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Circadian Transcription Profile of Mouse Breast Cancer Under Light-Dark and Dark-Dark Conditions

EUN-YOUNG OH[1,3*], XIAOMING YANG[1*], ALEX FRIEDMAN[2], CHRISTINE M. ANSELL[1], JOVELYN DU-QUITON[1], DINAH F. QUITON[1], PATRICIA A. WOOD[1,3] and WILLIAM J.M. HRUSHESKY[1,3,4,5]

[1]Medical Chronobiology Laboratory, Dorn Research Institute, WJB Dorn VA Medical Center, Columbia, SC 29209, U.S.A.; [2]Washington University, St. Louis, MO 63130 U.S.A.; [3]School of Medicine, [4]School of Public Health and [5]School of Engineering and Computer Science, University of South Carolina Columbia, SC 29209, U.S.A.

Abstract. *The circadian clock exists in virtually every cell and regulates key biological processes in cells and tissues. Even in cancer cells, DNA synthesis, cell division and tumor growth are gated by the circadian clock. This study examined the gene expression profiles of transplanted mouse breast tumor cells under normal light-dark (LD) as well as constant dark (DD) conditions. It was found that the overall percentage of rhythmic transcripts in breast tumor tissue was lower than that in normal tissue. Few transcripts had unaltered rhythmic expression patterns under both LD and DD conditions. Most rhythmic transcripts in DD displayed 12h or shorter periods. These results suggest that in addition to the circadian clock control of gene transcription, altering light, feeding, physical activity and other factors characteristically affect the expression of many genes.*

Circadian organization is highly conserved across species and is essential to an organism's well-being (1-3). Circadian rhythms are generated both directly and indirectly by a set of core circadian clock genes. The mammalian circadian clockwork mechanism includes both negative and positive transcription-translation feedback loops which generate approximately 24 h physiological cycles. Two transcription factors, CLK (or NPAS2), and BMAL1, form complexes that activate the transcription of *Per* (*Per1, 2*) and Cry (*Cry1, 2*) genes. The resultant PER and CRY proteins form dimers which, in turn, suppress the transcription of their own genes. As a result, the expression of the *Per* and *Cry* genes oscillates with a near 24 h periodicity (1, 3). The second circadian clock loop involves the rhythmic expression of the *Rorα* and *Rev-erbα* genes, whose expressions are also activated by CLK/BMAL1 and suppressed by PER/CRY. RORα and REV-ERBα control the transcription of the *Bmal1* gene (4). In this way, the rhythmic expression of the *Bmal1* gene stabilizes the circadian oscillator. Light is a potent and the most well studied environmental time cue that sets the circadian clock. Daily light-dark cycles entrain the mammalian clock by influencing directly the master clock located in the suprachiasmatic nuclei (SCN), which entrains the clocks within peripheral tissues. Circadian rhythmicity is a self-sustained process which remains organized under constant conditions, such as constant darkness (DD), in both the organism and its cells (1, 3).

Virtually all fundamental biological processes are circadian regulated. In proliferating tissues, DNA synthesis, cell division, DNA damage repair and apoptosis are gated by the circadian clock (5-9). In humans, the activity of thymidylate synthase (TS), one of the most frequently targeted enzymes in proliferation pathways that control cancers, peaks in the early afternoon in oral mucosa and gut epithelium (8, 10, 11). The expression of cell cycle genes and growth factor genes as well as tumor growth rates are also under the control of the circadian clock (12). In a mouse breast cancer model, the protein content of VEGF, tumor blood flow rate, TS enzyme activity, tumor cell mitotic index and tumor growth rate display robust and reproducible rhythmic daily variations (6, 7, 13).

Microarray data have indicated that more than 10% of the genes in peripheral tissues are expressed rhythmically each day (14-16). The majority of these rhythmically expressed genes are tissue specific, indicating that the circadian clock is tightly coupled to key tissue specific functions. Since it has been shown that tumor growth is gated by the circadian clock (17), this study examined the gene expression profiles of

*Both authors contributed equally to this work.

Correspondence to: William J.M. Hrushesky, WJB Dorn VA Medical Center, 6439 Garners Ferry Road, Columbia, SC 29209, U.S.A. Tel: +1 8036956825, Fax: +1 8036956829, e-mail: Williamhrushesky@gmail.com

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mouse breast cancer cells growing within the C3H mouse under normal 12 h light and 12 h dark (LD) as well as under constant darkness (DD) conditions. It was found that the expression of approximately 4% and 6% of total RNA transcripts within this breast cancer type varies in LD and DD conditions, respectively. However, most of them did not demonstrate a 24 h or a 12 h period. Of these transcripts with a 24 h or a 12 h period, very few demonstrated a constant expression pattern under both LD and DD conditions, indicating that in addition to the circadian clock control of gene transcription, altering light/dark also characteristically affects the expression of many breast cancer genes.

Materials and Methods

Animals and tumors. C3HeB/FeJ (C3H) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and kept under a 12 h light and 12 h dark (LD) schedule. When the mice were 13-15 weeks of age, 2×10^4 MTCL (murine mammary tumor cell line) cells were injected subcutaneously in the backs of the mice, as described previously (17, 18). When the tumor size reached 600-700 mm^3 (11 to 12 days after tumor inoculation), the mice were divided randomly into two groups, one of which was kept under LD conditions and the other under DD conditions. After 48 h, tumors from both groups were collected at six time points (zeitgeber time (zt) and circadian time (ct) 2, 6, 10, 14, 18, and 22) and flash frozen using liquid nitrogen. All experiments were performed in compliance with the NIH and VA guidelines for care and use of laboratory animals.

Reverse transcription and real-time PCR. Total RNA was isolated from tumors using Trizol. Two μg of RNA was reverse-transcribed by M-MLV reverse transcriptase using random primers (Invitrogen, Carlsbad, CA, USA). Real time quantitative PCR analyses were performed on an iCycler iQ PCR system (BioRad, CA, USA). PCR reactions were performed in triplicate. The amount of the target gene expression was computed with respect to the endogenous reference (*Gapdh* gene). The primers for each gene were reported previously (19).

Microarray experiment. RNA extraction, reverse transcription, and hybridization to the Affymetrix 430.20 mouse whole genome array were performed by Paradigm Array Labs (Morriville, NC, USA). The Affymetrix 430 2.0 array has probes for 45,101 mouse transcripts. Equal amounts of RNA from three tumors collected at the same time-point were pooled.

Data analysis. The microarray data were analyzed by Genespring GX software (Agilent Technologies, Santa Clara, CA, USA). Relative gene expression was determined by comparing intensity levels among samples collected at 6 time points (LD or DD). With respect to zt/ct 2, transcripts with fold-change >1.3, $\log_{10}$ intensity >–1.2 and $p<0.001$ within 6 time points were considered to be differentially expressed transcripts. Gene ontology analysis was performed to identify the biological processes associated with each differentially expressed transcript.

Study design and definition of rhythmic expression patterns. Figure 1 shows the study design used. The gene expressions were compared among tumor RNAs from six time points in LD and DD. The expression level of each transcript at zt/ct 2 was set as the reference point. Differentially expressed transcripts were indentified by comparing their RNA levels of zt/ct 6, 10 14, 18 and 22 with that of zt/ct 2. If the expression of a particular transcript differed only at one time point, this transcript was not considered to be differentially expressed within each 24 h period because it was not possible to determine what caused this singular alteration of expression. If the expression of a transcript significantly differed at two or more time points compared with its expression level at zt/ct 2, this transcript was considered to be differentially expressed within a 24 h period. Among these differentially expressed transcripts, if the expression levels displayed one peak and one trough during each 24 h, they were considered to have a 24 h circadian rhythm. If the expression levels had two peaks or two troughs within each 24 h, and the two peaks or the two troughs were 12 h apart, these transcripts were defined to have a 12 h rhythm.

