast and these pathways  
557 overlap to such a degree that it is likely that both are involved on some level. It is also important  
558 to note that a number of studies do see an increase in proliferation via MTT assay, colony  
559 formation assays or BrdU assays, but do not interrogate the exact mechanism for this increase,  
560 so we do not yet have an exhaustive list of what proteins and pathways are involved.

## 561 **Clocks in the clinic - How to tell if Tumours Tick?**

562 Knowledge of the mechanisms underlying clock influence on human tumours is  
563 important, but equally important is the ability to translate this into a clinical setting. As  
564 previously mentioned, it is crucial to know the degree of circadian function of a tumour.  
565 Understanding the expression of oscillating genes is not as simple as looking for genes that are  
566 up or down regulated. Instead, we must ascertain the presence or absence of oscillation, the  
567 amplitude and phase of the oscillation, and the abundance of clock genes relative to the point  
568 of the circadian cycle. In addition, it is clear that clock gene expression differs based on tumour  
569 type, tissue type and the individual. In an age of personalised medicine, bespoke knowledge of  
570 the rhythmic function within individual tumours will be paramount. However, taking a biopsy  
571 on the hour every hour for 24 hours to assess rhythmicity is unlikely to be a popular option. To  
572 state simply, we require an innovative way to know if tumours tick in individuals and what that  
573 ticking looks like.

574 Algorithms have been developed which aim to ascertain the biological time of single  
575 samples based on their transcriptome. Several supervised models have been trained using  
576 human circadian transcriptome datasets (Agostinelli et al., 2016, Hughey, 2017, Laing et al.,  
577 2017, Braun et al., 2018, Wittenbrink et al., 2018). Circadian transcriptomics experiments in  
578 mice have demonstrated that expression of each clock gene is usually synchronised in phase  
579 across different tissues, but the magnitude of expression and amplitude of oscillation can differ  
580 (Zhang et al., 2014). For example, the algorithms ZeitZeiger, PSLR, BIO\_CLOCK and  
581 BodyTime require that training and test datasets are tissue-matched, or even cell-matched  
582 (Wittenbrink et al., 2018). This means these time prediction algorithms are constrained to blood  
583 samples, for which training datasets around the clock are available.

584 Alternatively, other mathematical methods have been suggested that do not rely on  
585 serial samples of individuals. CYCLOPS is a largely unsupervised method which requires  
586 reasonably large transcriptomic datasets (ideally >250 samples) composed of single samples  
587 from different individuals over the course of the day (Anafi et al., 2017). CYCLOPS-ordered  
588 hepatocellular carcinoma (HCC) tumour biopsies demonstrated population-wide oscillations  
589 in 8 of 9 core clock genes, but showed a reduction in amplitude of oscillation and magnitude  
590 of expression relative to CYCLOPS-ordered HCC tumour margins (Lamb et al., 2011, Anafi  
591 et al., 2017). Unfortunately, since rhythmic gene expression is estimated from the population,

592 CYCLOPS cannot estimate the variation in rhythmic gene expression between individuals  
593 (Anafi et al., 2017), which limits its clinical use.

594 Another method that does not require tissue-matched, time-stamped training datasets,  
595 known as  $\Delta$ CCD, is based on calculating the pairwise Spearman correlation of the expressions  
596 of all 12 core clock genes previously used to build ZeitZeiger (Hughey et al., 2016, Shilts et  
597 al., 2018). For each sample the resulting correlation matrix is then compared to a mouse healthy  
598 tissue reference matrix. Using this method, 20 tumour biopsy datasets differed from non-  
599 tumour samples, indicating that core clock gene expression of the tumour samples was more  
600 different than clock gene expression of non-tumour samples, relative to the mouse reference.  
601 This suggests that core circadian gene expression is consistently disrupted in tumours over a  
602 population. Interestingly an unrelated algorithm, TimeTeller, trained using a circadian  
603 transcriptome data of healthy human oral mucosa biopsies, was used to predict the extent of  
604 molecular clock dysfunction in the biopsies of 226 breast cancer patients (Vlachou et al., 2019).  
605 Most importantly, clock dysfunction and overall survival correlated, suggesting a functional  
606 role for the tumour clock, which warrants further investigation.

607 Mathematical models have been used to predict population level dysregulation of  
608 rhythmic gene expression from human tumour biopsies and have provided some insight into  
609 individual clock variation (Vlachou et al., 2019, Anafi et al., 2017, Shilts et al., 2018). Yet,  
610 there is poor correlation in the degree of clock gene dysregulation between different tumour  
611 types or even between cohorts of the same tumour type (Shilts et al., 2018, Ye et al., 2018). It  
612 is unclear whether this is a result of individual molecular clock difference, intertumoral  
613 difference, sampling-time difference, or perhaps another factor such as tumour grade. This  
614 demonstrates the importance of well-annotated publicly available datasets, ideally samples  
615 would be time-annotated.

## 616 **Conclusion and Future Directions**

617 In conclusion, there is a wealth of evidence at many levels which links the expression  
618 of circadian clock proteins to tumour biology and tumourigenesis. Though some of this  
619 evidence is limited, such as a lack of timing information for human biospecimen, it still seems  
620 overwhelmingly likely that there is a link between circadian clock gene expression and tumour  
621 development or progression. There is, however, not a very detailed mechanistic understanding  
622 if any given circadian clock gene is pro- or anti-tumourigenic, as the effects of gene's products

623 appear cell-type specific. It is difficult to understand the cause of this cell specificity without  
624 greater knowledge of the underlying mechanisms of the role of circadian clock genes in  
625 tumourigenesis in these instances. Additionally, it is not known if the effect of circadian  
626 proteins on tumourigenesis is linked to their role in the clock or is a separate function regardless  
627 of oscillation.

628         Deciphering the role of circadian clocks in human tumours requires not only an  
629 understanding of the degree of clock function in the tumour, but also an understanding of the  
630 degree of clock function in the healthy tissue of the host, ideally in the context of the  
631 individual's overall behavioural rhythm. A number of ingenious mathematical models have  
632 been developed which have the potential to elucidate clock function from large transcriptomic  
633 datasets. However, the availability of timing information in the data, is important both for the  
634 training of these models and for their use in matching molecular clock status to clinical  
635 information. Important initiatives such as The Cancer Genome Atlas and the International  
636 Cancer Genome Consortium do not record time of sampling information, despite recording  
637 other important patient-specific metadata such as alcohol and smoking history. Time of  
638 sampling information would greatly improve the possible use of such datasets. An additional  
639 level of understanding about the patients' individual clocks could be gleaned from biopsies of  
640 healthy tissue similar to that of the tumour, this would be the gold standard for comparing the  
641 molecular clock of the healthy cells to those of the tumour. However, a more feasible and non-  
642 invasive way of gaining this information may be to use a short chronotype questionnaire to  
643 determine the patient's behavioural clock function (Ghotbi et al., 2020). This review in part  
644 serves as an appeal to patient-facing clinicians involved in research. If circadian-cancer studies  
645 in humans are to substantially improve, then more annotation of samples with as much time  
646 information as possible is paramount.

