Chronodisruption Alter Expression of Tumorigenesis Associated Transcripts in Mouse Brain

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Abstract: - Disturbances in "natural" daily light regulation, as frequently happen in modern life, may lead to the disruption of the circadian clock organization. Such chronodisruption had been associated with disturbances in cell cycle homeostasis and with malignancy. The International Agency for Research in Cancer (IARC) classified "shift work that involves circadian disruption" as possibly carcinogenic. Recently, I showed by microarray analysis that light pulse during the dark phase promptly affects transcription level of genes that directly control cell cycle progression in mouse brain. Here I specifically assess the microarray results with respect to transcripts that are associated with tumorigenesis. The analysis indicated that light promptly affects transcription level of a substantial number of genes that are associated with cell proliferation and tumorigenesis, as well as transcripts that control cell cycle progression. A considerable assembly of affected transcripts promotes cell proliferation. It includes tumor suppressors, oncogenes and genes that are involved in tumor growth and metastasis. The results suggest an association between light signal during the dark phase, obstruction of the cell cycle homeostasis and tumorigenesis.

Key-Words: - Chronodisruption, Circadian rhythm, Light entrainment, Cell cycle homeostasis, Tumorigenesis, microarray

1 Introduction

Modern life often encounters disturbances in "natural" light/dark organization, for example, by prolonging the normal light phase with light-at-night in urban settings, or by numerous occupations that include working at night or in shifts. These light-dark cycle disturbances can generate problems for circadian clock organization. Circadian rhythms are fundamental adaptations of living cells to the daily and seasonal fluctuations in light and temperature. Circadian oscillations persist in constant conditions, but they are also phase-adjusted (entrained) by environmental stimuli, with light being a key exogenous signal. Pulses of light during the dark phase reset the circadian pacemaker and elicit phase shifts (reviewed in [1]). In humans, the ability to correct the phase and to re-synchronize the circadian clock also enables the adjustment to changes in the environmental light/dark conditions that are imposed by traveling across time zones (jet-lag).

When illumination is repeatedly applied at unusual times it can disturb the seasonal and the circadian clock organization of physiology, endocrinology metabolism and behavior, and can lead to chronodisruption [2]. Chronodisruption had been associated with disturbance of cell cycle homeostasis and with malignancy [3-6]. The International Agency for Research in Cancer (IARC) classified "shift work that involves circadian disruption" as possibly carcinogenic [7]. However, there are no definitive studies that imply that light-at-night is directly related to ill-health and cancer rates among shiftworkers [8].

Chronodisruption *might* play a causal role for cancer growth and tumor progression, so it is important to enhance our understanding of the processes that may link the regulation of circadian

rhythms to cell cycle homeostasis. Considering the circadian mode of cell proliferation, an important question is whether altering circadian entrainment can also change the dynamics of cell division, with clear implications for tumor growth [9,10]. We recently showed that light, the major circadian clock entrainment signal, can operate as an environmental indicator and/or stressor which can promptly affect transcription levels of genes that directly control cell cycle progression in mouse brain [11,12]. The diversion from appropriate regulation of cell cycle may lead to malignant transformation. Here we specifically assess our results with respect to transcripts that are associated with tumorigenesis.

2 Methods

Changes in the expression of transcripts in mouse brain after administrating light pulses during the dark phase was investigated with microarray analysis [13]. Here, we re-examine the microarray results and specifically examined differential expression of transcripts associated with tumorigenesis (for microarray data analysis and validation, see [13]). Adult C57BL male mice were kept in 12:12 light:dark cycle. A 1 hr light pulse was administered at 2, 6 or 10 hr after onset of dark (i.e. ZT14, 18 or 22), while control animals remained in darkness. Whole brain total RNA was collected at 11 time points: 15 min, 1, 2, 3 and 4 hr after onset of the pulse at ZT14 or 18, and 1 hr after onset at ZT22, in experimental and time-matched control animals. At every time point RNA was collected from two experimental and two matched control mice (in all, 44 mice).

