In nature, all plants and animals demonstrate temporal coordination of cell division within the day (1,2). These daily patterns of cell division are tightly tied to the endogenous rhythmic circadian time structure of the animal (3). Murine experiments clearly indicate that, while toxicity and dose intensity each depend on the circadian timing of cytotoxic agent delivery, a second dose-intensity-independent relationship also exists between circadian drug timing and anticancer efficacy (4). Cells of the epithelial lining of the human gut and hematopoietic precursor cells residing within the bone marrow of healthy individuals undertake the process of DNA synthesis and cell division nonrandomly within the day (5,6). Single-institution and multicenter randomized clinical trials (7–11) have consistently demonstrated that cytotoxic drug toxicity can be diminished, dose intensity and objective tumor-response frequency can be increased, and cancer patient survival can be improved by means of optimal circadian drug timing.

If cell proliferation in spontaneous human cancer is coordinated within the day, then, since many cytotoxic drugs kill cells more or less effectively depending on the phase of the cell cycle, a finding of circadian rhythm in any aspect of cancer cell proliferation would provide a mechanism supporting the likelihood that cancer cell susceptibility itself waxes and wanes predictably throughout each day. Furthermore, if the normal endocrine-paracrine-autocrine loops controlling circadian gating of cell proliferation are intact in cancer cells, a novel paradigm for cancer control is opened that does not require cancer cell killing (12).

Determining the proportion of tumor cells residing within specific phases of the cell cycle at different times within the day currently demands frequent serial tumor sampling throughout at least one entire day. This feat has been accomplished in a few mouse tumor model systems (13,14). Data in cancer patients are understandably limited, but some relevant data have appeared. Voutilainen (15) and Tähti (16) each isolated biopsy specimens from a variety of cancer types, around the clock, and documented time-of-day-dependent peaks of cancer cell mitosis in the tumors of most patients and differing circadian patterns in cancers of different origin. On average, across all tumor types, two daily peaks of mitosis were found; the first occurred between midnight and 2 AM, and a second smaller peak occurred around noon (17). Smaaland et al. (18) found that malignant cells obtained, around the clock, from the involved lymph nodes of patients with various non-Hodgkin’s lymphomas, on average, organize their cell proliferation late each day, with a midnight peak in S and G2 phases in cancer cells. Klevecz et al. (19) concurrently sampled both malignant ovarian cells and nonmalignant, nonovarian mesothelial cells from the peritoneal cavities of patients with ovarian cancer and found different circadian organizations of these two cell populations arising within the identical physiologic milieu. A higher proportion of sampled ovarian cancer cells were in G2 and S phases in the late morning hours, while a larger proportion of benign peritoneal mesothelial cells inhabited these phases of the cell cycle in the late evening hours (19).

These data, limited though they are, en masse raise the possibility that the circadian time structure of DNA synthesis and/or mitosis is determined not by whether a cell is benign or malignant but rather by the tissue of origin of that cell, i.e., its ontogeny. To test this hypothesis, however, one must obtain benign and malignant tissue samples of the same cell type concurrently, around the clock.

In August 1978, a 50-year-old postmenopausal female dairy farmer had an epidermoid carcinoma on the pinna of her right ear excised. A right radical neck dissection did not demonstrate metastatic involvement of the regional lymph nodes, and she appeared otherwise healthy. Eight months later, a right supraclavicular lymph node biopsy revealed metastatic epidermoid carcinoma. This area and the right neck were then treated with external-beam radiation therapy. Ten months later, some 18 months after the initial diagnosis of the disease, an excised subcutaneous right breast mass and left axillary masses were found to harbor metastatic epidermoid carcinoma. During the next 1–2 months, the patient developed 100–150 rapidly growing subcutaneous tumor nodules (0.5–1.0 cm in diameter). She remained active and working, arising daily for morning milking (4–6 AM) and retiring between 8 PM and 10 PM each evening. Her only symptom was itching, which progressed throughout each day to burning pain in the nodules. Itching began each day in the early afternoon, and the “burning pain” was most severe between 6 PM and sleep onset (10 PM).