Results

Rhythmically expressed transcripts in mouse breast tumors under LD and DD conditions. The size and calculated growth rate of MTCL mouse breast tumor demonstrates a robust daily rhythm *in vivo* under 12 h light 12 h dark conditions (17). However, the tumor growth rate displays a two-peak instead of typical one-peak circadian pattern. One of the peak growth rates occurs in the middle of the day and the other in the middle of the night (17).

The pattern of a two-peak daily tumor size and growth rate prompted an examination of the *in vivo* circadian transcription profile of this MTCL mouse breast tumor in this study. An Affymetrix microarray was used to compare the relative RNA amount of 45,101 transcripts (22,000 genes) of MTCL tumors collected at six time points during a 24 h period. Approximately 1.8% of the transcripts (819 and 824 of 45,101 transcripts in total under LD and DD conditions, respectively) may be rhythmically expressed because their expressions vary at multiple time points (Table I, see Materials and Methods for the definition of rhythmic expression). Among these rhythmically expressed transcripts, some of them may have periods shorter than 12 h. However, sampling every 4 h does not allow a determination of whether these transcripts have a rhythm with a period of 4 h, 6 h or 8 h. Therefore, this study focused on the transcripts with either a 12 h or a 24 h rhythm (414 and 212 transcripts in LD and DD respectively). Among these, 38 transcripts had either a 12 h or a 24 h rhythm under both LD and DD conditions (Figure 2).

Expression patterns of transcripts only rhythmic in LD. A total of 376 transcripts displayed a 12 h or a 24 h expression rhythm only under the LD condition. Most of them (341 of 376) had a 24 h rhythm, while less than 10% (35 of 376) of the transcripts had a 12 h rhythm under the LD condition (Table II). Of those with a 24 h rhythm, more than half demonstrated a 'classic' circadian pattern, *i.e.* their highest expression and lowest expression points were 12 h apart. The

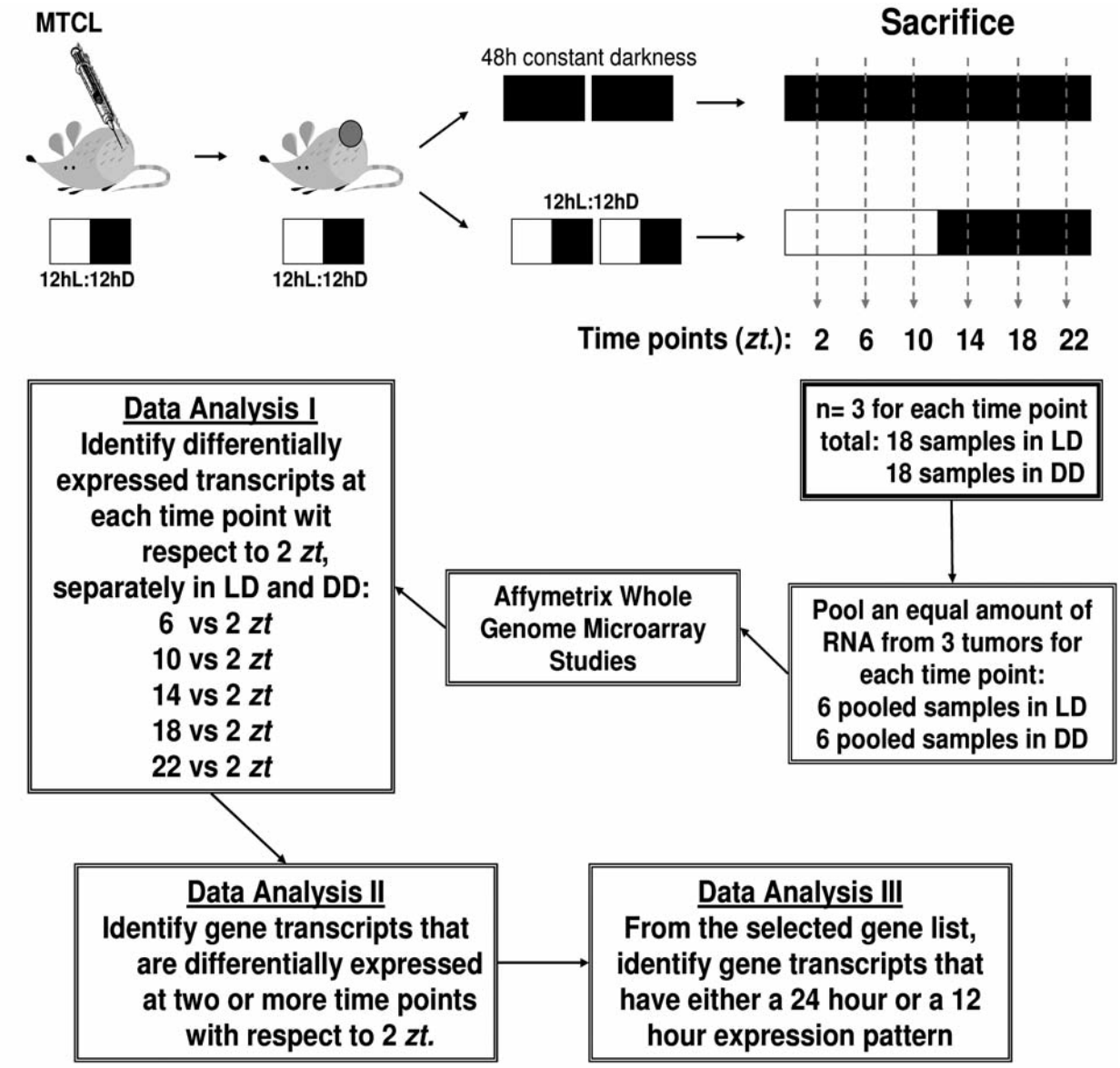

Figure 1. *Study design and data analysis*.

rest had a peak-trough either 8 h or 16 h apart. Most transcripts with 12 h expression patterns under LD conditions either had peaks at zt 2 and zt 14 or troughs at zt 2 and zt14 (Table II).

Further analysis reveals that the majority of these rhythmic transcripts in LD can be categorized into seven groups. The first two groups represented most of the classic 24 h circadian transcripts. They had peaks at zt2 and troughs at zt14 (Figure 3a) or peaks at zt14 and troughs at zt2 (Figure 3b). Unlike the expression patterns in the first two groups, the transcripts in the third and fourth group had the peak-trough in anti-phase, 8 h apart, either having the peak at zt2 and trough at zt10, or the peak at zt10 and trough at zt2 (Figure 3c, d). The expression patterns of the fifth and sixth group were also anti-phase. Their expression levels were highest at zt2 and lowest at zt18, or highest at zt18 and lowest at zt2 (Figure 3e, f). The transcripts of the last group displayed 12 h rhythms under LD conditions. These transcripts had two daily peaks at zt2 and zt14, and two troughs at zt6 and zt18 (Figure 3g).

