Gotta get that rhythm

Circadian timing systems and cancer

Anna Wagstaff

The role of circadian rhythms in cancer hit the headlines this year because of growing evidence that night shift work may increase the risk of breast cancer. But could a better understanding of the role of disruption to the daily rhythms that regulate the behaviour of cells open up new possibilities for treatment? One group of researchers has been arguing this case for decades.

In March 2009, the Danish national board of industrial injuries became the first in the world to recognise night shift work as a work-related hazard for breast cancer. The decision was provisional – each case has to be examined and adjudicated individually – but it was enough to ensure payments of between 30,000 and one million Danish kroner (4,000–134,500 euros) to 38 of the 75 women who applied for compensation. Successful applicants had typically worked nights at least once a week for 20–30 years and had no other obvious raised risk factors for breast cancer. Night shift was defined as at least seven hours' work, including the whole period from midnight until five o'clock in the morning, either as a permanent or rotating shift.

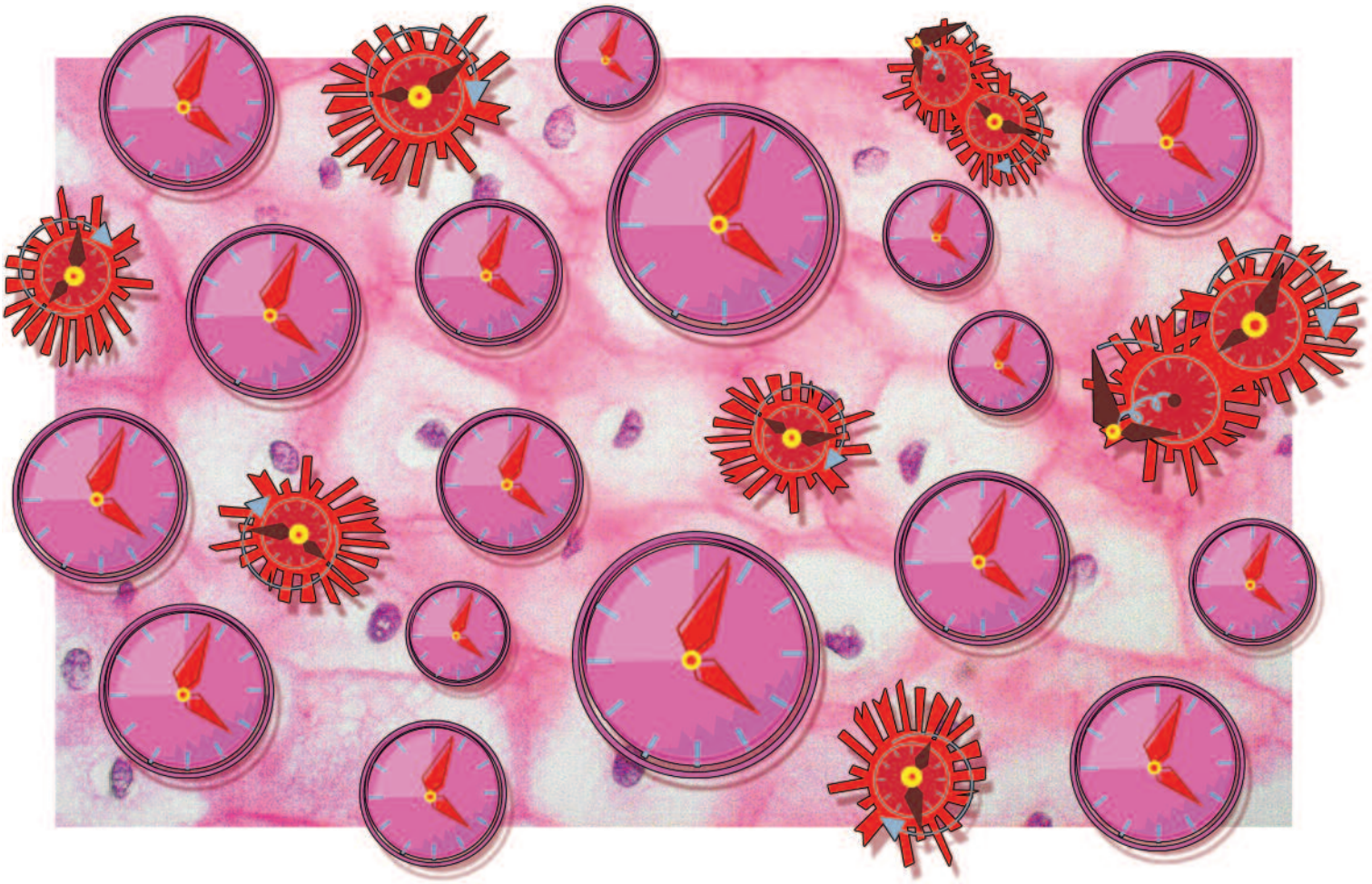
The Danish decision on compensation came as a surprise to many working in the field of cancer. It is true that since December 2007, the WHO International Agency for Cancer Research (IARC) has included "shiftwork that involves circadian disruption [disruption of the body's normal biological rhythms]" in its list of "probable" carcinogens. However, the epidemiological evidence for the effects of night work on the risk of cancer remains less than conclusive (see box). In particular, little is known for sure about what it is about night work that may be increasing cancer risk.