647         As for *in vitro* and *in vivo* modelling of the effects of clock genes on cancers, it will be  
648 important to strive for clinically relevant models. Effects of clock k/d *in vitro* do not always  
649 hold true *in vivo*. This is somewhat expected as, *in vivo*, tumour cells will be receiving  
650 synchronisation information from the SCN much like healthy cells. It is clear that the effects  
651 of circadian genes are highly cell type specific, and it is not yet clear what the reasons for this  
652 may be. The status of pathways such as p53 in the cells before the circadian aberration may  
653 play a key role. Testing circadian genes by a single knockdown in a single cell line gives  
654 interesting information but it is slow and cumbersome. A push towards screening more than  
655 one clock gene at a time and potentially testing the effects of the entire molecular clock at once

656 would allow a faster development of knowledge in this area. To ascertain if these effects are  
657 actually down to changes in the clock, or due to properties of clock genes which may have  
658 come uncoupled from their role in rhythms, it is useful to know the circadian health of a cell  
659 line before the modification and after. Some studies already test this but many do not. Better  
660 time matched human data and clinically relevant *in vivo* and *in vitro* analysis which takes into  
661 account the circadian system as a whole should go some way to allow us to pick apart the  
662 complex and varied roles that circadian genes appear to play in tumour biology.

- 664 AGOSTINELLI, F., CEGLIA, N., SHAHBABA, B., SASSONE-CORSI, P. & BALDI, P. 2016. What time is it?  
665 Deep learning approaches for circadian rhythms. *Bioinformatics*, 32, i8-i17.
- 666 ALTMAN, B. J., HSIEH, A. L., SENGUPTA, A., KRISHNANAIAH, S. Y., STINE, Z. E., WALTON, Z. E., GOUW,  
667 A. M., VENKATARAMAN, A., LI, B., GORAKSHA-HICKS, P., DISKIN, S. J., BELLOVIN, D. I., SIMON,  
668 M. C., RATHMELL, J. C., LAZAR, M. A., MARIS, J. M., FELSHER, D. W., HOGENESCH, J. B., WELJIE,  
669 A. M. & DANG, C. V. 2015. MYC Disrupts the Circadian Clock and Metabolism in Cancer Cells.  
670 *Cell metabolism*, 22, 1009-1019.
- 671 ANAFI, R. C., FRANCEY, L. J., HOGENESCH, J. B. & KIM, J. 2017. CYCLOPS reveals human transcriptional  
672 rhythms in health and disease. 114, 5312-5317.
- 673 ARAN, D., CAMARDA, R., ODEGAARD, J., PAIK, H., OSKOTSKY, B., KRINGS, G., GOGA, A., SIROTA, M. &  
674 BUTTE, A. J. 2017. Comprehensive analysis of normal adjacent to tumor transcriptomes.  
675 *Nature communications*, 8, 1077-1077.
- 676 ARYAL, R. P., KWAK, P. B., TAMAYO, A. G., GEBERT, M., CHIU, P.-L., WALZ, T. & WEITZ, C. J. 2017.  
677 Macromolecular Assemblies of the Mammalian Circadian Clock. *Molecular cell*, 67, 770-  
678 782.e6.
- 679 ASTIZ, M., HEYDE, I. & OSTER, H. 2019. Mechanisms of Communication in the Mammalian Circadian  
680 Timing System. *International journal of molecular sciences*, 20, 343.
- 681 BAE, S.-A., FANG, M. Z., RUSTGI, V., ZARBL, H. & ANDROULAKIS, I. P. 2019. At the Interface of Lifestyle,  
682 Behavior, and Circadian Rhythms: Metabolic Implications. 6.
- 683 BAGGS, J. E., PRICE, T. S., DITACCHIO, L., PANDA, S., FITZGERALD, G. A. & HOGENESCH, J. B. 2009.  
684 Network features of the mammalian circadian clock. *PLoS Biol*, 7, e52.
- 685 BALSALOBRE, A., BROWN, S. A., MARCACCI, L., TRONCHE, F., KELLENDONK, C., REICHARDT, H. M.,  
686 SCHÜTZ, G. & SCHIBLER, U. 2000. Resetting of circadian time in peripheral tissues by  
687 glucocorticoid signaling. *Science*, 289, 2344-7.
- 688 BASTI, A., FIOR, R., YALÇIN, M., PÓVOA, V., ASTABURUAGA, R., LI, Y., NADERI, J., GODINHO FERREIRA,  
689 M. & RELÓGIO, A. 2020. The Core-Clock Gene NR1D1 Impacts Cell Motility In Vitro and  
690 Invasiveness in A Zebrafish Xenograft Colon Cancer Model. *Cancers (Basel)*, 12.
- 691 BI, H., LI, S., QU, X., WANG, M., BAI, X., XU, Z., AO, X., JIA, Z., JIANG, X., YANG, Y. & WU, H. 2015. DEC1  
692 regulates breast cancer cell proliferation by stabilizing cyclin E protein and delays the  
693 progression of cell cycle S phase. *Cell death & disease*, 6, e1891-e1891.
- 694 BRAUN, R., KATH, W. L., IWANASZKO, M., KULA-EVERSOLE, E., ABBOTT, S. M., REID, K. J., ZEE, P. C. &  
695 ALLADA, R. 2018. Universal method for robust detection of circadian state from gene  
696 expression. *Proc Natl Acad Sci U S A*, 115, E9247-e9256.
- 697 BROWN, STEVEN A., KOWALSKA, E. & DALLMANN, R. 2012. (Re)inventing the Circadian Feedback  
698 Loop. *Developmental Cell*, 22, 477-487.
- 699 BROWN, S. A., RIPPERGER, J., KADENER, S., FLEURY-OLELA, F., VILBOIS, F., ROSBASH, M. & SCHIBLER,  
700 U. 2005. PERIOD1-Associated Proteins Modulate the Negative Limb of the Mammalian  
701 Circadian Oscillator. 308, 693-696.
- 702 BUNGER, M. K., WILSBACHER, L. D., MORAN, S. M., CLENDENIN, C., RADCLIFFE, L. A., HOGENESCH, J.  
703 B., SIMON, M. C., TAKAHASHI, J. S. & BRADFIELD, C. A. 2000. Mop3 is an essential component  
704 of the master circadian pacemaker in mammals. *Cell*, 103, 1009-17.
- 705 BUURMA, M., VAN DIEMEN, J. J. K., THIJIS, A., NUMANS, M. E. & BONTEN, T. N. 2019. Circadian Rhythm  
706 of Cardiovascular Disease: The Potential of Chronotherapy With Aspirin. 6.
- 707 CADENAS, C., VAN DE SANDT, L., EDLUND, K., LOHR, M., HELLWIG, B., MARCHAN, R., SCHMIDT, M.,  
708 RAHNENFUHRER, J., OSTER, H. & HENGSTLER, J. G. 2014. Loss of circadian clock gene  
709 expression is associated with tumor progression in breast cancer. *Cell Cycle*, 13, 3282-91.
- 710 CHEN, R., SCHIRMER, A., LEE, Y., LEE, H., KUMAR, V., YOO, S.-H., TAKAHASHI, J. S. & LEE, C. 2009.  
711 Rhythmic PER abundance defines a critical nodal point for negative feedback within the  
712 circadian clock mechanism. *Molecular cell*, 36, 417-430.

