Circadian Rhythms Play Role in Cancer Research

Over the years, studies have established that circadian clocks, the internal timekeepers that run many biological processes on a near 24-hour schedule, are associated in some way with tumor growth. What’s not known is precisely how the relationship works. But now researchers are uncovering some of the clock’s key cancer-related components and beginning to envision a time when manipulating or taking advantage of these internal mechanisms could play a role in treating or even preventing cancer.

“We are just learning which components of the circadian timing system are essential in [tumor development and progression]”, says Francis Lévi, M.D., Ph.D., of the Institut National de la Santé et de la Recherche Medicale in Villejuif, France.

We have “a long way to go to identify specific pathways that are controlled by the circadian clock,” says Marina Antoch, Ph.D., of the Lerner Research Institute in Cleveland, “but that is definitely the goal.”

On a molecular level, the mammalian circadian clock consists of a handful of proteins that, because of positive and negative interactions with each other, are expressed periodically with a 24-hour rhythm. These clock proteins in turn drive the cyclical expression of several hundred other proteins that regulate processes such as hormone release, the sleep–wake cycle, and cell division.

The clock can keep accurate time even in constant darkness, but it is also reset by light, so that an organism’s metabolism and behavior will quickly fall into sync with local environmental light conditions. The body’s master clock resides in a region of the brain known as the suprachiasmatic nuclei (SCN), but cells in most tissues express the clock proteins and can maintain a circadian rhythm in the absence of direction from the SCN.

Observational studies have shown that people and animals whose circadian rhythms are chronically disrupted, due to either environmental conditions or defects in clock components, are more likely to get cancer. In one well-known study, Eva S. Schernhammer, M.D., of Harvard Medical School in Boston and colleagues looked at the effects of night shifts on cancer risk among 85,000 participants in the Nurses’ Health Study. They found that nurses who worked for many years on rotating night shifts, and consequently experienced prolonged exposure to artificial light at night, were 36% more likely to develop breast cancer and 35% more likely to develop colon cancer than their peers who never worked at night. Other observational studies have supported these findings.

In the 5 years since publication of the Nurses’ Health Study finding, researchers have learned more about the specific genes and proteins involved in the circadian clock. Cheng Chi Lee, Ph.D., of the University of Texas Health Science Center in Houston discovered that mice lacking one of the clock genes, mPer2, are inordinately cancer prone. In an effort to generate mutations in new circadian genes, Lee had treated mPer2 mutant mice with a chemical mutagen that damages DNA. But what he found suggested that mPer2 functioned as a tumor suppressor.

“Most [mice] did not survive or recover from the standard dose [of mutagen] given to wild-type mice,” he said, suggesting that the mPer2 mutant mice couldn’t handle the stress of DNA damage. Lee’s postdoctoral fellow, Loning Fu, Ph.D., followed up on the observation by examining the effects of radiation on mPer2 mutant mice. Nearly three-quarters of the mutant mice developed lymphoma within 16 months after radiation exposure compared with only 5% of normal mice.

Another tantalizing observation is that among the hundreds of proteins whose expression oscillates in sync with the circadian clock, several are key elements of
the cell division machinery. Thus, disruption of the clock may alter the delicate balance between factors that promote and restrain cell division, leaving cells more prone to becoming cancerous.

“There is an integration between circadian regulation and cell division and the biological processes that maintain such events,” Lévi said. He found, for example, that the tumor suppressor gene p53, which helps limit cell division in the face of DNA damage, was severely deficient in his mPer2 mutant mice. “mPer2 or the circadian clock plays a very important role in the DNA damage response,” he said.

Another circadian clock component that intrigues cancer researchers is melatonin. This protein, which appears to suppress tumor growth in animals, is secreted by the pineal gland in the brain only in darkness. Even brief exposure to light at night can shut down melatonin production.