Transcript expression patterns of transcripts only rhythmic in DD. The gene expressions of DD samples displayed unique circadian patterns. Unlike the rhythmically expressed transcripts

Table I. *The results of Data Analysis I.*

	No. of gene transcripts that are statistically differentially expressed at each time point with respect to 2 *zt/ct*. (p<0.001, fold-change ≥1.3, and $\log_{10}$ intensity >–1.2)					No. of gene transcripts that are statistically differentially expressed at multiple time points with respect to 2 *zt/ct*.				Summary		
	6 *vs*. 2	10 *vs*. 2	14 *vs*. 2	18 *vs*. 2	22 *vs*. 2	2 t.p.	3 t.p.	4 t.p.	5 t.p.	*d.e*. ≥2 t.p	*d.e*. 1 t.p. only	Total no. of unique transcripts
LD	554	493	1107	854	227	611	26	141	41	819	967	1786
DD	492	311	1857	417	1060	467	308	149	103	824	1931	2755

Comparison analysis using the resolver error method. zt=Zeitgeber time; ct, circadian time; d.e., differentially expressed; t.p., time point.

in LD, most of the transcripts that were rhythmic only in DD had 12 h rhythms (113 out of 174 transcripts, 65%) while 35% of them (61 out of 174) had 24 h rhythms (Table II). The expression patterns of these DD samples can be characterized into six main cluster patterns (Figure 4). The first two groups had typical 24 h circadian rhythms with their peak-trough 12 h apart. They either had peaks at ct2 and troughs at ct14 (Figure 4a), or peaks at ct14 and troughs at ct2 (Figure 4b). The next two groups had two high expressions at ct2 and ct14. They also had two low expressions at ct6 and ct18 (Figure 4c) or at ct10 and ct22 (Figure 4d). These two groups represented most of the transcripts with 12 h rhythms in DD. The transcripts in the fifth group had peaks at ct10 and ct22, and the transcripts in the sixth group had peaks at ct6 and ct18. These two groups of transcripts both had troughs at ct2 and ct14 (Figure 4e, f).

Expression patterns of transcripts that have rhythms under both LD and DD. Among the 38 transcripts that displayed 12 h or 24 h rhythms under both LD and DD conditions, 16 of them (42%) had 24 h rhythms and 12 of them (31.6%) had 12 h rhythms. The expression patterns of those transcripts that maintained their 24 h rhythms in LD and DD can be categorized into 3 groups (Figure 5, Panel A). The first group of transcripts had peaks at the day/night transition time (zt/ct 10, 14) and troughs at night/day transition time (zt/ct 2, 22). The second group had the anti-phase expression pattern of the first group. The transcripts in the third group displayed anti-phase expression patterns in LD and DD. The transcripts that had 12h rhythms in both LD and DD all had low expression levels in the middle of the day and in the middle of the night, and had high expression levels at day/night and night/day transition times (Figure 5 Panel B). Ten transcripts (26.4 %) had different time structures in LD and DD. Nine of them demonstrated 24 h rhythms in LD, with expression peaks at the day/night transition time, troughs at the night/day transition time, and 12 h rhythms in DD. Only one transcript demonstrated a 12 h rhythm in LD but a 24 h rhythm in DD (Figure 5 Panel C).

Table II. *Summary of different 24 h and 12 h expression patterns of MTCL mammary tumor gene transcripts identified in each group.*

	LD		DD	
24-Hour expression patterns				
I. Classic (Peak and Trough 12 h apart)				
P2,T14	97	(46.20%)	27	(57.45%)
P6,T18	3	(1.43%)	0	
P10,T22	4	(1.90%)	2	(4.26%)
P14,T2	96	(45.71%)	17	(36.17%)
P18,T6	7	(3.33%)	0	
P22,T10	3	(1.43%)	1	(2.13%)
Total	210	(100%)	47	(100%)
II. Variants (Peak and Trough 8 or 16 h apart)				
P2,T10	20	(15.27%)	6	(42.86%)
P2,T18	31	(23.66%)	2	(14.29%)
P6,T14	1	(0.76%)	1	(7.14%)
P6,T22	1	(0.76%)	0	
P10,T2	15	(11.45%)	3	(21.43%)
P10,T18	3	(2.29%)	0	
P14,T6	4	(3.05%)	1	(7.14%)
P14,T22	0		0	
P18,P2	53	(40.46%)	0	
P18,T10	3	(2.29%)	0	
P22,T6	0		0	
P22,T14	0		1	(7.14%)
Total	131	(100%)	14	(100%)
Combined Total	341		61	
12-Hour expression patterns				
Peaks 2, 14				
T6,18	13	(37.14%)	73	(64.60%)
T10,22	6	(17.14%)	23	(20.35%)
Troughs 2, 14				
P6,18	2	(5.72%)	8	(7.08%)
P10,22	4	(11.43%)	9	7.97%
Other		10		(28.57%)
Total	35	(100%)	113	(100%)

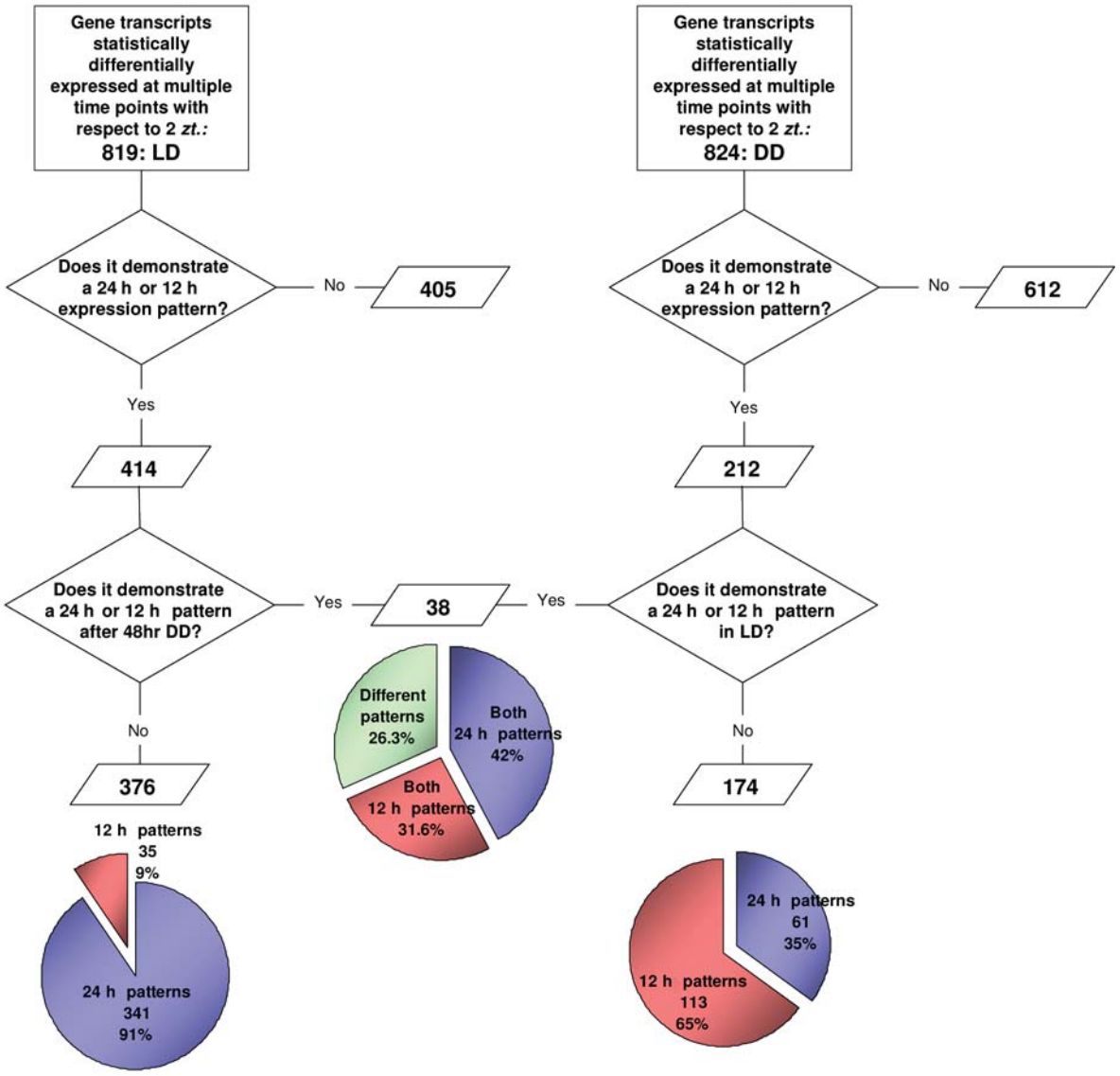

Figure 2. *Flowchart depiction of the process and results of Data Analysis II.*

Pattern analysis revealed another feature of these rhythmic transcripts in LD and DD. The overall amplitude of rhythmic transcripts under the DD condition was higher than that under the LD condition. The amplitudes of these 38 transcripts were compared with daily rhythms in both LD and DD. These transcripts demonstrated more robust rhythms under constant darkness (Figure 6).