Well-respected advocacy groups advise caution. Olivia Marks-Woldman, head of Policy and Campaigns at Breast Cancer

Shift work and breast cancer

An estimated one in five workers in Europe does some form of shift work, concentrated in healthcare, industry, transportation, communications and the hospitality sector. Epidemiological evidence for a link between night working and breast cancer has been building over decades, from studies on nurses, flight attendants, radio and telegraph operators and other occupational groups. A study of 78,562 US nurses, for instance, found 36% higher incidence of breast cancer over a 10-year follow-up than would normally have been expected. However, questions have been raised about whether the increased risk might be due to other (confounding) factors. Patterns of childbirth, for instance, are known to affect cancer risk and are also likely to differ between night shift and day workers. Alcohol consumption is known to be higher in some occupational groups than others. Night work may require less activity than day work – also a known risk factor.

No guidelines exist – even in Denmark – on how to mitigate the possible increase in cancer risk, because too little is known about exactly what it is about shiftwork that might be causing it.



Care in the UK, stressed that age, gender and family history remain the major risk factors. "There is some evidence to show that reduced melatonin levels, as a result of night working, may increase a woman's risk of developing breast cancer, however, this is one of many contributing life style elements, such as diet and exercise, that could increase an individual's risk."

The US National Breast and Ovarian Cancer Center gave a similar response. "The risk associated with nightshift work needs to be reviewed in the context of other known modifiable risk factors for which there is strong evidence, such as alcohol consumption and postmenopausal weight gain."

The Director of the Breast Cancer Institute in New South Wales, John Boyages, went so far as to call the Danish decision "puzzling", and said they had "gone out on a limb". So far, no other national authority has followed Denmark's lead.

The Danish decision has, however, been welcomed by some – not least by a small and determined network of lab and clinical researchers who have spent decades studying the link between the body's 24-hour biological clocks and cancer.

Specifically, they are interested in the role that faulty molecular time clocks may play in driving the erratic behaviour of cancer cells. As these molecular clocks take their cues from the body's overall circadian timing system – the one that governs feeling sleepy at night and active in the day – it could make sense that if the circadian timing system is disrupted, molecular clocks could get confused, and the risk of cancer could then be raised.

Francis Lévi, a medical oncologist at the Paul Brousse hospital in Villejuif, and head of the Circadian Rhythms and Cancer unit at the French medical and health research unit INSERM, has been researching this topic since 1975. In line with the IARC position, he argues that it is probably not nightwork per se that heightens cancer risk so much as the disruption caused by rotating shifts.

"When you shift the circadian time structure by eight hours, it takes about three days to adjust the rest/activity rhythm. If you change shift every three days, you don't have time to adjust your circadian time structure. But if you change your shift every week, you are always in the middle of adjustment, and this has been shown to be more disruptive."

FRED VAN DEELEN / SCIENCE PHOTO LIBRARY

Molecular clocks are now known to play a role in most of the cell processes that are key for cancer

Quite how this disruption links to cancer remains to be proved, but there is strong evidence to show that disrupted time clocks can affect apoptosis – the mechanism by which cells stop dividing after DNA damage. Research by Loning Fu and colleagues from the human genetics department at Baylor College of Medicine, Houston (Fu et al *Cell* 111:1055), has shown that mice lacking the Per2 gene – key to controlling their circadian system – are prone to cancer. "We found that the response of normal cells to DNA damage is time dependent in a live organism. When the circadian gene is mutated, the cells become resistant to radiation-induced apoptosis," said Fu. Indeed molecular clocks are now known to play a role in most of the cell processes that are key for cancer, including cellular proliferation, DNA damage sensing and repair.

As a doctor, Lévi's interest in all this focuses not so much on cancer prevention, but on how understanding the role of daily rhythm, and the loss of daily rhythm, in cancer cells can help us treat the disease. Until recently, the bulk of this work has centred on the technique of chronotherapy – timing the administration of anti-cancer treatments to do minimum damage to healthy cells and maximum damage to malignant cells.