For this study, after University of Minnesota Investigational Review Board-approved written informed consent was obtained from the patient, she became resident at the University of Minnesota General Clinical Research Center for 6 days between March 7 and March 13, 1979. During this span, she arose daily between 5 AM and 7 AM and retired at 10 PM. Her oral temperature was recorded, and fractional urine collections were obtained every 4 hours throughout her stay. The patient underwent only these baseline measurements for 2 days. Subsequently, two separate

**Evidence for an Ontogenetic Basis for Circadian Coordination of Cancer Cell Proliferation**

William J. M. Hrushesky, Donald Lannin, Erhard Haus

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tumor nodules and at least one piece of apparently uninvolved skin were excised, under local anesthesia, from her back or flank every 4 hours during a 28-hour period. Twenty-one blocks of tissue—14 tumor nodules and seven grossly normal skin biopsy specimens—were submitted in formalin. The tissue was embedded in paraffin. Two slides were prepared from each tissue block containing gross tumor (28 sections), with the two sections from each specimen taken from different levels of the tissue blocks. Four slides were prepared from each block thought by the surgeon to contain only normal skin (28 sections). The slides were coded, numbered 1–56, randomized for cell counting, and counted "blindly" by the same pathologist (E. Haus). The number of mitotic figures was recorded for every 2000 basal cells of the epidermis and for every 2000 tumor cells examined. In sections in which no tumor was present, only the basal cells were counted. After completion of all counting, it was found that tumor cells were present in several tissue blocks in which no tumor had been clinically suspected, i.e., distant from a gross metastasis. The number of mitoses in the basal cells of the epidermis in sections with tumor and without tumor in the underlying dermis did not differ statistically. During the remainder of the 6-day hospitalization, the patient was administered circadian-timed chemotherapy (8), and she was then discharged to her home.

Urinary cortisol excretion: The hypothalamic–pituitary–adrenal axis is one of the most important and thoroughly studied mammalian circadian pacemakers. The urinary cortisol excretion pattern, specifically the time of day of daily peak cortisol excretion and the amplitude of the daily surge in cortisol excretion, is an excellent measure of the circadian orientation of an

![Fig. 1. Circadian urinary cortisol excretion pattern of our 50-year-old female patient with metastatic epidermoid carcinoma compared with that of a group of 19 apparently healthy younger women aged 20–30 years. During a 3-day prechemotherapy monitoring span, 24 timed fractional urine samples were obtained, their volumes were measured and recorded, and the cortisol concentration in each was determined by use of a standard radioimmunoassay. These data were then arithmetically converted to fractional cortisol excretions in micrograms (mcg) per hour during each of the 24 collection spans. In the main figure, the fractional circadian cortisol excretion pattern for this patient can be contrasted with the pattern of cortisol excretion similarly obtained from each member of the group of 19 putatively healthy, young adult women. These hormone concentration determinations were performed in the same laboratory, using identical methodology (25). Our patient’s average 24-hour cortisol excretion was some threefold higher than the average for these apparently healthy women (mean ± standard error [SE] = 8.60 ± 1.62 versus 2.42 ± 0.19; see table at the bottom of the Fig.). The circadian waveforms describing these 24-hour patterns are otherwise similar. The 95% confidence intervals (CIs) of the timing of the daily peak excretions of these two time series overlap entirely. The amplitudes of the circadian waveforms describing one half of the average daily peak-to-trough difference are virtually identical when expressed relative to the respective 24-hour mean cortisol excretion values (64% versus 68%; see table at the bottom of the Fig.). Inset depicts the two circadian rhythms with their amplitudes normalized to the 24-hour mean values of each series (cancer patient, thick line; healthy women, thin line). The similarities and differences between these two circadian waveforms demonstrate that our patient was substantially "stressed" by bearing a widely metastatic cancer, resulting in the unusually enhanced adrenocortical activity (20). They also indicate that, although "stressed," our patient maintained fundamentally normal, environmentally entrained, circadian temporal hypothalamic–pituitary–adrenal orientation. The statistical test employed, cosinor analysis, fits the raw data by least squares to all possible cosine functions. The zero amplitude hypothesis is tested. If this hypothesis is rejected with 95% probability, then there is only a 5% probability that any straight line fits the data better than the fitted cosine curve, i.e., $P < .05$ (27).
individual. Our patient’s circadian cortisol excretion dynamics support the likelihood that this woman remained coordinated with her circadian environment (Fig. 1). As expected for this patient with a life-threatening chronic illness, in this case metastatic cancer, her overall 24-hour mean cortisol excretion was some threefold higher than that observed for a group of 19 apparently healthy, somewhat younger women (20). The circadian cortisol excretion pattern (see inset in Fig. 1) is otherwise, however, quite normal.