Functional categories of rhythmically expressed transcripts. The transcripts with 12 h and 24 h daily rhythms were grouped according to their major associated functions (Figure 7, and data not shown). Most of the transcripts that cycle daily in LD, DD, or both are involved in regulating cell cycle, cellular metabolism, cytoskeleton and immune function (data not shown). There were more rhythmically expressed cell cycle regulators in LD conditions, while more cytoskeleton and structure related genes had circadian rhythms in DD. Compared with those cell cycle regulators that were rhythmic only in LD or DD, a larger proportion of rhythmic genes that had rhythms in both LD and DD were immune related. Genes involved in angiogenesis are directly related to tumor cell proliferation. There were three and five angiogenesis-related genes that had daily rhythms in LD and DD respectively. Of them, two were rhythmic in both LD and DD.

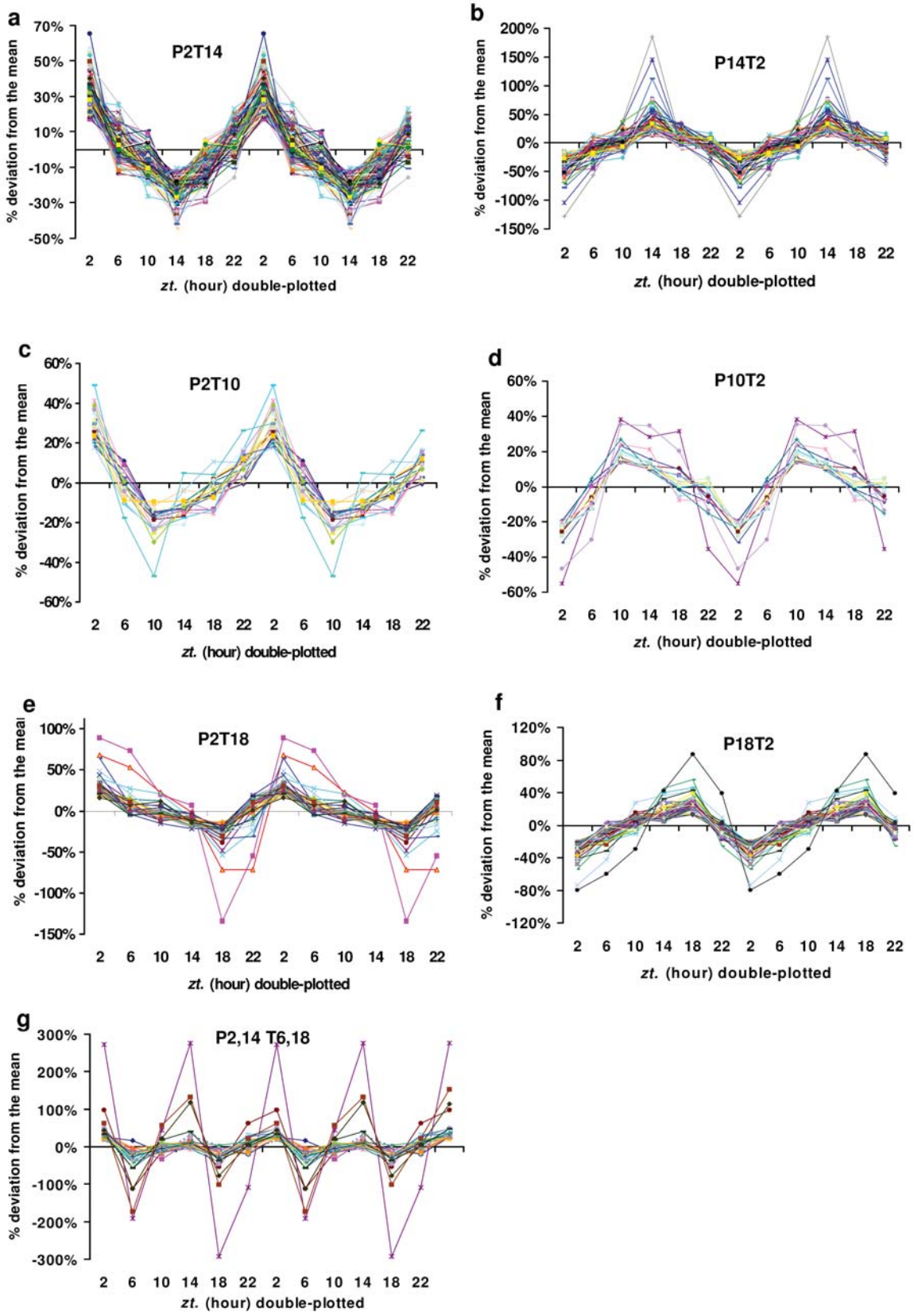

Figure 3. *24 h and 12 h gene expression patterns that are predominant during LD.*