713 CHEN, S. T., CHOO, K. B., HOU, M. F., YEH, K. T., KUO, S. J. & CHANG, J. G. 2005. Deregulated expression  
714 of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis*, 26, 1241-6.

715 CHEN, Z., LIU, P., LI, C., LUO, Y., CHEN, I., LIANG, W., CHEN, X., FENG, Y., XIA, H. & WANG, F. 2013.  
716 Deregulated expression of the clock genes in gliomas. *Technol Cancer Res Treat*, 12, 91-7.

717 CHI, L., ZOU, Y., QIN, L., MA, W., HAO, Y., TANG, Y., LUO, R. & WU, Z. 2017. TIMELESS contributes to  
718 the progression of breast cancer through activation of MYC. *Breast Cancer Res*, 19, 53.

719 COMAS, M., GORDON, C. J., OLIVER, B. G., STOW, N. W., KING, G., SHARMA, P., AMMIT, A. J.,  
720 GRUNSTEIN, R. R. & PHILLIPS, C. L. 2017. A circadian based inflammatory response –  
721 implications for respiratory disease and treatment. *Sleep Science and Practice*, 1, 18.

722 DE MAIRAN, J. 1729. Observation botanique. *Histoire de l'Academie Royale des Sciences*, 35-36.

723 DEBRUYNE, J. P., WEAVER, D. R. & REPERT, S. M. 2007. CLOCK and NPAS2 have overlapping roles in  
724 the suprachiasmatic circadian clock. *Nat Neurosci*, 10, 543-5.

725 DIERICKX, P., EMMETT, M. J., JIANG, C., UEHARA, K., LIU, M., ADLANMERINI, M. & LAZAR, M. A. 2019.  
726 SR9009 has REV-ERB-independent effects on cell proliferation and metabolism. 116, 12147-  
727 12152.

728 DOI, M., HIRAYAMA, J. & SASSONE-CORSI, P. 2006. Circadian regulator CLOCK is a histone  
729 acetyltransferase. *Cell*, 125, 497-508.

730 DONEHOWER, L. A., SOUSSI, T., KORKUT, A., LIU, Y., SCHULTZ, A., CARDENAS, M., LI, X., BABUR, O.,  
731 HSU, T.-K., LICHTARGE, O., WEINSTEIN, J. N., AKBANI, R. & WHEELER, D. A. 2019. Integrated  
732 Analysis of TP53 Gene and Pathway Alterations in The Cancer Genome Atlas. *Cell Reports*, 28,  
733 1370-1384.e5.

734 DONG, Z., ZHANG, G., QU, M., GIMPLE, R. C., WU, Q., QIU, Z., PRAGER, B. C., WANG, X., KIM, L. J. Y.,  
735 MORTON, A. R., DIXIT, D., ZHOU, W., HUANG, H., LI, B., ZHU, Z., BAO, S., MACK, S. C., CHAVEZ,  
736 L., KAY, S. A. & RICH, J. N. 2019. Targeting Glioblastoma Stem Cells through Disruption of the  
737 Circadian Clock. *Cancer discovery*, 9, 1556-1573.

738 DUFFY, J. F. & CZEISLER, C. A. 2009. Effect of Light on Human Circadian Physiology. *Sleep medicine  
739 clinics*, 4, 165-177.

740 DUN, A., ZHAO, X., JIN, X., WEI, T., GAO, X., WANG, Y. & HOU, H. 2020. Association Between Night-  
741 Shift Work and Cancer Risk: Updated Systematic Review and Meta-Analysis. *Front Oncol*, 10,  
742 1006.

743 EL-ATHMAN, R. & RELÓGIO, A. 2018. Escaping Circadian Regulation: An Emerging Hallmark of Cancer?  
744 *Cell Systems*, 6, 266-267.

745 ELGOHARY, N., PELLEGRINO, R., NEUMANN, O., ELZAWAHRY, H. M., SABER, M. M., ZEENELDIN, A. A.,  
746 GEFFERS, R., EHEMANN, V., SCHEMMER, P., SCHIRMACHER, P. & LONGERICH, T. 2015.  
747 Protumorigenic role of Timeless in hepatocellular carcinoma. *Int J Oncol*, 46, 597-606.

748 ELSHAZLEY, M., SATO, M., HASE, T., YAMASHITA, R., YOSHIDA, K., TOYOKUNI, S., ISHIGURO, F., OSADA,  
749 H., SEKIDO, Y., YOKOI, K., USAMI, N., SHAMES, D. S., KONDO, M., GAZDAR, A. F., MINNA, J. D.  
750 & HASEGAWA, Y. 2012. The circadian clock gene BMAL1 is a novel therapeutic target for  
751 malignant pleural mesothelioma. *Int J Cancer*, 131, 2820-31.

752 FARSHADI, E., VAN DER HORST, G. T. J. & CHAVES, I. 2020. Molecular Links between the Circadian Clock  
753 and the Cell Cycle. *Journal of Molecular Biology*.

754 FEKRY, B., RIBAS-LATRE, A., BAUMGARTNER, C., DEANS, J. R., KWOK, C., PATEL, P., FU, L., BERDEAUX,  
755 R., SUN, K., KOLONIN, M. G., WANG, S. H., YOO, S. H., SLADEK, F. M. & ECKEL-MAHAN, K. 2018.  
756 Incompatibility of the circadian protein BMAL1 and HNF4  $\alpha$  in hepatocellular carcinoma. *Nat  
757 Commun*, 9, 4349.

