Cancer and the Circadian Clock: Has the Time Finally Come?

By Karyn Hede

Without the concept of time, there would be no physics, chemistry, or geology, but in medicine, time often gets short shrift. Yet in recent decades a small cadre of clinical investigators has found associations between the body’s daily rhythms and the effectiveness of cancer treatment.

Working in tandem, biologists have dissected the molecular underpinnings of the human circadian clock, a feedback loop that controls the sleep–wake cycle and coordinates physiological processes.

Now a series of recent discoveries has implicated the circadian clock in cancer development, reinvigorating the field of chronobiology, a subspecialty that has been operating at the fringes of cancer research.

Most recently, Aziz Sancar, M.D., Ph.D., and his colleagues at the University of North Carolina, Chapel Hill, showed that highly cancer-prone mice missing the master tumor suppressor gene p53 lived 50% longer when a circadian clock gene called cryptochrome (Cry) was also deleted. In the study, published in February in the Proceedings of the National Academy of Sciences, the p53-mutant mice developed lymphomas and lymphosarcomas and died by 19 weeks. By contrast, the mice missing Cry as well as p53 lived for a median of 28 weeks.

These mice did eventually succumb to cancer, raising the question of whether the cancer was delayed or whether another protective system was activated. That question was answered, the researchers said, when skin fibroblasts isolated from the mice showed identical signs of oncogenic transformation, indicating that the mice missing Cry did not have delayed onset of cancer. Instead, they found that isolated cells from the p53-Cry–knockout mice were more sensitive to radiation-induced apoptosis, or programmed cell death. They concluded that eliminating the two genes had increased apoptosis, lengthening the animals’ lifespan by

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eliminating genetically damaged precancerous cells.

**p53 and Circadian Clock Gene**

“What this really does now is open up the field for targeted inquiry because you have a direct genetic interaction between p53 and cryptochrome,” said Russell Van Gelder, M.D., Ph.D., professor of ophthalmology at the University of Washington Harborview Medical Center in Seattle and an expert on cryptochrome. “Now we have hypotheses to go on, and you could immediately go to the clinic and ask in 100 breast cancers or 100 colon cancers: ‘What is the prognostic significance of loss of cryptochrome function with respect to p53 status?’”

Indeed, a research team led by Eloisa Jantus Lewintre, Ph.D., from the Prince Felipe Research Centre in Valencia, Spain, reported in February in the journal *Haematologica* that cryptochrome gene expression can serve as a prognostic marker in newly diagnosed, early-stage chronic lymphocytic leukemia. Patients with this disease fall into two groups based on the mutational status of the immunoglobulin heavy chain variable region (IgVH) gene: those with IgVH gene mutations have better treatment outcomes and survival. However, testing for the IgVH gene is difficult and expensive, and the Prince Felipe investigators hoped to identify a prognostic marker that would be easier to use. In a study of 70 patients, they found that high Cry levels did serve as a surrogate for the IgVH unmutated status, thus predicting a poorer outcome.

“This finding suggests cryptochrome is as good a prognostic marker as some of the other prognostic markers in this particular hematologic malignancy,” said Van Gelder. “Now you can take this a step farther and say maybe, if you combine that with p53 status, you may get a really complete picture of what that tumor progression risk is.”

However, the new findings raise many new questions. For instance, they run counter to epidemiologic research that suggests perturbations in the circadian clock increase cancer risk. In the Nurses’ Health Study, one of the largest and most complex epidemiological studies ever completed, researchers reported that nurses on the night shift had a substantially elevated risk of developing breast cancer. Similar findings have been reported for flight attendants.

Questions remain on the mechanistic level, as well. Another, similar circadian clock protein, Period 2 (Per2), has been implicated in cancer risk, but unlike Cry, it appears to increase rather than lower risk when missing. Mice without the Per2 gene develop spontaneous and radiation-induced lymphomas at a much higher rate than control animals.

Per2 and Cry are both central transcriptional regulators of the internal clock in humans and might be expected to have the same effect. But these findings are not necessarily incompatible, according to William Hrushesky, M.D., senior clinician investigator and director of the medical chronobiology laboratory at the W.J.B. Dorn Department of Veterans Affairs Medical Center in Columbia, S.C.

“The really simple and important thing that has happened lately is to actually tie the clocks to the therapeutic targets directly through clock control genes [Cry and Per] that then influence proliferation, that gait it, and that influence apoptosis,” he said.