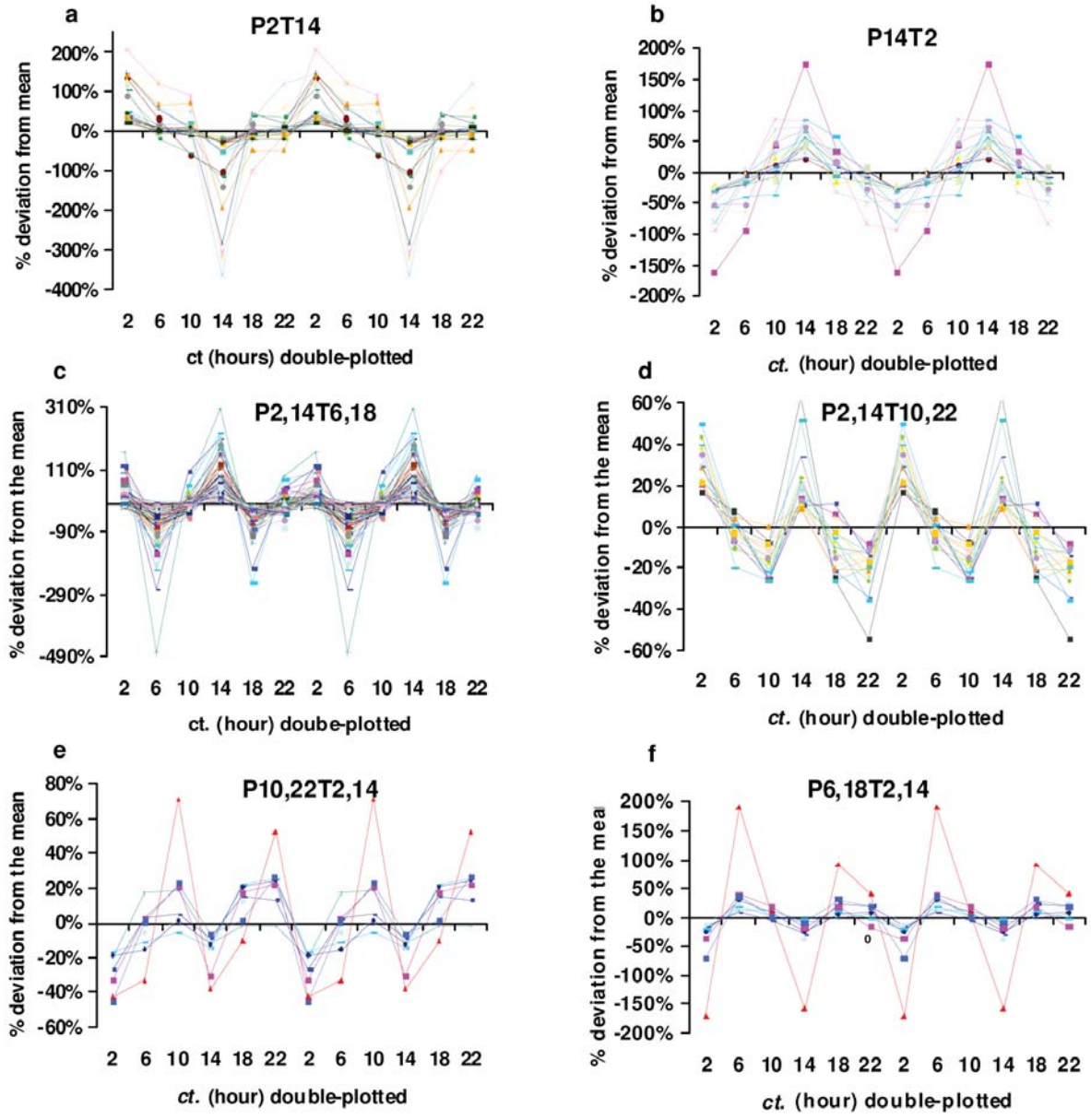

Figure 4. *Predominant 24 h and 12 h gene expression patterns during DD.*

Expression of core clock genes in tumors under LD and DD conditions. Because the circadian clock genes cycle with lower amplitudes in this tumor (19), the microarray analysis did not identify the rhythmic expression of core circadian genes in either LD or DD tumor samples. Real-time PCR, a more sensitive method, was used to compare the expression patterns of core clock genes and a clock output gene, *Dbp*, in LD and DD tumor samples (Figure 8). *Per1, Per2, Cry1, Bmal1, Rev-erbα* and *Dbp* have a damped but statistically significant 24 h rhythm in LD tumor samples. The expression patterns with respect to peak timing of these clock genes were very similar to those reported in normal tissues (20). In DD tumor samples, *Bmal1* expression lost its circadian rhythm. *Dbp*, a clock controlled gene, tended to have a 12 h circadian rhythm under DD condition compared to a 24 h rhythm in LD. The circadian rhythms of *Per1* and *Rev-erbα* in DD were slightly damped compared with those in LD samples. *Per2* expressions had identical patterns in LD and DD tumor samples. Increased amplitudes of clock gene expressions were not seen in DD.

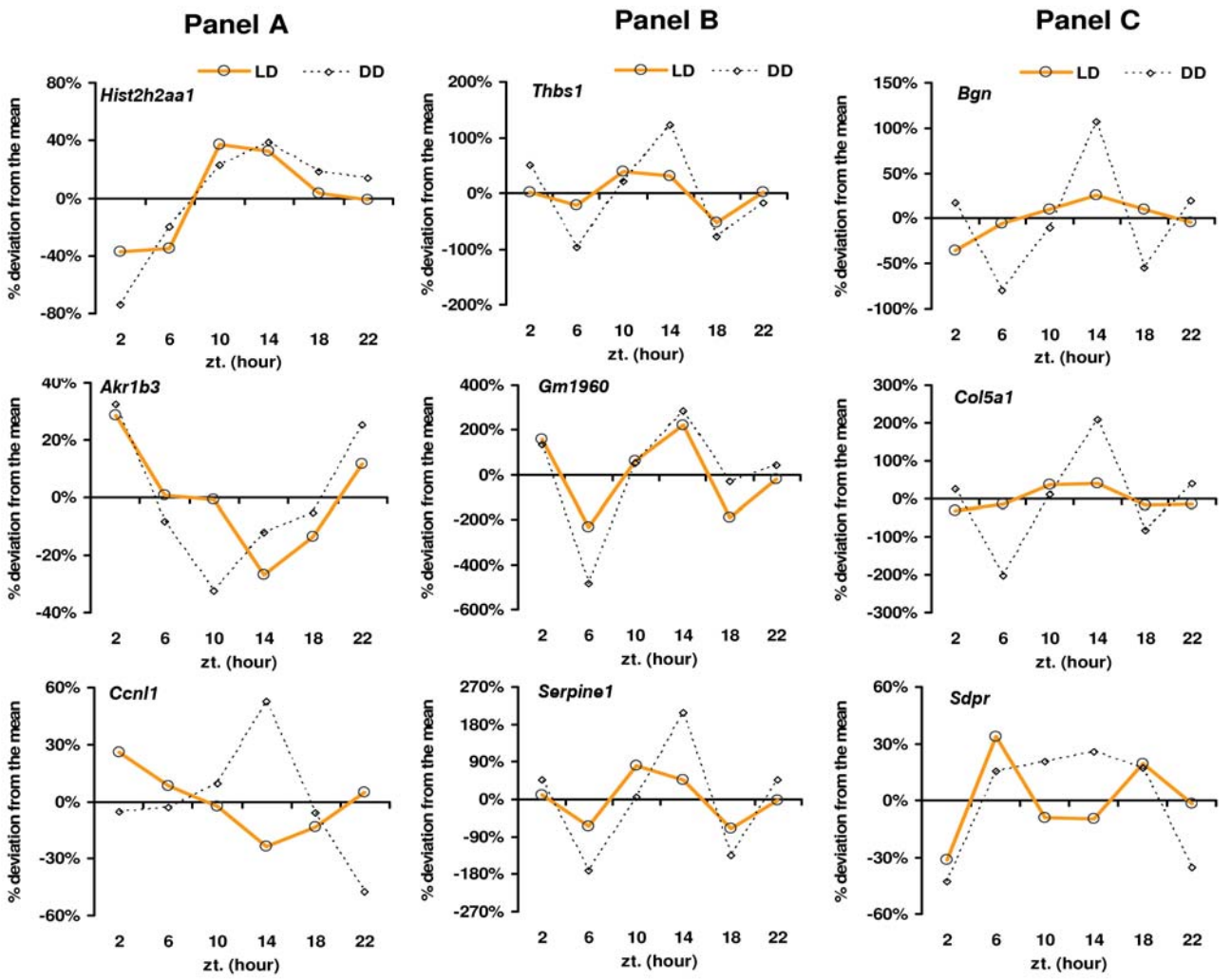

Figure 5. *Examples of gene transcripts that sustained 24 h expression patterns during both LD and DD (Panel A), sustained 12 h patterns during both LD and DD (Panel B), and demonstrated different time structures (i.e. 24 h pattern during LD but 12 h pattern during DD, and vice versa) (Panel C).*

Discussion

In this study, daily rhythmic gene expression patterns of transplanted mouse breast tumors were compared under LD and DD conditions. This is the first demonstration of the circadian gene expression profile in mouse breast cancer under both LD and DD conditions. It has been previously demonstrated that despite the fact that cancer cells proliferate rapidly, tumor growth *in vivo* is still under the control of the circadian clock (7, 13, 17). However, the rhythmic expressions of clock genes were not detectable by the microarray analysis due to their low amplitudes. The percentage of transcripts with daily rhythms in the tumor was somewhat lower than that reported in normal peripheral tissues (14). This may reflect the fact that the circadian clock is not well organized in tumors compared to normal tissues. Interestingly, the number of differentially expressed tumor transcripts was higher in DD than in LD. However, more than half of these LD transcripts and more than two third of these DD transcripts were only differently expressed at one time point during the day. Their expressions did not differ among the other five time points within a 24 h period. It is unclear what causes these singular altered expressions. Therefore, these transcripts were not considered to have circadian expression patterns. The numbers of transcripts that were differently expressed at multiple time points in these 6 time points under LD and DD conditions were similar. Some of them may have had ultradian expression patterns with shorter periods that could not be defined accurately because the sampling frequency was not high enough to determine the existence of the ultradian rhythm. Therefore, this study only focused on the transcripts with either a 12 h or a 24 h rhythm.