3 Results

Our results indicated that light promptly affected the transcription level of genes that are associated with tumorigenesis, as well as transcripts that control cell cycle progression and apoptosis. Of 1787 cDNAs on the arrays found suitable for analysis (consistently up- or down-regulated hybridization signal in at least 8 tests), 73 (~4% of the cDNAs that were examined) representing 51 different genes, are associated with cell proliferation and tumorigenesis (Table 1; Figure 1). Thirty of these transcripts were up-regulated and 21 down-regulated.

Within these 51 genes there are oncogenes, tumor suppressors and genes that are causally associated with tumorigenesis and metastasis, as well as genes that show alter expression patterns in various malignancies (Table 1). Considering the direction of changes in gene regulation, a single light pulse given to healthy mice induced changes in transcription level of 16 genes (>30%) that promotes cell proliferation and malignancy in various cancers, while 11 transcripts may inhibit cell proliferation. The activity of four of the genes affected by light pulse (*Bid, Fgf15, Notch2l* and *Sparc*) is context- and cell-type-dependent because they are involved in both oncogenic or tumor inhibitor in various cancers (Table 1; Figure 1).

Table 1:
Genes that are associated with tumorigenesis (U = up-regulated, D = down-regulated)

U/D	GENE*	Directly Promotes	Associated with	Inhibits
U	*Aplp2*		Detected in several tumors	
U	*Atf4*	Overexpressed in hypoxic areas; plays a role in tumor hypoxic adaptation		
U	*Atp2a2*		Displays altered expression patterns in various malignancies	

D	*Bid*	Promotes tumorigenesis in myeloid cells. Overexpression can facilitate apoptotic death of hepatocellular carcinoma [24]		Inhibits carcinogenesis in the liver
D	*Ccnd1*			Overexpression is linked to tumorigenesis of different cancer types
U	*Cdc2l1*		Deregulated in human tumors. Inhibition may induce p53 protein [25]	
U	*cdc42*		Participates in the regulation of tumorigenesis	
U	*Clu*		Overexpressed in various malignancies	
D	*Cspg2*		Expression is associated with various cancers; often secreted in response to tumor signals	
U	*Ctsb*	Causally associated with various aspects of tumorigenesis including metastasis		
U	*Dad1*	Inhibit apoptosis		
U	*Dag1*		Deregulated in a variety of human malignancies. Related to tumor aggressiveness	
U	*Dcn*	Enhances proliferation		
U	*Drg1*			Down-regulated in malignancy. Putative suppressor of metastases
U	*Eef2*		Overexpressed in several cancers	
U	*Egr1*	Essential for cancer proliferation and survival		

D	*Eif4ebp1*		Increased expression in a number of solid tumors	
D	*Epor*			Blockade may inhibit various cancer types growth [26]
D	*Ets2*			Oncogene
U	*Fasn*	Displays oncogenic properties; highly expressed in several human cancers		
D	*Fgf15*	Suppresses proliferation in cortical cultures [27]		Plays an important roles in oncogenesis
U	*Hmgb1*	Involved in tumor growth and metastasis		
U	*Hsp60*		Associated with carcinogenesis, specifically with tumor cell survival and proliferation	
U	*Hsp70-2*	Promotes tumor cell growth		
D	*Hyal2*	Tumor suppressor		
D	*Kif1a*		Deregulated in the endometrial cancer	
D	*Kras2*			Oncogene
D	*Lamc2*		A specific marker of invasive tumors	
U	*Lasp1*	Plays a functional role in migration and proliferation of certain cancer cells		
U	*Mif*	Over-expressed in tumors. May serve as an important link between chronic inflammation and cancer development		
U	*Mt1*			Acts as an oncosuppressor
D	*Myd118*	Play important roles in growth suppression and apoptosis		