**Body temperature:** The daily shape of our patient’s oral temperature rhythm, in the spans before and during the biopsies, appeared very much like that of 15 normal infants and adult men (22,23). Highly malignant metastatic and soon-to-be-lethal cancer cells also demonstrate cell-division coordination within the day. In normal skin and epidermoid cancer, the number of mitoses decreases prominently throughout the night, reaching a low point in the morning hours, 6 AM to 8 AM, and then rises throughout the rest of the day to a peak value in the evening. The analog scale at the top of the Fig. represents self-reported, subjective symptom quality and severity in the areas of the skin involved with metastatic nodules. Itching began daily after noon and increased in subjective severity until concurrent burning pain supervened. This burning pain worsened steadily until the patient finally fell asleep. She would awaken each morning between 5 AM and 7 AM without itching or pain, only to begin this symptom cycle anew. Radiation therapy and the majority of useful cytotoxic drugs damage cells that are actively dividing more than cells that are residing in the resting phase of the cell cycle. Furthermore, many of these treatments selectively damage cells most effectively as they traverse specific phases of the cell cycle. External-beam γ-irradiation, for example, damages cells that are undergoing mitosis more than cells that are inhabiting other stages of the cell cycle. If radiation were chosen as a primary treatment for our patient, one might expect different outcomes depending on when in the day this woman received daily radiation fractions. Treatment at approximately 8 AM each day (Rx-1) would result in therapy being given when relatively few cancer cells and small numbers of surrounding normal skin cells were inhabiting the sensitive “M (mitotic) phase” of the cell cycle. Little damage to both skin and cancer might result from therapeutic irradiation at this particular time of day. Conversely, if the same daily dose of irradiation were administered at approximately 4 PM (Rx-2), very different cell killing might be expected to result. In this case, a greater proportion of cancer cells would be undergoing mitosis, thereby being relatively more sensitive to lethal radiation damage. Although normal skin cells are also more frequently dividing at this time of day, mitoses are some threefold more frequent in the malignant than in the nonmalignant epidermoid cells between 2 PM and 4 PM. Thus, optimal timing of radiation treatments, or any therapy more effectively damaging cells undergoing mitosis, might result in temporal selectivity and a potentially enhanced therapeutic index, i.e., a temporal window of opportunity. See legend to Fig. 1 for explanation of cosinor analysis.

<table>
<thead>
<tr>
<th>2 way Analysis of Variance:</th>
<th>Sample Time</th>
<th>Cancer Mitoses*</th>
<th>Skin Mitoses*</th>
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</thead>
<tbody>
<tr>
<td>Cancer vs. Skin F = 44.5 p=.0001</td>
<td>06:00</td>
<td>14.5 ± 0.5</td>
<td>11.6 ± 1.05</td>
</tr>
<tr>
<td>Time of Day F = 12.0 p=.0001</td>
<td>07:00</td>
<td>15.4 ± 2.9</td>
<td>9.59 ± 1.5</td>
</tr>
<tr>
<td>Interaction F = 0.1 p=.0001</td>
<td>08:00</td>
<td>2.00 ± 0.0</td>
<td>4.25 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>09:00</td>
<td>2.10 ± 0.7</td>
<td>8.89 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>10:00</td>
<td>27.3 ± 5.6</td>
<td>12.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>11:00</td>
<td>25.1 ± 2.9</td>
<td>12.5 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>12:00</td>
<td>23.1 ± 2.9</td>
<td>12.5 ± 1.9</td>
</tr>
</tbody>
</table>

