Effects of Chronic Jet Lag on Tumor Progression in Mice

Elisabeth Filipski,1 Franck Delaunay,2 Verdun M. King,3 Ming-Wei Wu,1 Bruno Claustret,4 Aline Gréchez-Cassiau,2 Catherine Guettier,5 Michael H. Hastings,6 and Lévi Francis1

1INSERM U0354 Cancer chronotherapeutics, Hôpital Paul Brousse, Villejuif Cedex, France; 2CNRS UMR 6078 Université de Nice-Sophia Antipolis, Villefranche sur mer, France; 3Department of Anatomy, University of Cambridge, Cambridge, United Kingdom; 4Service de Radioanalyse, Hôpital Neurocardiologique, Lyon, France; 5Laboratory of Anatomy and Pathologic Cytology, Hôpital Paul Brousse, Villejuif, France; and 6Laboratory of Molecular Biology, MRC Centre, Cambridge, United Kingdom

ABSTRACT

Frequent transmeridian flights or predominant work at night can increase cancer risk. Altered circadian rhythms also predict for poor survival in cancer patients, whereas physical destruction of the suprachiasmatic nuclei (SCN), the hypothalamic circadian pacemaker, accelerates tumor growth in mice. Here we tested the effect of functional disruption of circadian system on tumor progression in a novel experimental model of chronic jet lag. B6D2F1 mice were synchronized with 12 hours of light and 12 hours of darkness or underwent repeat 8-hour advances of the light/dark cycle every 2 days before inoculation of Glasgow osteosarcoma. The 24-hour changes were assessed for plasma corticosterone, clock protein mPER1 expression in the SCN, and mRNA expression of clock genes mPer2 and mRev-era in liver and tumor. Time series were analyzed by spectral analysis and/or Cosinor. Differences were compared with analysis of variance (ANOVA). The 24-hour rest/activity cycle was ablated, and the rhythms of body temperature, serum corticosterone, and mPER1 protein expression in the SCN were markedly altered in jet-lagged mice as compared with controls (ANOVA, \( P < 0.001 \) for corticosterone and \( P = 0.01 \) for mPER1). Tumor grew faster in the jet-lagged animals as compared with controls (ANOVA, \( P < 0.001 \)), whereas exposure to constant light or darkness had no effect (ANOVA, \( P = 0.66 \) and \( P = 0.8 \), respectively). The expression of mPer2 and mRev-era mRNAs in controls showed significant circadian rhythms in the liver (\( P = 0.006 \) and \( P = 0.003 \), respectively, Cosinor) and in the tumor (\( P = 0.04 \) and \( P < 0.001 \)). Both rhythms were suppressed in the liver (\( P = 0.2 \) and \( P = 0.1 \), respectively, Cosinor) and in the tumor (\( P = 0.5 \)) of jet-lagged mice. Altered environmental conditions can disrupt circadian clock molecular coordination in peripheral organs including tumors and play a significant role in malignant progression.

INTRODUCTION

Cancer patients with altered 24-hour rhythms had poorer survival as compared with those with nearly normal rhythms in two prospective studies (1, 2). Severe alterations in the rest/activity circadian rhythm predicted for a 5-fold increase in the risk of death in patients with metastatic colorectal cancer as compared with those with normal rest/activity patterns (1). Similarly, an abnormal cortisol rhythm predicted for a doubling of the risk of death in patients with metastatic breast cancer as compared with those with a normal cortisol pattern (2). Most importantly, the predictive value of alterations in these rhythms for poor survival outcome was independent from all known clinical factors in both studies (reviewed in ref. 3).

Circadian rhythms are generated within most mammalian cells by interconnected molecular loops involving specific genes that consti-

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Results

The rest/activity and temperature rhythms of all of the mice kept in LD12:12 in experiments 1 and 2 were marked and regular from one day to the next (Fig. 1A, left panels, top and bottom, respectively). Spectral analysis indicated a dominant 24-hour period for both variables (Fig. 1A, right panels, top and bottom, respectively). Repeated light advances suppressed circadian rhythmicity of activity in 2 of 12 mice and temperature in 1 of 12 mice in experiment 1 (Fig. 1B). In all of the other animals, the period of both activity and temperature rhythms was lengthened (mean ± SE, 24.6 ± 0.1 and 25.0 ± 0.2 hours, respectively), and the relative amplitudes decreased by 50% and 30%, respectively (Table 1).