758 FU, L., PELICANO, H., LIU, J., HUANG, P. & LEE, C. C. 2002. The Circadian Gene Period2 Plays an  
759 Important Role in Tumor Suppression and DNA Damage Response In Vivo. *Cell*, 111, 41-50.

760 GERY, S., KOMATSU, N., BALDJYAN, L., YU, A., KOO, D. & KOEFFLER, H. P. 2006. The circadian gene per1  
761 plays an important role in cell growth and DNA damage control in human cancer cells. *Mol  
762 Cell*, 22, 375-82.

763 GHOTBI, N., PILZ, L. K., WINNEBECK, E. C., VETTER, C., ZERBINI, G., LENSSEN, D., FRIGHETTO, G.,  
764 SALAMANCA, M., COSTA, R., MONTAGNESE, S. & ROENNEBERG, T. 2020. The  $\mu$ MCTQ: An  
765 Ultra-Short Version of the Munich ChronoType Questionnaire. *J Biol Rhythms*, 35, 98-110.

766 GOOLEY, J. J., LU, J., CHOU, T. C., SCAMMELL, T. E. & SAPER, C. B. 2001. Melanopsin in cells of origin of  
767 the retinohypothalamic tract. *Nature Neuroscience*, 4, 1165.

768 GOTOH, T., VILA-CABALLER, M., SANTOS, C. S., LIU, J., YANG, J. & FINKIELSTEIN, C. V. 2014. The  
769 circadian factor Period 2 modulates p53 stability and transcriptional activity in unstressed  
770 cells. *Molecular biology of the cell*, 25, 3081-3093.

771 GOTTER, A. L., MANGANARO, T., WEAVER, D. R., KOLAKOWSKI, L. F., JR., POSSIDENTE, B., SRIRAM, S.,  
772 MACLAUGHLIN, D. T. & REPERT, S. M. 2000. A time-less function for mouse timeless. *Nat*  
773 *Neurosci*, 3, 755-6.

774 GWON, D. H., LEE, W.-Y., SHIN, N., KIM, S. I., JEONG, K., LEE, W.-H., KIM, D. W., HONG, J. & LEE, S. Y.  
775 2020. BMAL1 Suppresses Proliferation, Migration, and Invasion of U87MG Cells by  
776 Downregulating Cyclin B1, Phospho-AKT, and Metalloproteinase-9. *International journal of*  
777 *molecular sciences*, 21, 2352.

778 HATTAR, S., LIAO, H. W., TAKAO, M., BERSON, D. M. & YAU, K. W. 2002. Melanopsin-containing retinal  
779 ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science (New York,*  
780 *N.Y.)*, 295, 1065-1070.

781 HIDA, A., KITAMURA, S., KATAYOSE, Y., KATO, M., ONO, H., KADOTANI, H., UCHIYAMA, M., EBISAWA,  
782 T., INOUE, Y., KAMEI, Y., OKAWA, M., TAKAHASHI, K. & MISHIMA, K. 2014. Screening of Clock  
783 Gene Polymorphisms Demonstrates Association of a PER3 Polymorphism with Morningness–  
784 Eveningness Preference and Circadian Rhythm Sleep Disorder. *Scientific Reports*, 4, 6309.

785 HOFFMAN, A. E., ZHENG, T., BA, Y., STEVENS, R. G., YI, C. H., LEADERER, D. & ZHU, Y. 2010. Phenotypic  
786 effects of the circadian gene Cryptochrome 2 on cancer-related pathways. *BMC Cancer*, 10,  
787 110.

788 HSU, C. M., LIN, S. F., LU, C. T., LIN, P. M. & YANG, M. Y. 2012. Altered expression of circadian clock  
789 genes in head and neck squamous cell carcinoma. *Tumour Biol*, 33, 149-55.

790 HU, M.-L., YE, K.-T., LIN, P.-M., HSU, C.-M., HSIAO, H.-H., LIU, Y.-C., LIN, H. Y.-H., LIN, S.-F. & YANG,  
791 M.-Y. 2014. Deregulated expression of circadian clock genes in gastric cancer. *BMC*  
792 *gastroenterology*, 14, 67-67.

793 HUA, H., WANG, Y., WAN, C., LIU, Y., ZHU, B., WANG, X., WANG, Z. & DING, J. M. 2007. Inhibition of  
794 tumorigenesis by intratumoral delivery of the circadian gene mPer2 in C57BL/6 mice. *Cancer*  
795 *Gene Ther*, 14, 815-8.

796 HUA, H., WANG, Y., WAN, C., LIU, Y., ZHU, B., YANG, C., WANG, X., WANG, Z., CORNELISSEN–  
797 GUILLAUME, G. & HALBERG, F. J. C. S. 2006. Circadian gene mPer2 overexpression induces  
798 cancer cell apoptosis. 97, 589-596.

799 HUBER, A.-L., PAPP, S. J., CHAN, A. B., HENRIKSSON, E., JORDAN, S. D., KRIEBS, A., NGUYEN, M.,  
800 WALLACE, M., LI, Z., METALLO, C. M. & LAMIA, K. A. 2016. CRY2 and FBXL3 Cooperatively  
801 Degrade c-MYC. *Molecular cell*, 64, 774-789.

802 HUGHEY, J. J. 2017. Machine learning identifies a compact gene set for monitoring the circadian clock  
803 in human blood. *Genome Medicine*, 9, 19.

804 HUGHEY, J. J., HASTIE, T. & BUTTE, A. J. 2016. ZeitZeiger: supervised learning for high-dimensional data  
805 from an oscillatory system. *Nucleic Acids Res*, 44, e80.

806 HUNTER, C. M. & FIGUEIRO, M. G. 2017. Measuring Light at Night and Melatonin Levels in Shift  
807 Workers: A Review of the Literature. *Biological research for nursing*, 19, 365-374.

808 IARC, M. V. G. 2019. Carcinogenicity of night shift work. *Lancet Oncol*, 20, 1058-1059.

809 JIANG, W., ZHAO, S., JIANG, X., ZHANG, E., HU, G., HU, B., ZHENG, P., XIAO, J., LU, Z., LU, Y., NI, J., CHEN,  
810 C., WANG, X., YANG, L. & WAN, R. 2016. The circadian clock gene Bmal1 acts as a potential  
811 anti-oncogene in pancreatic cancer by activating the p53 tumor suppressor pathway. *Cancer*  
812 *Lett*, 371, 314-25.