U	*Nedd8*	Essential for the regulation of protein degradation pathways involved in tumorigenesis.		
U	*Nme1*		Reduced mRNA transcript levels in highly metastatic cells	
D	*Nmyc1*			Oncoprotein
U	*Notch2l*	Oncogene or tumor inhibitor in various cancers		Oncogene or tumor inhibitor in various cancers
U	*Pftk1*		Overexpression may confer a motile phenotype in malignant hepatocytes	
U	*Ppp2ca*			Downregulation may take part in carcinogenesis.
D	*Rb1*	Tumor suppressor		
U	*S100b*		Differentially underexpressed in cancers	
D	*Sipa1 (SPA1)*		Metastasis susceptibility gene	
U	*Smarcb1 (SNF5)*			Tumor suppressor
U	*Sparc*	The activity is context- and cell-type-dependent. Shows contradictory effects on tumor progression		The activity is context- and cell-type-dependent. Shows contradictory effects on tumor progression
D	*Stat5a*			Activation is essential for tumorigenesis
D	*Tgfb2*		Suppressed early in breast carcinogenesis	
D	*Tgfbr2*		Suppressed early in breast carcinogenesis	
U	*Thra*		May be involved in human cancer	
D	*Tmpo (LAP2)*		Overexpression in a significant percentage of cancer tissues	

D	*Tnfrsf1a*			Major receptors for the tumor necrosis factor
U	*tsg101*	Expression may be necessary for tumor progression		
D	*Zfp144*	Tumor suppressor		

*** Gene's full names and alternative symbols are in appendix 1**

4 Discussion

A light signal during the dark phase represents a temporal cue that has an acute effect on the level of components of the circadian clock, leading to synchronization of the circadian oscillator with the external environment. The circadian oscillator may also be involved in the major cellular pathways of cell division [14-16] and may possibly be implicated in gating the entry (or exit) of cell division [17,18].

Light signals during the dark phase represent environmental stressors, that consistently activate several stress proteins (*Hsp60*, *Hsp70-2* and *Egr1*, Table 1), which are involved in the regulation of cell cycle progression and play an indispensable role in cancer proliferation and survival. These proteins act as a primary line of cellular defense and are directly involved in malignancy. Recently the tumor specific functions of the *Hsps* have been suggested as possible markers to target cancer [19].

The adult brain is characterized by a relatively low mitotic index. Nonetheless, we recently found that a single light pulse during the dark phase affects transcripts that are involved the control of cell cycle progression in the mouse brain, and may possibly lead to cell cycle arrest [11]. If the light signal during the dark phase also disrupts the homeostatic control of cell division, it may affect cell proliferation and malignancy. Our microarray results indicated that a light pulse during the dark phase swiftly affects a substantial assembly of transcripts that promote proliferation. It includes tumor suppressors, oncogenes and genes that are involved in tumor growth and metastasis (Table 1; Figure 1B). The light pulse consistently down-regulated expression of three tumor suppressors: *Hyaluronidase 2 (Hyal2), Retinoblastoma (Rb1*) and *Zinc finger protein 144 (Zfp144).* Consistent up-regulation was observed among other transcript such as: (i) *macrophage migration inhibitory factor* (*Mif*) that may serve as an important link between chronic inflammation and cancer development. (ii) *activating transcription factor 4* (*Atf4*) that plays a role in tumor hypoxic adaptation. (iii) *cathepsin B* (*Ctsb*) whose expression has been implicated in tumor invasion and metastasis. (iv) *fatty acid synthase* (*Fasn*) that is highly expressed in several human cancers and displays oncogenic properties such as resistance to apoptosis and induction of proliferation when overexpressed [20] (v) *LIM and SH3 protein 1* (*Lasp1*) that plays an important functional role in migration and proliferation of certain cancer cells. (vi) *high mobility group protein 1* (*Hmgb1*), a chromatin architectural protein reported to be involved in tumor growth and metastasis and (vii) *tumor susceptibility gene 101* (*tsg101*) whose expression may be necessary for activities associated with aspects of tumor progression [21].

Among these transcripts there are also some that may inhibit proliferation, (Table 1) and may lead to a suppression of tumor growth [10,22]. These transcripts include: (i) down-regulation of several oncogenes like *E26 avian leukemia oncogene 2,3' domain* (*Ets2*), *Kirsten rat sarcoma oncogene 2* (*Kras2*) and *Neuroblastoma myc-related oncogene 1* (*Nmyc1*); (ii) up-regulation of the tumor suppressor *SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1* (*Smarcb1*), and (iii) up-regulation of *Developmentally regulated GTP-binding protein 1*(*Drg1*) a putative suppressor of metastases.