In experiment 2, chronic jet lag suppressed the activity rhythm in 3 of 16 mice and the temperature rhythm in 4 of 16 mice, before tumor inoculation. In the other animals, it lengthened the period of both activity and temperature rhythms (25.4 ± 0.3 and 25.3 ± 0.3 hours, respectively) and decreased the relative amplitude by 80% and 37%, respectively (Table 1). Both the period lengths and the relative amplitudes of both rhythms differed significantly in mice subjected to chronic jet lag as compared with those kept in LD12:12 (Table 1).

In experiment 4, both temperature and activity rhythms were robustly circadian in animals in constant darkness or constant light, with mean ± SE periods of 23.4 ± 0.2 and 25.6 ± 0.2 hours, respectively. Mice in LD12:12 had a period of 24.0 ± 0.1 hours, and there were no

by 8 hours every 2 days for 10 days was the most disruptive schedule for the locomotor activity rhythm among those tested, consistent with the observation that circadian function is more severely altered during adaptation to advances rather than delays in local time (21). Here we show that chronic jet lag accelerated initial tumor growth and shortened mouse survival, in contrast to constant light or darkness, which is known to maintain circadian coordination (4–6, 22). We relate this effect of chronic jet lag to the disruption produced in the circadian timing system at several levels of organization including behavioral and hormonal rhythms as well as clock gene expression in the SCN, the liver, and the tumor itself.

MATERIALS AND METHODS

Study Design. The study was conducted in accordance with the guidelines approved for animal experimental procedures by the French Ethical Committee (Decree 87-848) and the guidelines for the Welfare of Animals in Experimental Neoplasia from the United Kingdom Co-ordinating Committee in Cancer Research.

Four experiments were performed in a total of 208 mice.

Experiment 1 assessed the effects of chronic jet lag on circadian physiology and SCN function in non–tumor-bearing mice. The rhythms in rest/activity, body temperature, plasma corticosterone concentration, and clock protein mPER1 expression in the SCN were studied in 120 male 6-week-old B6D2F1 mice (Charles Rivers, L’Arbresle, France). Twenty-four of them had a radio transmitter (Physio Tel, TA 10 TA-F20; Data Sciences, St. Paul, MN) implanted into the peritoneal cavity, which recorded locomotor activity and body temperature every 10 minutes throughout the experiment. The mice were synchronized to standard lighting conditions of LD12:12, with lights on from 6 a.m. (Zeitgeber time 0) to 6 p.m. (Zeitgeber time 12), for 3 weeks and then randomly assigned to either remain in this lighting regimen or undergo experimental chronic jet lag produced by 10 days of serial 8-hour advances of light/dark cycle every 2 days. The animals were then exposed to constant darkness for 2 days before sacrifice at one of six CTs at 4-hour intervals. The CT corresponding to light onset was defined as CT0. CT0-recurs at the same clock hour over the initial 3 days in constant darkness. These procedures avoid any masking effect of light on circadian rhythmic patterns (23). Blood samples were taken, and serum corticosterone concentration was measured by radioimmunoassay (9). The expression of mPER1 was measured in the SCN with immunocytochemistry using anti-mPER1 antibody nuclear staining (24).

Experiment 2 investigated the effect of chronic jet lag on tumor growth and clock gene expression patterns in healthy and malignant tissues. Two groups of 16 mice each were randomly assigned to remain in standard lighting (LD12:12) or to be exposed to experimental chronic jet lag. Their locomotor activity and clock gene expression patterns were monitored with a radiotransmitter as described in experiment 1. Ten days after the start of light/dark cycle advances, animals in both groups were inoculated subcutaneously with a 3-mm fragment of transplantable Glasgow osteosarcoma (Aventis Pharma S.A.; Vitry sur Seine, France; ref. 9). Three mice in each group served as non–tumor-bearing controls. Survival was checked daily, and tumor size was measured with a caliper three times per week. Tumor weight was computed as follows:

\[ \text{tumor weight} = \frac{(\text{length} \times \text{width})^2}{2} \]

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significant differences in relative amplitudes among the three groups (data not shown).

Plasma corticosterone concentration (Fig. 2A) in control mice kept in LD12:12 showed a marked circadian rhythm ($P < 0.001$, Cosinor) peaking at the subjective light/dark transition (CT12, in reference to previous time of light onset CT0). The mean value at peak was 19-fold greater than the mean trough value. The mice with experimental jet lag exhibited a severely disrupted, biphasic corticosterone profile that
resulted in a statistically significant 12-hour periodic component (P < 0.001, Cosinor) with a major peak at CT4 and a lower one at CT16. The mean value at peak was nearly 7-fold greater than the mean trough value at CT12. Two-way ANOVA validated statistically significant effects of CT (P = 0.01), whereas the 24-hour mean level was not affected by the lighting schedule (P = 0.21). A significant (time × lighting schedule) interaction (P < 0.001) further validated that the circadian patterns in mean plasma corticosterone were different in control mice and in jet-lagged ones.