813 KASTENHUBER, E. R. & LOWE, S. W. 2017. Putting p53 in Context. *Cell*, 170, 1062-1078.

814 KONOPKA, R. J. & BENZER, S. 1971. Clock mutants of *Drosophila melanogaster*. *Proceedings of the*  
815 *National Academy of Sciences of the United States of America*, 68, 2112-2116.

816 KOURTIDIS, A., JAIN, R., CARKNER, R. D., EIFERT, C., BROSNAN, M. J. & CONKLIN, D. S. 2010. An RNA  
817 interference screen identifies metabolic regulators NR1D1 and PBP as novel survival factors  
818 for breast cancer cells with the ERBB2 signature. *Cancer research*, 70, 1783-1792.

819 KURIEN, P., HSU, P.-K., LEON, J., WU, D., MCMAHON, T., SHI, G., XU, Y., LIPZEN, A., PENNACCHIO, L. A.,  
820 JONES, C. R., FU, Y.-H. & PTÁČEK, L. J. 2019. TIMELESS mutation alters phase responsiveness  
821 and causes advanced sleep phase. 116, 12045-12053.

822 LAING, E. E., MÖLLER-LEVET, C. S., POH, N., SANTHI, N., ARCHER, S. N. & DIJK, D.-J. 2017. Blood  
823 transcriptome based biomarkers for human circadian phase. *eLife*, 6, e20214.

824 LAMB, J. R., ZHANG, C., XIE, T., WANG, K., ZHANG, B., HAO, K., CHUDIN, E., FRASER, H. B., MILLSTEIN,  
825 J., FERGUSON, M., SUVER, C., IVANOVSKA, I., SCOTT, M., PHILIPPAR, U., BANSAL, D., ZHANG,  
826 Z., BURCHARD, J., SMITH, R., GREENAWALT, D., CLEARY, M., DERRY, J., LOBODA, A., WATTERS,  
827 J., POON, R. T. P., FAN, S. T., YEUNG, C., LEE, N. P. Y., GUINNEY, J., MOLONY, C., EMILSSON, V.,  
828 BUSER-DOEPNER, C., ZHU, J., FRIEND, S., MAO, M., SHAW, P. M., DAI, H., LUK, J. M. & SCHATZ,  
829 E. E. 2011. Predictive genes in adjacent normal tissue are preferentially altered by sCNV during  
830 tumorigenesis in liver cancer and may rate limiting. *PLoS one*, 6, e20090-e20090.

831 LANDGRAF, D., WANG, L. L., DIEMER, T. & WELSH, D. K. 2016. NPAS2 Compensates for Loss of CLOCK  
832 in Peripheral Circadian Oscillators. *PLoS Genet*, 12, e1005882.

833 LEE, C., WEAVER, D. R. & REPERT, S. M. 2004. Direct association between mouse PERIOD and  
834 CKIepsilon is critical for a functioning circadian clock. *Mol Cell Biol*, 24, 584-94.

835 LEE, J. H. & SANCAR, A. 2011. Regulation of apoptosis by the circadian clock through NF-kappaB  
836 signaling. *Proceedings of the National Academy of Sciences of the United States of America*,  
837 108, 12036-12041.

838 MAO, Y., FU, A., LEADERER, D., ZHENG, T., CHEN, K. & ZHU, Y. 2013. Potential cancer-related role of  
839 circadian gene TIMELESS suggested by expression profiling and in vitro analyses. *BMC cancer*,  
840 13, 498-498.

841 MICHAEL, A. K., FRIBOURGH, J. L., VAN GELDER, R. N. & PARTCH, C. L. 2017. Animal Cryptochromes:  
842 Divergent Roles in Light Perception, Circadian Timekeeping and Beyond. *Photochemistry and*  
843 *photobiology*, 93, 128-140.

844 MIKI, T., MATSUMOTO, T., ZHAO, Z. & LEE, C. C. 2013. p53 regulates Period2 expression and the  
845 circadian clock. *Nat Commun*, 4, 2444.

846 MURE, L. S., LE, H. D., BENEGLIAMO, G., CHANG, M. W., RIOS, L., JILLANI, N., NGOTHO, M., KARIUKI, T.,  
847 DKHISSI-BENYAHYA, O., COOPER, H. M. & PANDA, S. 2018. Diurnal transcriptome atlas of a  
848 primate across major neural and peripheral tissues. 359, eaao0318.

849 NA, H., HAN, J., KA, N.-L., LEE, M.-H., CHOI, Y.-L., SHIN, Y. K. & LEE, M.-O. 2019. High expression of  
850 NR1D1 is associated with good prognosis in triple-negative breast cancer patients treated with  
851 chemotherapy. *Breast Cancer Research*, 21, 127.

852 NAKASHIMA, A., KAWAMOTO, T., HONDA, K. K., UESHIMA, T., NOSHIRO, M., IWATA, T., FUJIMOTO, K.,  
853 KUBO, H., HONMA, S., YORIOKA, N., KOHNO, N. & KATO, Y. 2008. DEC1 modulates the  
854 circadian phase of clock gene expression. *Molecular and cellular biology*, 28, 4080-4092.

855 NOH, J. 2018. The Effect of Circadian and Sleep Disruptions on Obesity Risk. *Journal of obesity &*  
856 *metabolic syndrome*, 27, 78-83.

857 NUMATA, A., KWOK, H. S., KAWASAKI, A., LI, J., ZHOU, Q. L., KERRY, J., BENOUKRAF, T., BARARIA, D.,  
858 LI, F., BALLABIO, E., TAPIA, M., DESHPANDE, A. J., WELNER, R. S., DELWEL, R., YANG, H., MILNE,  
859 T. A., TANEJA, R. & TENEN, D. G. 2018. The basic helix-loop-helix transcription factor SHARP1  
860 is an oncogenic driver in MLL-AF6 acute myelogenous leukemia. *Nat Commun*, 9, 1622.

861 ODA, A., KATAYOSE, Y., YABUUCHI, S., YAMAMOTO, K., MIZUMA, M., SHIRASOU, S., ONOGAWA, T.,  
862 OHTSUKA, H., YOSHIDA, H., HAYASHI, H., RIKIYAMA, T., KIM, H., CHOE, Y., KIM, K., SON, H.,  
863 MOTOI, F., EGAWA, S. & UNNO, M. 2009. Clock gene mouse period2 overexpression inhibits

864 growth of human pancreatic cancer cells and has synergistic effect with cisplatin. *Anticancer*  
865 *Res*, 29, 1201-9.