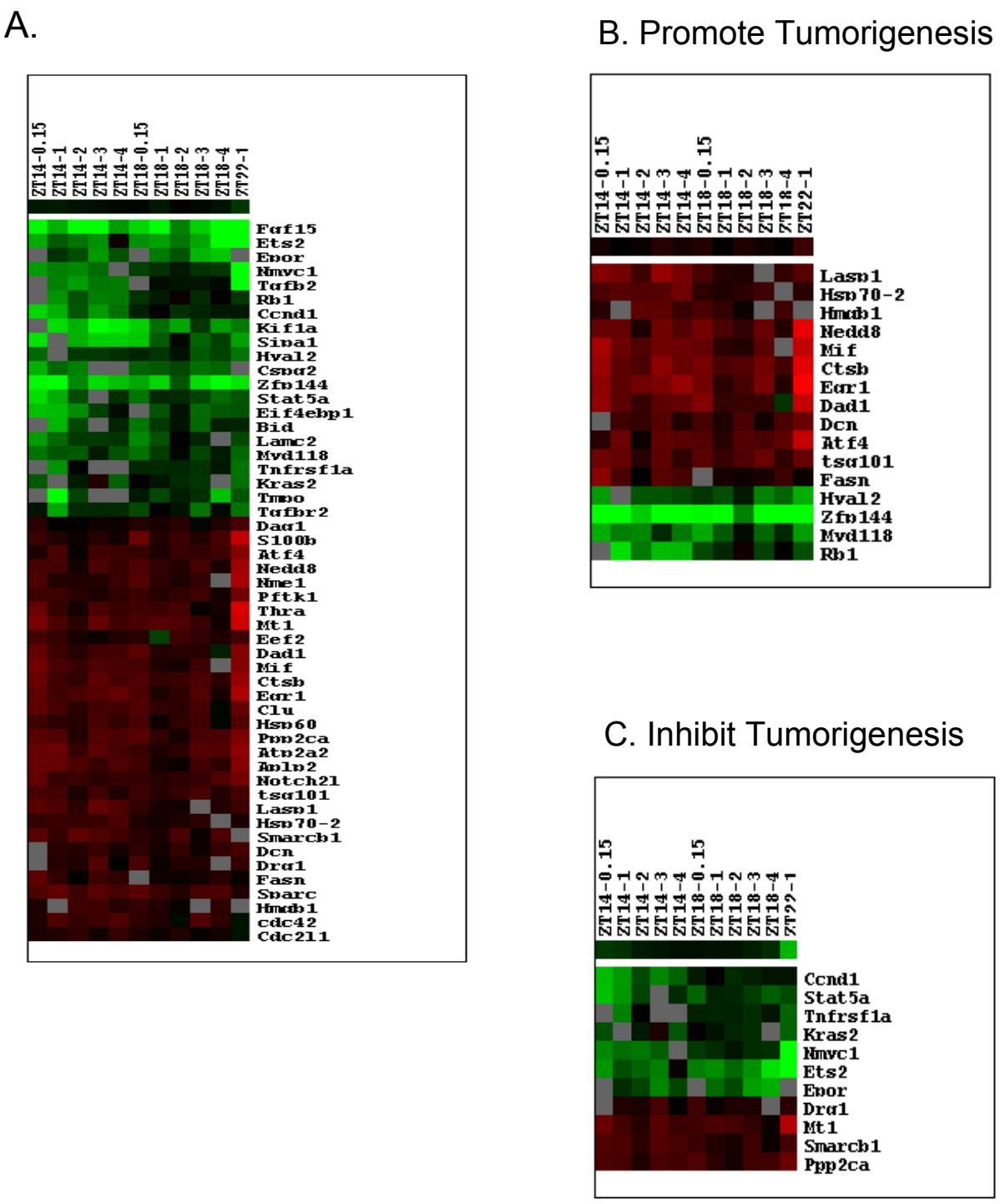

Figure 1:
A. Cluster analysis of changes in tumorigenesis associated transcripts resulting from a light pulse during the dark phase. Each column represent the time of light pulse (ZT 14, 18, or 22) and the time of RNA collection after the pulse. Thus, ZT14–0.15 represents administration of light pulse at ZT14, and RNA collection 15 min after the pulse. Red represents up-regulation of a transcript, and green down-regulation. Gray represents missing data. Cluster analysis of transcripts that promote tumorigenesis. C. Cluster analysis of transcripts that inhibit tumorigenesis.