Researchers have speculated that reduced melatonin levels might be responsible for the higher rates of cancer among night workers, but so far the epidemiological data are mixed. Delving again into the Nurses’ Health Study data, Harvard’s Schernhammer and Susan Hankinson, Sc.D., found that women with the lowest levels of a melatonin metabolic product in their morning urine were approximately 70% more likely to later develop invasive breast cancer than those with the highest levels. However, in a similar study done on British women living on the island of Guernsey, Ruth Travis, M.Sc., of Oxford University and colleagues found no such correlation. Many researchers are now working to find a molecular mechanism that explains the apparent connection between melatonin and breast cancer risk.

As researchers learn more about the internal workings of the circadian clock, they are also exploring its possible role in managing cancer. Lévi disrupted the circadian rhythms of mice with tumors, both physically, by destroying the master clock neurons in the SCN, and environmentally, by turning on the lights 8 hours earlier every 2 days, a model of chronic jet lag. Tumors in these mice grew more rapidly than normal and caused death sooner.

Lévi’s group found evidence for a similar phenomenon in humans. They measured the circadian rhythms of patients with advanced colorectal cancer by using activity monitors that the patients wore on their wrists. Patients with the most aberrant circadian behavior patterns fared far worse than those with normal patterns.

Loss of circadian rhythmicity is fairly common in patients with advanced cancer and may be both a cause and an effect of the disease, according to Lévi.

“Circadian disruption can be caused by many factors—clock gene mutations, lifestyle, treatments—but also by the tumor [itself],” he said. “This could be a mechanism though which the tumor accelerates its own growth.” Lévi hopes that restoring circadian rhythms in cancer patients might improve their prognoses.

**Chronotherapy**

Keeping an eye on the circadian clock may also benefit chemotherapy patients. The effects of administering anticancer drugs on a circadian schedule, a strategy called chronotherapy, have been investigated extensively in mice and rats. Researchers have found that the toxic effects of more than 30 drugs varies according to the timing of administration.

Unfortunately, the least toxic administration times, even for similar drugs, are scattered around the clock, suggesting that many different mechanisms are involved. Toxic effects could be influenced by circadian variations in drug metabolism. Or drugs may be less toxic if given when the cells of healthy tissues are in the least vulnerable stage of the cell division cycle.

Recently, Antoch and colleagues investigated one anticancer drug, cyclophosphamide, whose toxic effects vary strongly with circadian time. Using mouse strains with mutations in circadian clock genes, the researchers expected to find that the changing toxicity was tied to differences in the activities of drug-metabolizing enzymes. “That was the most obvious hypothesis,” said Antoch.

But they got a surprise. The toxic effects varied not because of the enzymes but because of a difference in the sensitivity of B cells, Antoch said. They found that when clock-controlled genes were expressed at high levels, B cells were more resistant to cyclophosphamide.

Antoch hopes that one day there will be drugs that can “shift the clock temporarily … to a functional state where it will promote higher resistance of normal cells.”

Several small studies have suggested that chronotherapy has promise in humans. Recently, Lévi and his colleagues completed a large phase III clinical trial on chronotherapy in patients with metastatic colon cancer. Patients in the trial received two drugs commonly used to treat colon cancer at 4 a.m. and a third drug at 4 p.m.

The results of the smaller trials testing this regimen of chronotherapy in colon cancer patients have been encouraging—patients experienced fewer side effects and were able to receive higher medication doses than they could with constant-rate infusions of the same drugs. The chronotherapy regimen outperformed nearly all other treatments previously tested for this type of cancer both in terms of the fraction of patients whose tumors got smaller and the average survival time, reports Lévi.

However, chronotherapy is still uncommon, limited to only 50 cancer centers throughout the world. “For chronotherapy to become widely accepted … additional randomized clinical trials with adequate support [will need to be] conducted,” Lévi said. “Methods and technology that allow routine and reliable assessment of the status of the circadian timing system in an individual patient need be developed.”

—Karen Ross