866 OSHIMA, T., TAKENOSHITA, S., AKAIKE, M., KUNISAKI, C., FUJII, S., NOZAKI, A., NUMATA, K.,  
867 SHIOZAWA, M., RINO, Y., TANAKA, K., MASUDA, M. & IMADA, T. 2011. Expression of circadian  
868 genes correlates with liver metastasis and outcomes in colorectal cancer. *Oncol Rep*, 25, 1439-  
869 46.

870 OZTURK, N., LEE, J. H., GADDAMEEDHI, S. & SANCAR, A. 2009. Loss of cryptochrome reduces cancer  
871 risk in p53 mutant mice. *Proceedings of the National Academy of Sciences of the United States*  
872 *of America*, 106, 2841-2846.

873 PAPAGIANNAKOPOULOS, T., BAUER, M. R., DAVIDSON, S. M., HEIMANN, M., SUBBARAJ, L., BHUTKAR,  
874 A., BARTLEBAUGH, J., VANDER HEIDEN, M. G. & JACKS, T. 2016. Circadian Rhythm Disruption  
875 Promotes Lung Tumorigenesis. *Cell Metab*, 24, 324-31.

876 PENDERGAST, J. S., NISWENDER, K. D. & YAMAZAKI, S. 2012. Tissue-specific function of Period3 in  
877 circadian rhythmicity. *PLoS one*, 7, e30254-e30254.

878 PETRENKO, V. & DIBNER, C. 2017. Circadian orchestration of insulin and glucagon release. *Cell cycle*  
879 *(Georgetown, Tex.)*, 16, 1141-1142.

880 PHILPOTT, J. M., NARASIMAMURTHY, R., RICCI, C. G., FREEBERG, A. M., HUNT, S. R., YEE, L. E.,  
881 PELOFSKY, R. S., TRIPATHI, S., VIRSHUP, D. M. & PARTCH, C. L. 2020. Casein kinase 1 dynamics  
882 underlie substrate selectivity and the PER2 circadian phosphoswitch. *eLife*, 9, e52343.

883 PRASAI, M. J., PERNICOVA, I., GRANT, P. J. & SCOTT, E. M. 2011. An Endocrinologist's Guide to the  
884 Clock. *The Journal of Clinical Endocrinology & Metabolism*, 96, 913-922.

885 PURAM, R. V., KOWALCZYK, M. S., DE BOER, C. G., SCHNEIDER, R. K., MILLER, P. G., MCCONKEY, M.,  
886 TOTOHOVA, Z., TEJERO, H., HECKL, D., JARAS, M., CHEN, M. C., LI, H., TAMAYO, A., COWLEY, G.  
887 S., ROZENBLATT-ROSEN, O., AL-SHAHROUR, F., REGEV, A. & EBERT, B. L. 2016. Core Circadian  
888 Clock Genes Regulate Leukemia Stem Cells in AML. *Cell*, 165, 303-16.

889 QIN, T., LU, X. T., LI, Y. G., LIU, Y., YAN, W., LI, N. & SUN, Y. Y. 2018. Effect of Period 2 on the  
890 proliferation, apoptosis and migration of osteosarcoma cells, and the corresponding  
891 mechanisms. *Oncol Lett*, 16, 2668-2674.

892 RAHMAN, S. A., GRANT, L. K., GOOLEY, J. J., RAJARATNAM, S. M. W., CZEISLER, C. A. & LOCKLEY, S. W.  
893 2019. Endogenous Circadian Regulation of Female Reproductive Hormones. *The Journal of*  
894 *Clinical Endocrinology & Metabolism*, 104, 6049-6059.

895 RAMOS, C. A., OUYANG, C., QI, Y., CHUNG, Y., CHENG, C.-T., LABARGE, M. A., SEEWALDT, V. L. & ANN,  
896 D. K. 2020. A Non-canonical Function of BMAL1 Metabolically Limits Obesity-Promoted Triple-  
897 Negative Breast Cancer. *iScience*, 23, 100839-100839.

898 RELLES, D., SENDECKI, J., CHIPITSYNA, G., HYSLOP, T., YEO, C. J. & ARAFAT, H. A. 2013. Circadian gene  
899 expression and clinicopathologic correlates in pancreatic cancer. *J Gastrointest Surg*, 17, 443-  
900 50.

901 RIVERA, A. S., AKANBI, M., O'DWYER, L. C. & MCHUGH, M. 2020. Shift work and long work hours and  
902 their association with chronic health conditions: A systematic review of systematic reviews  
903 with meta-analyses. *PLoS One*, 15, e0231037.

904 ROENNEBERG, T. & MERROW, M. 2016. The Circadian Clock and Human Health. *Current Biology*, 26,  
905 R432-R443.

906 ROSBASH, M. 2009. The implications of multiple circadian clock origins. *PLoS biology*, 7, e62-e62.

907 ROSSETTI, S., CORLAZZOLI, F., GREGORSKI, A., AZMI, N. H. A. & SACCHI, N. 2012. Identification of an  
908 estrogen-regulated circadian mechanism necessary for breast acinar morphogenesis. *Cell*  
909 *Cycle*, 11, 3691-3700.

910 SATO, F., NAGATA, C., LIU, Y., SUZUKI, T., KONDO, J., MOROHASHI, S., IMAIZUMI, T., KATO, Y. & KIJIMA,  
911 H. 2009. PERIOD1 is an anti-apoptotic factor in human pancreatic and hepatic cancer cells. *J*  
912 *Biochem*, 146, 833-8.

913 SEHGAL, A., PRICE, J., MAN, B. & YOUNG, M. 1994. Loss of circadian behavioral rhythms and per RNA  
914 oscillations in the *Drosophila* mutant timeless. 263, 1603-1606.