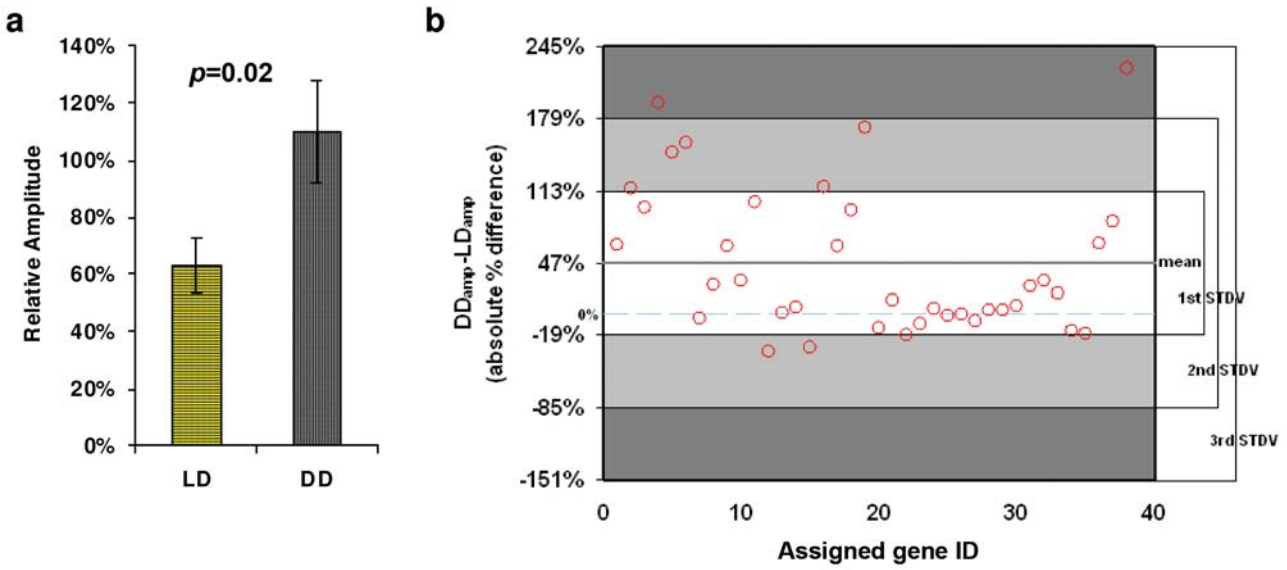

Figure 6. *The average amplitude of 38 transcripts that demonstrated a 24 h or 12 h expression pattern during both the LD and DD was statistically higher during DD than LD (a). The amplitude difference between DD and LD for each of the 38 transcripts is shown in (b). The average amplitude difference between DD and LD was 47%, with a standard deviation (STDV) of 66%.*

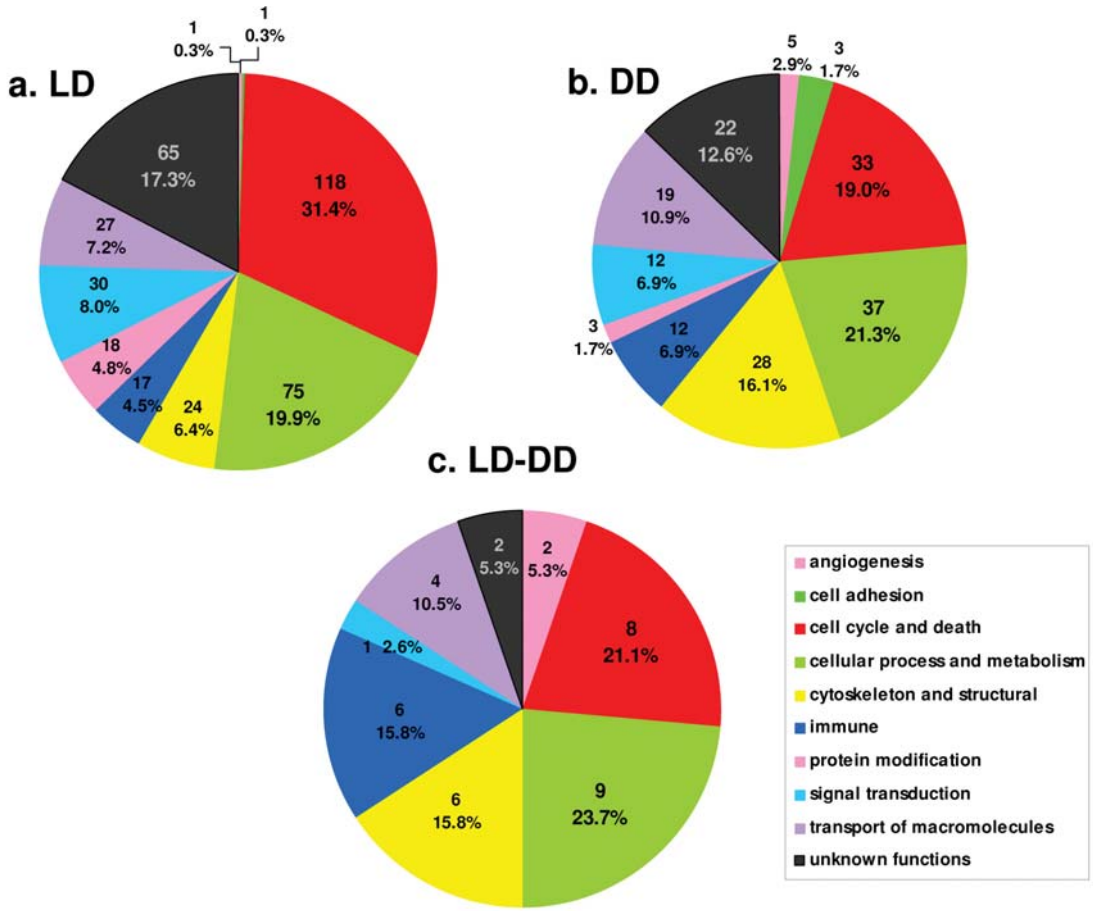

Figure 7. *Distribution of cellular functions of gene transcripts that demonstrated 24 h or 12 h expression patterns in LD (a), DD (b), or both in LD and DD (c).*