Cosinor Analysis (24 Hour Period):

<table>
<thead>
<tr>
<th>Cancer Mitosis</th>
<th>#pts</th>
<th>PR</th>
<th>p value</th>
<th>MESOR*</th>
<th>Amplitude*</th>
<th>Acrophase*</th>
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<tbody>
<tr>
<td>26</td>
<td>22.5</td>
<td>0.0003</td>
<td>25.08 ± 1.97</td>
<td>3.11 ± 0.9</td>
<td>18.45 ± 01.08</td>
<td></td>
</tr>
</tbody>
</table>

* ± standard error of the mean
apparently healthy age-matched women (data not shown) (21). The robust amplitude and day-to-day reproducibility of this cancer patient’s temperature and cortisol excretion patterns tentatively confirm the presence of relatively normal, environmentally entrained, endocrine and metabolic circadian time structures.

Mitotic indices of skin and epidermoid cancer: The usual circadian pattern of mitosis in the epidermis, first documented for humans by Cooper (22) and confirmed by Scheving (23), shows that the greatest amount of daily cell division occurs in the evening and that the level falls each day to a minimum in the morning. The mitotic index of normal epidermoid skin cells and of epidermoid cancer cells of skin origin in our patient fell throughout the night and early morning hours to very low values at 6–8 AM and then rose again (Fig. 2). While the daily peaks and nadirs of mitosis occurred in our patient at very similar times of day for both normal cells and tumor cells, the 24-hour mean mitotic rate in the tumor cells, as well as its daily amplitude, was substantially higher in the malignant cells. Thus, in this patient, both normal cells and metastatic carcinoma cells originating from the same tissue showed a prominent and almost identical circadian rhythm in cell proliferation. It should be noted, however, that multiple sections of one of the tumor biopsy specimens obtained at 8 AM was found to contain no identifiable cancer. Therefore, the 8-AM data point shown for cancer cells in Fig. 2 is based on enumeration of mitotic figures from 2000 cancer cell nuclei observed in two sections of a single biopsy specimen.

These data demonstrate that the proliferation of metastatic cancerous tissue in this patient apparently maintained its capacity to sense and respond to the usual daily regulators of cell division. These signals, whatever they are, command this patient’s normal skin cells to stop dividing in the night, and they stop dividing. These same commands are apparently also heeded by soon-to-be-lethal metastatic epidermoid cancer cells of the same ontogenetic origin. These data indicate that cancerous cells may well maintain the circadian chronobiologic literacy of the cells from which they arose. This observation suggests that, if the circadian signals responsible for circadian cell division control can be defined, it may be possible to control cancer growth without resorting to cytotoxic therapeutic strategies. Even in the absence of therapies that can reconstitute natural circadian controls of malignant cell proliferation, there remain implications of the demonstrated circadian coordination of cancer cell division for many currently available cancer treatments. Cell cycle-active and/or cell cycle stage-specific cancer chemotherapy given to cancer patients at optimal times of day has been shown in randomized prospective trials to enhance the therapeutic index of these treatments. Circadian-based treatment strategies have enhanced ovarian cancer survival (8,10), doubled objective tumor response frequency in colorectal cancer (9), and enhanced the frequency of cure of acute lymphoblastic leukemia of childhood (24).

In summary, we have provided evidence that the mitotic figures of benign and malignant epidermoid cells of skin origin from an environmentally synchronized patient with metastatic cancer appeared and disappeared nonrandomly throughout the day. This finding is consistent with the hypothesis that the proliferation of human cancer cells is organized within the day, and it is in keeping with the four relevant series in the literature. To our knowledge, these limited data are the first to indicate directly that the cell type of cancer origin may confer specific circadian cytokinetic time-keeping characteristics on the analogous cancer cell. Such circadian cytokinetic coordination may be an important cause of the therapeutic advantage observed by optimally timing cytotoxic cancer therapy within the day (25). This circadian cytokinetic organization is likely to be relevant to the toxic–therapeutic ratio of irradiation and cytotoxic, biologic, and many, if not all, other molecular strategies with the potential to control cancer (26).

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NOTES

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