Association between disruption of the circadian clock, interference in the regulation of cell cycle, and malignancy has been known for several years [3,4]. The results presented here suggest that there may be a causative association between the light signal during the dark phase, and the disruption of the cell cycle homeostasis and tumorigenesis. This clearly has implications for the issue of whether there is an increased relative risk for cancer among shiftworkers [7].

Chronodisruption of the circadian timing system may also change the pace of tumor growth via modification of the host clock, which then may accelerate abnormal proliferation [9]. In contrast, a complementary approach would be to consider circadian down-regulation of malignant growth factors in any chronotherapeutic intervention [23]. Our results provide an entrée into enhancing our understanding of the processes that may link the regulation of circadian rhythms to that of cell cycle homeostasis.

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Appendix 1: The full name of the genes that are associated with tumorigenesis

Gene symbol	Full Name
Aplp2 (CDEBP) *	Amyloid beta (A4) precursor-like protein 2
Atf4 (CREB2)	Activating transcription factor 4
Atp2a2 (SERCA2)	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
Bid	BH3 interacting domain death agonist
Ccnd1	Cyclin D1
Cdc2l1 (Cdk11)	PITSLRE serine/threonine-protein kinase. CDC2L1 Cell division cycle 2-like protein kinase 1
cdc42	Cell division control protein 42 homolog
Clu	Clusterin
Cspg2	Chondroitin sulfate proteoglycan 2 (Versican core protein)
Ctsb	Cathepsin B
Dad1	Defender against cell death 1
Dag1	Dystroglycan 1
Dcn	Decorin
Drg1	Developmentally regulated GTP-binding protein 1
Eef2	eukaryotic translation elongation factor 2
Egr1	Early growth response 1
Eif4ebp1	Eukaryotic translation initiation factor 4E binding protein
Epor	Erythropoietin receptor
Ets2	E26 avian leukemia oncogene 2, 3' domain
Fasn	Fatty acid synthase
Fgf15	Fibroblast growth factor 15
Hmgb1	High mobility group protein 1
Hsp60	Heat shock protein, 60 kDa
Hsp70-2	Heat shock protein, 70 kDa 2
Hyal2	Hyaluronidase 2
Kif1a	Kinesin heavy chain member 1A
Kras2	Kirsten rat sarcoma oncogene 2, expressed
Lamc2	Laminin, gamma 2
Lasp1	LIM and SH3 protein 1
Mif	Macrophage migration inhibitory factor
Mt1	Metallothionein 1
Myd118	Myeloid differentiation primary response gene 118
Nedd8	Neural precursor cell expressed, developmentally down-regulated gene 8
Nme1	Expressed in non-metastatic cells 1, protein (NM23A)
Nmyc1	Neuroblastoma myc-related oncogene 1
Notch2l	Notch2-like
Pftk1 (CDK)	PFTAIRE protein kinase 1
Ppp2ca	Protein phosphatase 2a, catalytic subunit, aCTha isoform
Rb1	Retinoblastoma 1
S100b	S100 protein, beta polypeptide, neural
Sipa1 (SPA1)	Signal-induced proliferation associated gene 1
Smarcb1 (SNF5; INI1)	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
Sparc	Secreted acidic cysteine rich glycoprotein
Stat5a	Signal transducer and activator of transcription 5A
Tgfb2	Transforming growth factor, beta 2
Tgfbr2	Transforming growth factor, beta receptor II
Thra	Thyroid hormone receptor aCTha
Tmpo	Thymopoietin
Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1a
tsg101	Tumor susceptibility gene 101
Zfp144	Zinc finger protein 144

* In parenthesis alternative symbols