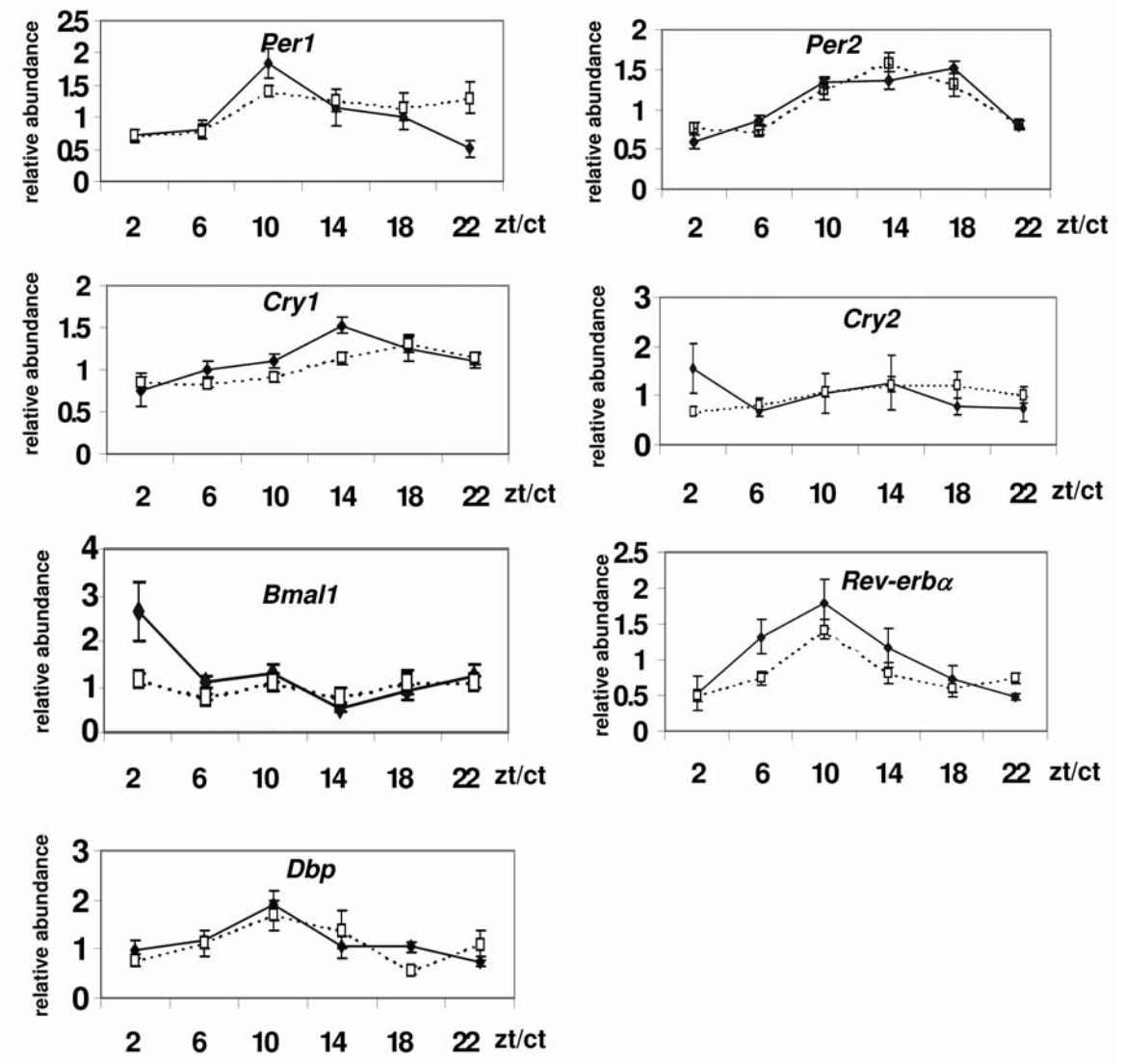

Figure 8. *The expression of core clock genes in tumors under LD (solid line) and DD (dashed line) conditions. The average abundance is set as 1.*

It is provocative that only a few of these oscillated under both LD and DD conditions. The differential daily gene expression pattern in LD and DD is not unique to this tumor; similar results have been reported in chick pineal gland and *Drosophila* head (21-23). The expression of those genes that had 24 h circadian rhythms only under LD may have been driven solely by the light (24-27). On the other hand, the expression of those genes that had 24 h rhythms only in the DD condition may have been controlled by the endogenous circadian clock and masked by the daily light cycle. It is also possible that the expression of some genes was induced by darkness, which also acts as a circadian cue (28).

One major difference between the rhythmic expression pattern of LD and DD tumor samples in this study was that the amplitudes of many rhythmic genes were increased under the DD condition. It is possible that the expression of such genes was controlled mainly by the clock, and light may have suppressed or stimulated their expressions. Therefore, their expressions will have had more robust and uniform rhythms without the interference of light.

Most cyclic genes under LD conditions have a single peak within each 24 h period, but a significant proportion of cycling gene transcripts (9% in this study) have two daily peaks. In fact, the 12 h rhythm is fairly common in normal tissues under normal light and dark conditions. Gene expressions in normal chick pineal gland and mouse distal colon under LD conditions exhibit both 24 h and 12 h rhythms (29). Under constant conditions (DD), most rhythmically expressed genes have two

peaks within every 24 h (65%). Similar expression patterns have been demonstrated in normal tissues under DD conditions (30). One possibility is that the expression of these clock-controlled genes may respond to other stimuli such as light, feeding and physical activity. The effects of these stimuli and endogenous clock may then generate gene expression patterns with 12 h, 8 h or 6 h periods. Since light is such a potent environmental cue, under LD conditions, it can mask or attenuate the effects of other stimuli. Therefore, when light is removed, the expression of these genes is controlled by the combination of multiple factors including the circadian clock, feeding, physical activity etc. which may lead to ultradian expression patterns under DD conditions.

It is known that cell division can desynchronize the circadian clock and tissues containing rapidly dividing cells have less robust rhythms of clock gene expression as a population (20, 31). Even though the expressions of core circadian clock genes in tumors have damped 24 h rhythms, tumor growth has a robust 12 h rhythm. Several genes related to cell proliferation and apoptosis display 12 h rhythmic expression patterns under LD conditions, including *Ephb3, Ctnnd2, Rbm3, Plrg1, Kif20a, Ccng2* and *Casp6*. EPHB3 is one of the Eph receptor tyrosine kinases that regulate tumor growth and angiogenesis *in vivo* (32). CTNND2, or delta-catenin, is required for vascular assembly and tumor growth (33). The RNA-binding protein RBM3 is required for cell proliferation and cell death inhibition under serum starvation (34, 35). PLRG1 is a component of splicesome and is essential for cell proliferation (36). KIF20A belongs to the kinesin family of motor protein. Down-regulation of *Kif20a* has been shown to inhibit the growth of pancreatic cancer cells (37). *Ctnnd2* gene encodes cyclin G2 which is a negative regulator of cell growth (38, 39). Caspase 6 is one of major caspases functioning in apoptosis (40, 41). It is unclear whether there is a causal relationship between the expression pattern of these genes and the tumor growth pattern. At the molecular level, it has been shown that other cell cycle indicators such as the tumor mitotic index, thymidylate synthase activity (S phase-specific), and cyclin E protein also clearly have 2 daily peaks (7, 13). Since this study identified more rhythmic genes with 2 or 3 peaks per 24 h under constant dark conditions, it would be important to determine the tumor growth rate and rhythm under the DD condition *in vivo*. A careful review of other circadian gene expression patterns in both normal and malignant tissues confirms the primacy of 12 h periodicity. It is possible that the unimodal circadian clock gene rhythm can generate and coordinate multimodal gene expressions. Finally, this study revealed that the variation of gene expression occurs most prominently at the light-dark and dark-light transition zone.

In summary, this study indicates that in tumors, as in normal tissues, the expression of a subset of genes is controlled by the daily light-dark cycle, by the endogenous circadian clock, or both. This finding should provide information for the further study of the role of circadian clock in tumor biology.

References

1 Reppert SM and Weaver DR: Coordination of circadian timing in mammals. Nature *418*: 935-941, 2002.

2 Takahashi JS, Hong HK, Ko CH and McDearmon EL: The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet *9*: 764-775, 2008.

3 Ko CH and Takahashi JS: Molecular components of the mammalian circadian clock. Hum Mol Genet 15 Spec. No. *2*: R271-R277, 2006.

4 Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitGerald GA, Kay SA and Hogenesch JB: A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron *43*: 527-537, 2004.

5 Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F and Okamura H: Control mechanism of the circadian clock for timing of cell division *in vivo*. Science *302*: 255-259, 2003.

6 Wood PA, Hrushesky WJ and Klevecz R: Distinct circadian time structures characterize myeloid and erythroid progenitor and multipotential cell clonogenicity as well as marrow precursor proliferation dynamics. Exp Hematol *26*: 523-533, 1998.

7 Wood PA, Du-Quiton J, You S and Hrushesky WJ: Circadian clock coordinates cancer cell cycle progression, thymidylate synthase, and 5-fluorouracil therapeutic index. Mol Cancer Ther *5*: 2023-2033, 2006.

8 Smaaland R, Laerum OD, Lote K, Sletvold O, Sothern RB and Bjerknes R: DNA synthesis in human bone marrow is circadian stage dependent. Blood *77*: 2603-2611, 1991.

9 Kang TH, Reardon JT, Kemp M and Sancar A: Circadian oscillation of nucleotide excision repair in mammalian brain. Proc Natl Acad Sci USA *106*: 2864-2867, 2009.