Hrushesky said interest in chronobiology and cancer is growing and will be the subject of an upcoming 1-day meeting at the New York Academy of Sciences on June 19.
**An Ancient Clock**

In mammals, the master circadian clock resides within the suprachiasmatic nucleus of the hypothalamus. The clock is set by light received directly from the retina and is synchronized daily to maintain a 24-hour cycle. Without a light-dark cycle, the circadian clock runs on a nearly 25-hour cycle. A key function of the clock appears to be maintaining synchronicity; surgical experiments in mice, in which the suprachiasmatic nucleus is removed, demonstrate that even without a central clock, peripheral tissues such as the liver and skeletal muscle maintain circadian rhythms, although they are out of sync with one another.

Work in the laboratory of Joseph Takahashi, Ph.D., and colleagues at Northwestern University in Evanston, Ill., along with collaborators at several other institutions, demonstrated that the circadian clock in mice controls many genes that are associated with cell division and proliferation in tissues such as liver and skeletal muscle.

The circadian protein cryptochrome, which was discovered in 1998 by Michael Rosbash, Ph.D., and Jeffrey Hall, Ph.D., at Brandeis University, is found in species ranging from plants to microbes to fruit flies to humans. It is in the family of proteins that also includes the DNA repair protein photolyase. The Cry protein detects light in the violet-blue spectra, which is most abundant at dawn and dusk. Cry’s central role in gating the clock is well established, although definitive proof that Cry is the photoreceptor for the central circadian clock is lacking, according to Van Gelder.

The bottom line, Hrushesky said, is that “clocks gate cellular processes that are essential to the cancer process and also that are essential targets for most common and developing therapies.”

**Into the Clinic**

Some clinical investigators have advocated giving chemotherapy agents in sync with the body’s circadian rhythms since the mid-1980s, when a clinical trial conducted by Hrushesky’s group demonstrated that 5-year survival of ovarian cancer patients increased from 11% to 44% depending on the time of day they received cisplatin and doxorubicin. Since then, other groups have published similar results from clinical trials showing that time of day of drug administration makes a measurable difference. However, chronotherapy has been applied sporadically and has not gained widespread acceptance.

Despite some positive trials, “there is more and more evidence that circadian function is very individual,” said Marina Antoch, Ph.D., a molecular biologist who studies circadian clock proteins at Roswell Park Cancer Institute in Buffalo, N.Y. “People know that some people are morning larks and some are night owls, which means that their circadian system is functioning on a different schedule. I think the entire concept of timed therapy could be successful only when applied as individual therapy.”

Antoch points to chronotherapy trials conducted by the European Organisation for the Research and Treatment of Cancer chronotherapy study group and published in the *Journal of Clinical Oncology* in 2006. The group reported that timed administration of fluorouracil, leucovorin, and oxaliplatin as first-line chemotherapy for...
metastatic colon cancer benefited men but not women.

The result, she said, demonstrates that “we are far from understanding the mechanism, and there is no good consensus.”

But Sancar is optimistic. “Until 1998, the clock was really a mystery,” he said. “In 1998, all of a sudden there was an explosion in the field. … Now you can ask if chronobiology works, you can adjust the clock, and it should have predictable effects.”

Looking for such effects, Sancar and his colleagues have found that the enzymes responsible for DNA excision repair, which in humans repair the bulky lesions created by chemotherapy drugs such as cisplatin, are controlled in a circadian expression pattern in mice (see box).

The finding, published in January in *PNAS*, is the first direct molecular evidence demonstrating that an enzyme known to reverse the action of common chemotherapy agents is controlled in a timed circadian pattern, Sancar said. The implication is that one would want to administer chemotherapeutic drugs when these enzymes, which can reverse the drugs’ actions, are at their lowest levels in the body.

Sancar believes the finding also has implications for cancer prevention. The excision repair proteins identified in the study also repair damage caused by ultraviolet light, and Sancar is now studying whether the DNA repair ability in human skin oscillates as well. If the findings in mice extrapolate to humans, the safest time to be in the sun would be in the late afternoon, and the time to avoid exposure would be not only mid-day when the sun’s rays are most direct, but also in the morning, when repair ability is lower.

“Now we have an idea of where in the clock to push,” said John Hogenesch, Ph.D., a circadian clock researcher and associate professor of pharmacology at the Institute for Translational Medicine and Therapeutics at the University of Pennsylvania, Philadelphia. “Before, we were really working blind. Now I think we have a better idea of where to target the clock to try to do some of this from a rational design standpoint.”