- 915 SHEN, W., ZHANG, W., YE, W., WANG, H., ZHANG, Q., SHEN, J., HONG, Q., LI, X., WEN, G., WEI, T. &  
916 ZHANG, J. 2020. SR9009 induces a REV-ERB dependent anti-small-cell lung cancer effect  
917 through inhibition of autophagy. *Theranostics*, 10, 4466-4480.
- 918 SHILTS, J., CHEN, G. & HUGHEY, J. J. 2018. Evidence for widespread dysregulation of circadian clock  
919 progression in human cancer. *PeerJ*, 6, e4327.
- 920 STEVENS, R. G. 1987. Electric power use and breast cancer: a hypothesis. *Am J Epidemiol*, 125, 556-61.
- 921 SULLI, G., ROMMEL, A., WANG, X., KOLAR, M. J., PUCA, F., SAGHATELIAN, A., PLIKUS, M. V., VERMA, I.  
922 M. & PANDA, S. 2018. Pharmacological activation of REV-ERBs is lethal in cancer and  
923 oncogene-induced senescence. *Nature*, 553, 351-355.
- 924 SUN, C. M., HUANG, S. F., ZENG, J. M., LIU, D. B., XIAO, Q., TIAN, W. J., ZHU, X. D., HUANG, Z. G. &  
925 FENG, W. L. 2010. Per2 inhibits k562 leukemia cell growth in vitro and in vivo through cell cycle  
926 arrest and apoptosis induction. *Pathol Oncol Res*, 16, 403-11.
- 927 SUZUKI, T., SATO, F., KONDO, J., LIU, Y., KUSUMI, T., FUJIMOTO, K., KATO, Y., SATO, T. & KIJIMA, H.  
928 2008. Period is involved in the proliferation of human pancreatic MIA-PaCa2 cancer cells by  
929 TNF- $\alpha$ . *Biomedical Research*, 29, 99-103.
- 930 TABEBI, M., SÖDERKVIST, P. & JENSEN, L. D. 2018. Hypoxia Signaling and Circadian Disruption in and  
931 by Pheochromocytoma. *Frontiers in endocrinology*, 9, 612-612.
- 932 TAKAHASHI, J. S. 2016. Transcriptional architecture of the mammalian circadian clock. *Nature Reviews*  
933 *Genetics*, 18, 164.
- 934 TAO, L., YU, H., LIANG, R., JIA, R., WANG, J., JIANG, K. & WANG, Z. 2019. Rev-erb  $\alpha$  inhibits proliferation  
935 by reducing glycolytic flux and pentose phosphate pathway in human gastric cancer cells.  
936 *Oncogenesis*, 8, 57.
- 937 TONG, H., LIU, X., LI, T., QIU, W., PENG, C., SHEN, B. & ZHU, Z. 2020. NR1D2 Accelerates Hepatocellular  
938 Carcinoma Progression by Driving the Epithelial-to-Mesenchymal Transition. *OncoTargets and*  
939 *therapy*, 13, 3931-3942.
- 940 TYNES, T., HANNEVIK, M., ANDERSEN, A., VISTNES, A. I. & HALDORSEN, T. 1996. Incidence of breast  
941 cancer in Norwegian female radio and telegraph operators. *Cancer Causes Control*, 7, 197-  
942 204.
- 943 VAN DER HORST, G. T., MUIJTJENS, M., KOBAYASHI, K., TAKANO, R., KANNO, S., TAKAO, M., DE WIT,  
944 J., VERKERK, A., EKER, A. P., VAN LEENEN, D., BUIJS, R., BOOTSMA, D., HOEIJMAKERS, J. H. &  
945 YASUI, A. 1999. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms.  
946 *Nature*, 398, 627-30.
- 947 VLACHOU, D., BJARNASON, G., GIACCHETTI, S., LEVI, F. & RAND, D. 2019. *TimeTeller: a New Tool for*  
948 *Precision Circadian Medicine and Cancer Prognosis*.
- 949 WAGNER, P. M., MONJES, N. M. & GUIDO, M. E. 2019. Chemotherapeutic Effect of SR9009, a REV-ERB  
950 Agonist, on the Human Glioblastoma T98G Cells. *ASN neuro*, 11, 1759091419892713-  
951 1759091419892713.
- 952 WANG, F., LI, C., YONGLUO & CHEN, L. 2016. The Circadian Gene Clock Plays an Important Role in Cell  
953 Apoptosis and the DNA Damage Response In Vitro. *Technol Cancer Res Treat*, 15, 480-6.
- 954 WANG, J., LI, S., LI, X., LI, B., LI, Y., XIA, K., YANG, Y., AMAN, S., WANG, M. & WU, H. 2019. Circadian  
955 protein BMAL1 promotes breast cancer cell invasion and metastasis by up-regulating matrix  
956 metalloproteinase9 expression. *Cancer cell international*, 19, 182-182.
- 957 WANG, S., LI, F., LIN, Y. & WU, B. 2020. Targeting REV-ERB  $\alpha$  for therapeutic purposes: promises and  
958 challenges. *Theranostics*, 10, 4168-4182.
- 959 WANG, Y., KOJETIN, D. & BURRIS, T. P. 2015a. Anti-proliferative actions of a synthetic REV-ERB  $\alpha$  /  $\beta$   
960 agonist in breast cancer cells. *Biochemical pharmacology*, 96, 315-322.
- 961 WANG, Y., QIAN, R., SUN, N., LU, C., CHEN, Z. & HUA, L. 2015b. Circadian gene hClock enhances  
962 proliferation and inhibits apoptosis of human colorectal carcinoma cells in vitro and in vivo.  
963 *Mol Med Rep*, 11, 4204-10.
- 964 WANG, Y., SUN, N., LU, C., BEI, Y., QIAN, R. & HUA, L. 2017. Upregulation of circadian gene 'hClock'  
965 contribution to metastasis of colorectal cancer. *Int J Oncol*, 50, 2191-2199.

966 WEGRZYN, L. R., TAMIMI, R. M., ROSNER, B. A., BROWN, S. B., STEVENS, R. G., ELIASSEN, A. H., LADEN,  
967 F., WILLET, W. C., HANKINSON, S. E. & SCHERNHAMMER, E. S. 2017. Rotating Night-Shift  
968 Work and the Risk of Breast Cancer in the Nurses' Health Studies. *Am J Epidemiol*, 186, 532-  
969 540.

970 WINTER, S. L., BOSNOYAN-COLLINS, L., PINNADUWAGE, D. & ANDRULIS, I. L. 2007. Expression of the  
971 circadian clock genes Per1 and Per2 in sporadic and familial breast tumors. *Neoplasia (New  
972 York, N.Y.)*, 9, 797-800.

973 WITTENBRINK, N., ANANTHASUBRAMANIAM, B., MÜNCH, M., KOLLER, B., MAIER, B., WESCHKE, C.,  
974 BES, F., DE ZEEUW, J., NOWOZIN, C., WAHNSCHAFFE, A., WISNIEWSKI, S., ZALESKA, M.,  
975 BARTOK, O., ASHWAL-FLUSS, R., LAMMERT, H., HERZEL, H., HUMMEL, M., KADENER, S., KUNZ,  
976 D. & KRAMER, A. 2018. High-accuracy determination of internal circadian time from a single  
977 blood sample. *J Clin Invest*, 128, 3826-3839.