The expression of clock protein mPER1 in the SCN of control animals exhibited a marked circadian rhythm (P < 0.0001, Cosinor), with values nearly 6-fold as high as at the peak (at CT12) as compared with trough (at CT0; Fig. 2B). In contrast, the rhythm was damped in the SCN of mice submitted to chronic jet lag (P = 0.11, Cosinor), with a mean value at peak (at CT12) only twice as high as that at trough (at CT8).

Two-way ANOVA validated statistically significant effects of CT (P < 0.001) and time × lighting schedule interaction (P = 0.01), whereas no effect of lighting schedule on the 24-hour mean of mPER1 protein expression was found (P = 0.27).

Relevance of Circadian Coordination for Tumor Growth

Tumor progressed significantly faster in the desynchronised animals undergoing jet lag as compared with those kept in LD12:12 in both experiment 2 (ANOVA, P < 0.001; Fig. 3A) and experiment 3 (ANOVA, P = 0.002; Fig. 3B). Both experiments indicated that chronic jet lag accelerated tumor growth rate mostly between the 8th and 11th day after tumor inoculation. In experiment 2, mean tumor weight ± SE on day 11 (i.e., before the death of the first animal) was 1330 ± 151 mg in jet-lagged mice and 647 ± 56 mg in controls (t test, P = 0.001). In experiment 3, it was 1376 ± 131 and 847 ± 107 mg in jet-lagged and control mice, respectively (t test, P = 0.005).

The survival curves further differed with statistical significance as a function of lighting schedule, with poorest survival in jet-lagged mice, in each experiment considered separately (log-rank test, P = 0.013 for experiment 2 and P = 0.0025 for experiment 3) or pooled (P < 0.0001; Fig. 4).

Conversely, exposure to constant darkness or constant light had no significant effect on tumor growth (ANOVA, P = 0.8) or survival (log-rank test, P = 0.66) as compared with mice kept in LD12:12 (Fig. 3C).

**Circadian Gene Expression**

**Control Mice.** In the liver, the mRNA expression of mPer2 and that of mRev-erbα showed significant circadian variations, with respective peak times occurring 6 hours apart (Fig. 5A and B). Mean mPer2 expression tripled from CT6 (trough) to CT12 (peak) (Cosinor, P = 0.006; ANOVA, P = 0.005). Mean mRev-erbα expression reached a maximum at CT6, with a value 36-fold as high as that measured at nadir at CT18 (Cosinor, P = 0.003; ANOVA, P < 0.001).

In tumor, the expression of both clock genes was also rhythmic, but with minor alterations (Fig. 5A and D). Mean mPer2 expression also peaked at CT12, yet the nadir was found at CT0 rather than CT6 (Cosinor, P = 0.04; ANOVA, P = 0.09). High values of mRev-erbα expression plateaued from CT6 to CT12, with a nadir occurring at CT18 and a 30% reduction in amplitude as compared with liver (Cosinor, P < 0.001; ANOVA, P < 0.001).

**Jet-lagged Mice.** The 24-hour rhythms of mPer2 or mRev-erbα mRNAs were markedly altered in both liver and tumor. In liver, the peak of mPer2 expression was advanced to the light/dark transition (CT0) as compared with control. No significant rhythm was found (Cosinor, P = 0.22; ANOVA, P = 0.41). This was also the case for...
mean mRev-erb expression, despite the fact that it peaked at CT12, as seen in control mice (Cosinor, $P = 0.13$; ANOVA, $P = 0.23$; Fig. 5A and C). In tumor, no rhythmic pattern was obvious or statistically validated for expression of either clock gene [mPer2, $P = 0.54$ (Cosinor) and $P = 0.74$ (ANOVA); mRev-erb, $P = 0.58$ (Cosinor) and $P = 0.24$ (ANOVA); Fig. 5A and E].