10 Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothern RB, Hrushesky WJ and Ben-David Y: Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. Am J Pathol *158*: 1793-1801, 2001.

11 Buchi KN, Moore JG, Hrushesky WJ, Sothern RB and Rubin NH: Circadian rhythm of cellular proliferation in the human rectal mucosa. Gastroenterology *101*: 410-415, 1991.

12 Sothern RB, Levi F, Haus E, Halberg F and Hrushesky WJ: Control of a murine plasmacytoma with doxorubicin-cisplatin: dependence on circadian stage of treatment. J Natl Cancer Inst *81*: 135-145, 1989.

13 You S, Wood PA, Xiong Y, Kobayashi M, Du-Quiton J and Hrushesky WJ: Daily coordination of cancer growth and circadian clock gene expression. Breast Cancer Res Treat *91*: 47-60, 2005.

14 Miller BH, McDearmon EL, Panda S, Hayes, KR, Zhang J, Andrews JL, Antoch MP, Walker JR, Esser KA, Hogenesch JB and Takahashi JS: Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. Proc Natl Acad Sci USA *104*: 3342-3347, 2007.

15 Oishi K, Miyazaki K, Kadota K, Kikuno R, Nagase T, Atsumi G, Ohkura N, Azama T, Mesaki M, Yukimasa S, Kobayashi H, Litaka C, Umehara T, Horikoshi M, Kudo T, Shimizu Y, Yano M, Monden M, Machida K, Matsuda J, Horie S, Todo T and Ihida N: Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. J Biol Chem *278*: 41519-41527, 2003.

16 McCarthy JJ, Andrews JL, McDearmon EL, Campbell KS, Barber BK, Miller BH, Walker JR, Hogenesch JB, Takahashi JS and Esser KA: Identification of the circadian transcriptome in adult mouse skeletal muscle. Physiol Genomics *31*: 86-95, 2007.

17 Yang X, Wood PA, Oh EY, Du-Quiton J, Ansell CM and Hrushesky WJ: Down regulation of circadian clock gene Period 2 accelerates breast cancer growth by altering its daily growth rhythm. Breast Cancer Res Treat *117*: 423-431, 2009.

18 Oh EY, Wood PA, Yang X and Hrushesky WJ: Discovery of candidate genes and pathways that may help explain fertility cycle stage-dependent post-resection breast cancer outcome. Breast Cancer Res Treat *118*: 345-359, 2008.

19 Yang X, Wood PA, Ansell CM, Quiton DF, Oh EY, Du-Quiton J and Hrushesky WJ: The circadian clock gene *Per1* suppresses cancer cell proliferation and tumor growth at specific times of day. Chronobiol Int *26*: 1323-1339, 2009.

20 Yamamoto T, Nakahata Y, Soma H, Akashi M, Mamine T and Takumi T: Transcriptional oscillation of canonical clock genes in mouse peripheral tissues. BMC Mol Biol *5*: 18, 2004.

21 Karaganis SP, Kumar V, Beremand PD, Bailey MJ, Thomas TL and Cassone VM: Circadian genomics of the chick pineal gland *in vitro*. BMC Genomics *9*: 206, 2008.

22 Ueda HR, Matsumoto A, Kawamura M, Iino M, Tanimura T and Hashimoto S: Genome-wide transcriptional orchestration of circadian rhythms in Drosophila. J Biol Chem *277*: 14048-14052, 2002.

23 Lin Y, Han M, Shimada B, Wang L, Gibler TM, Amarakone A, Awad TA, Stormo GD, Van Gelder RN and Taghert PH: Influence of the period-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*. Proc Natl Acad Sci US *99*: 9562-9567, 2002.

24 Dziema H, Oatis B, Butcher GQ, Yates R, Hoyt KR and Obrietan K: The ERK/MAP kinase pathway couples light to immediate-early gene expression in the suprachiasmatic nucleus. Eur J Neurosci *17*: 1617-1627, 2003.

25 Porterfield VM and Mintz EM: Temporal patterns of light-induced immediate-early gene expression in the suprachiasmatic nucleus. Neurosci Lett *463*: 70-73, 2009.

26 Tischkau SA, Mitchell JW, Tyan SH, Buchanan GF and Gillette MU: Ca^{2+}/cAMP response element-binding protein (CREB)-dependent activation of Per1 is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. J Biol Chem *278*: 718-723, 2003.

27 Porterfield VM, Piontkivska H and Mintz EM: Identification of novel light-induced genes in the suprachiasmatic nucleus. BMC Neurosci *8*: 98, 2007.

28 Zhang J, Kaasik K, Blackburn MR and Lee CC: Constant darkness is a circadian metabolic signal in mammals. Nature *439*: 340-343, 2006.

29 Dekens MP, Santoriello C, Vallone D, Grassi G, Whitmore D and Foulkes NS: Light regulates the cell cycle in zebrafish. Curr Biol *13*: 2051-2057, 2003.

30 Hoogerwerf WA, Sinha M, Conesa A, Luxon BA, Shahinian VB, Cornelissen G, Halberg F, Bostwick J, Timm J and Cassone VM: Transcriptional profiling of mRNA expression in the mouse distal colon. Gastroenterology *135*: 2019-2029, 2008.

31 Nagoshi E, Saini C, Bauer C, Laroche T, Naef F and Schibler U: Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. Cell *119*: 693-705, 2004.

32 Pasquale EB: Eph receptors and ephrins in cancer: bidirectional signalling and beyond. Nat Rev Cancer *10*: 165-180, 2010.

33 DeBusk LM, Boelte K, Min Y and Lin PC: Heterozygous deficiency of delta-catenin impairs pathological angiogenesis. J Exp Med *207*: 77-84, S1-4, 2010.

34 Wellmann S, Truss M, Bruder E, Tornillo L, Zelmer A, Seeger K and Buhrer C: The RNA-binding protein RBM3 is required for cell proliferation and protects against serum deprivation-induced cell death. Pediatr Res *67*: 35-41, 2010.

35 Sureban SM, Ramalingam S, Natarajan G, May R, Subramaniam D, Bishnupuri KS, Morrison AR, Dieckgraefe BK, Brackett DJ, Postier RG, Houchen CW and Anant S: Translation regulatory factor *RBM3* is a proto-oncogene that prevents mitotic catastrophe. Oncogene *27*: 4544-4556, 2008.

36 Kleinridders A, Pogoda HM, Irlenbusch S, Smyth N, Koncz C, Hammerschmidt M and Bruning JC: PLRG1 is an essential regulator of cell proliferation and apoptosis during vertebrate development and tissue homeostasis. Mol Cell Biol *29*: 3173-3185, 2009.

37 Taniuchi K, Nakagawa H, Nakamura T, Eguchi H, Ohigashi H, Ishikawa O, Katagiri T and Nakamura Y: Down-regulation of RAB6KIFL/KIF20A, a kinesin involved with membrane trafficking of discs large homologue 5, can attenuate growth of pancreatic cancer cell. Cancer Res *65*: 105-112, 2005.

38 Choi MG, Noh JH, An JY Hong SK, Park SB, Baik YH, Kim KM, Sohn TS and Kim S: Expression levels of cyclin G2, but not cyclin E, correlate with gastric cancer progression. J Surg Res *157*: 168-174, 2009.

39 Xu G, Bernaudo S, Fu G, Lee DY, Yang BB and Peng C: Cyclin G2 is degraded through the ubiquitin-proteasome pathway and mediates the antiproliferative effect of activin receptor-like kinase 7. Mol Biol Cell *19*: 4968-4979, 2008.

40 Cohen GM: Caspases: the executioners of apoptosis. Biochem J 326: 1-16, 1997.

41 Faleiro L, Kobayashi R, Fearnhead H and Lazebnik Y: Multiple species of CPP32 and *MCH2* are the major active caspases present in apoptotic cells. EMBO J *16*: 2271-2281, 1997.

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