978 WU, Y., SATO, H., SUZUKI, T., YOSHIZAWA, T., MOROHASHI, S., SEINO, H., KAWAMOTO, T., FUJIMOTO,  
979 K., KATO, Y. & KIJIMA, H. 2015. Involvement of c-Myc in the proliferation of MCF-7 human  
980 breast cancer cells induced by bHLH transcription factor DEC2. *Int J Mol Med*, 35, 815-20.

981 XIA, H. C., NIU, Z. F., MA, H., CAO, S. Z., HAO, S. C., LIU, Z. T. & WANG, F. 2010. Deregulated expression  
982 of the Per1 and Per2 in human gliomas. *Can J Neurol Sci*, 37, 365-70.

983 XIAO, L., CHANG, A. K., ZANG, M.-X., BI, H., LI, S., WANG, M., XING, X. & WU, H. 2014. Induction of the  
984 CLOCK gene by E2-ER  $\alpha$  signaling promotes the proliferation of breast cancer cells. *PLoS one*,  
985 9, e95878-e95878.

986 YE, F., CHEN, C., QIN, J., LIU, J. & ZHENG, C. 2015. Genetic profiling reveals an alarming rate of cross-  
987 contamination among human cell lines used in China. *Faseb j*, 29, 4268-72.

988 YE, R., SELBY, C. P., CHIOU, Y.-Y., OZKAN-DAGLIYAN, I., GADDAMEEDHI, S. & SANCAR, A. 2014. Dual  
989 modes of CLOCK:BMAL1 inhibition mediated by Cryptochrome and Period proteins in the  
990 mammalian circadian clock. *Genes & development*, 28, 1989-1998.

991 YE, Y., XIANG, Y., OZGUC, F. M., KIM, Y., LIU, C. J., PARK, P. K., HU, Q., DIAO, L., LOU, Y., LIN, C., GUO,  
992 A. Y., ZHOU, B., WANG, L., CHEN, Z., TAKAHASHI, J. S., MILLS, G. B., YOO, S. H. & HAN, L. 2018.  
993 The Genomic Landscape and Pharmacogenomic Interactions of Clock Genes in Cancer  
994 Chronotherapy. *Cell Syst*, 6, 314-328.e2.

995 YU, H., MENG, X., WU, J., PAN, C., YING, X., ZHOU, Y., LIU, R. & HUANG, W. 2013. Cryptochrome 1  
996 overexpression correlates with tumor progression and poor prognosis in patients with  
997 colorectal cancer. *PLoS One*, 8, e61679.

998 YU, Y., LI, Y., ZHOU, L., YANG, G., WANG, M. & HONG, Y. 2018. Cryptochrome 2 (CRY2) Suppresses  
999 Proliferation and Migration and Regulates Clock Gene Network in Osteosarcoma Cells. *Med  
1000 Sci Monit*, 24, 3856-3862.

1001 ZENG, Z. L., WU, M. W., SUN, J., SUN, Y. L., CAI, Y. C., HUANG, Y. J. & XIAN, L. J. 2010. Effects of the  
1002 biological clock gene Bmal1 on tumour growth and anti-cancer drug activity. *J Biochem*, 148,  
1003 319-26.

1004 ZHANG, R., LAHENS, N. F., BALLANCE, H. I., HUGHES, M. E. & HOGENESCH, J. B. 2014. A circadian gene  
1005 expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci U S A*,  
1006 111, 16219-24.

1007 ZHANG, Y. & PAPANTONIOU, K. 2019. Night shift work and its carcinogenicity. *The Lancet Oncology*,  
1008 20, e550.

1009 ZHOU, L., YU, Y., SUN, S., ZHANG, T. & WANG, M. 2018. Cry 1 Regulates the Clock Gene Network and  
1010 Promotes Proliferation and Migration Via the Akt/P53/P21 Pathway in Human Osteosarcoma  
1011 Cells. *J Cancer*, 9, 2480-2491.

1012 ZHOU, M., KIM, J. K., ENG, G. W., FORGER, D. B. & VIRSHUP, D. M. 2015. A Period2 Phosphoswitch  
1013 Regulates and Temperature Compensates Circadian Period. *Mol Cell*, 60, 77-88.

1014 ZHU, Y., BROWN, H. N., ZHANG, Y., STEVENS, R. G. & ZHENG, T. 2005. Period3 structural variation: a  
1015 circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol  
1016 Biomarkers Prev*, 14, 268-70.

1017 Figure 1. A general overview of the molecular circadian clock. CLOCK/BMAL1 binds  
1018 to E-box elements in clock-controlled genes (CCGs). CCGs are varied, producing important  
1019 proteins such as p53, as well as circadian proteins which enter into feedback loops with  
1020 CLOCK/BMAL1. In the primary circadian loop PER and CRY proteins are translated and  
1021 accumulate in the cytoplasm. Here they form macromolecular cytoplasmic complexes with  
1022 other proteins including GAPVD1, who's role in circadian biology remains largely elusive,  
1023 and CK1 $\epsilon/\delta$ . During this cytoplasmic phase a number of post-translational modifications occur  
1024 which modify the activity of the PERs and CRYs. PER2 phosphorylation is pictured as an  
1025 example, CK1 $\epsilon/\delta$  phosphorylates PER2 on either the FASP site or the degron site leading to  
1026 PER2 stabilisation or  $\beta$ -TrCP mediated degradation respectively. Stabilised PER2, along with  
1027 other members of the cytoplasmic circadian complex can be translocated to the nucleus. Here  
1028 a nuclear complex of PERs and CRYs can repress CLOCK/BMAL1 mediated transcriptional  
1029 activation and therefore, repress their own transcription. In the secondary loop RORs and REV-  
1030 ERBs, who's transcription is regulated by E-boxes, compete for access to ROR response  
1031 elements (RREs) with RORs promoting *BMAL1* transcription and REV-ERBs repressing it.  
1032 Pictured here are two of the proposed sub loops. DEC1 and DEC2 repress *PER1* transcription.  
1033 DBP mediates oscillatory transcription via D-box elements and is repressed by NFIL3, which  
1034 itself is transcribed via ROR activity at RREs.

1035

1036

1037 Figure 2. An overview of the known data linking circadian genes to cancer which will  
1038 be discussed in this review. Green fill denotes an anti-tumourigenic effect of the gene, red fill  
1039 denotes a pro-tumourigenic effect of the gene, yellow fill denotes conflicting evidence with  
1040 some studies suggesting the gene to be anti- and others pro-tumourigenic. † denotes a cell type  
1041 whos identity was potentially misattributed.