**DISCUSSION**

Here we demonstrate that a severe functional disruption of the circadian system was produced by environmental factors and resulted in accelerated malignant progression in a mouse tumor model. The results are in line with our previous findings regarding accelerated tumor progression in mice with physically ablated hypothalamic SCN (9). Similarly, a constitutive mutation of clock gene mPer2 markedly altered circadian function, increased the incidence of cancers in mice, and shortened their survival (10). In our study, exposure of the mice to constant darkness or constant light exerted no effect on tumor growth. Both of these conditions altered the period length of the circadian rhythms in activity and body temperature, which maintained their physiologic phase relations, however. Indeed the integrity of circadian coordination has long been known to persist in mice exposed to constant light or constant darkness (28). Conversely, an abrupt phase shift in the day/night cycle disrupted the synchronous oscillations of clock gene expression in the central circadian pacemaker of mice or rats (24, 29) and disrupted the synchronous oscillations of clock gene expression even more in the case of an advance of light onset by 8 hours (24). Such a disrupting regimen for the CT structure was chronically applied here to mice, thereby constituting an experimental model of chronic jet lag or shift work. Indeed, the mice subjected to repeat advances of light onset by 8 hours every 2 days had ablated or severely altered physiologic outputs of the circadian pacemaker, including rest/activity, body temperature, and corticosterone secretion, as compared with the mice kept in 24-hour photoperiodic synchronization. The disruptive effects of chronic jet lag were further documented at a molecular level both in the SCN, the central pacemaker, and in the liver, a host tissue well characterized as a peripheral circadian oscillator (4, 5, 7). Thus, clock protein mPER1 displayed a clear-cut circadian pattern in the SCN of synchronized mice, similar to that described previously (21, 30). This was also the case for the mRNA expression of clock genes mPer2 and mRev-erb in liver, with 24-hour patterns similar to those reported previously (4, 5, 29, 31). In contrast, chronic jet lag ablated the mPER1 rhythm in the SCN and the rhythmic transcriptional activity of both clock genes in the liver. However, a severe alteration of the rhythm rather than mere ablation of the rhythm could not be ruled out because such a distinction would require further increase in statistical power.

We further demonstrated for the first time that the tumor was
equipped with a molecular clock because the circadian expression of mPer2 and mRev-erbα persisted with minor alterations in synchronized mice. The similarity of tumor and host clock gene transcription patterns suggested the synchronization of tumor rhythms by the SCN, potentially via adrenocortical secretion, as is known for normal peripheral tissues (32). Indeed, most experimental tumor models displayed a significant circadian rhythm in the proportion of mitotic cells, with a maximum usually occurring near the end of darkness (33).

Chronic jet lag ablated both rhythms in clock gene transcription in the tumor. This finding indicated that the negative control exerted by the host circadian timing system on the initial phase of cancer growth could be achieved via an effect on temporal structure within the tumor. The latter might relate to the control exerted by the molecular clock on key genes for cell proliferation in peripheral organs, such as c-myc, wee1, cdc2, cyclin B1, gadd45α, and mdm2 (10, 34). This possibly resulted in a shorter cell cycle length during exponential growth in jet-lagged mice as compared with mice in LD12:12. However, the circadian clock also regulates tumor angiogenesis (35). Thus, circadian clock disruption could also facilitate energy supply to the tumor, a factor known to contribute to tumor progression. Finally, cell death and/or cell quiescence could result from poor nutrient supply and/or mechanical constraints and contribute to differences in tumor growth rate (36, 37). In a separate study, food availability was restricted to 4 or 6 hours during darkness in mice with Glasgow osteosarcoma. Tumor grew more slowly during the exponential stage in the meal-fed mice as compared with those fed ad libitum (38). Taken together, the results from both studies have pinpointed the exponential stage of malignant growth as that mostly influenced by circadian physiology. It supports the view that the better the circadian coordination, the stronger its negative effect on malignant cell cycle progression.

In conclusion, we demonstrate for the first time that imposing iterative alterations of lifestyle severely disturbed the physiologically coordinated transcription of circadian clock genes in both normal peripheral tissues and tumor and that this functional alteration of normal physiology was accompanied by a significant increase in the rate of initial malignant progression. Circadian disruption by surgical ablation of the SCN also accelerated tumor progression in mice (9), emphasizing the generality of the effects reported here. Circadian dysfunctions can also result from tumor products8 or from wrongly timed therapy (39, 40). Several clinical studies have shown the relevance of applying circadian biology concepts to the treatment of cancer patients (reviewed in ref. 3; ref. 41). The current findings warrant the development of preventive or therapeutic anticancer strategies targeted at the circadian clock, especially in the early stages of malignant development.

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