An intriguing concept

The conceptual underpinning of cancer chronotherapy can be traced back to the early 20th century, with the discovery that cell division in healthy proliferating tissue does not occur at random, but according to a daily rhythm. In humans, most cells synthesise DNA near the middle of the day; most cells undergo mitosis near the beginning of the night. Then in the 1970s evidence began to emerge from animal studies that cell division in tumours tends not to follow the normal circadian rhythm. It either follows no rhythm at all, synthesising DNA and undergoing mitosis at random, or it shadows the normal rhythm, but out of phase with cell division in normal proliferating tissue – principally bone marrow, gut, oral mucosa and skin.

This latter finding opened up the intriguing possibility that the delivery of cytotoxic medicines – which aim to kill cells that are dividing – could be timed to coincide with a period in the cycle of normal cells when they are least vulnerable to damage, while still being able to damage the cancer cells.

In 1975, Lévi, then a young medical student, opted to write his thesis on circadian rhythms in cancer treatments. This was virgin territory: "I'm probably among the very few to have had this idea at that time," he says. He went on to become one of the great pioneers of chronotherapy.

He and his colleagues started by proving the concept in mice, using 5-fluorouracil (5-FU), an antimetabolite that kills cells by interfering with the process of DNA synthesis – the so-called S-phase of the cell cycle. Mice are nocturnal animals and well-regulated cells in normal proliferating mouse tissue tend to be in the S-phase during the middle of the night, with very little S-phase activity in the early morning, when they rest. Sure enough, they found that a potentially lethal dose of 5-FU was tolerated between three and eight times better when the drug was delivered in the early morning compared to the middle of the night.

Different anti-cancer drugs work in different ways, and each has its own optimal time of administration. "There are now 40 agents on which we have information from animal studies on differences in toxicity as a function of circadian timing," says Lévi. "We also have information of large differences in efficacy for 28 of these drugs." Encouragingly and perhaps surprisingly, in 90% of the drugs they tested for toxicity and efficacy, the time of least toxicity coincides with the time of greatest efficacy.

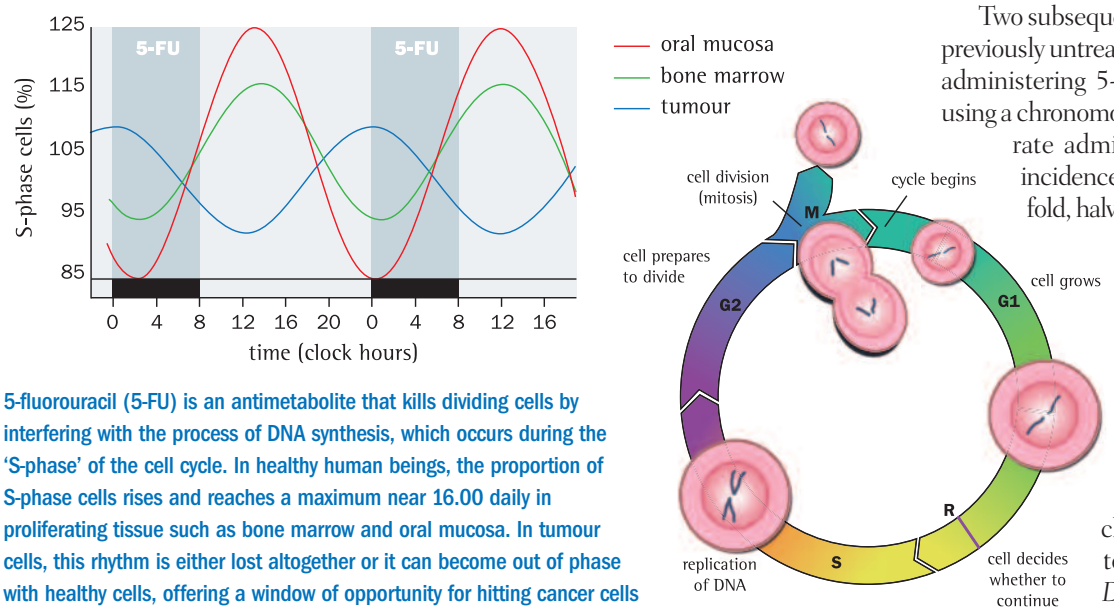
The reasons for this happy coincidence remain unclear. "We have wondered about this for a long time," he says. "There are two possible reasons. Where tumour cells are no longer synchronised, and are living at their own pace, if we hit at the best tolerated time, we can increase the dose and be even more effective. The alternative possibility is that the tumour sensitivity window is out of phase with the normal cells, so if we hit at a time normal cells tolerate best, cancer cells are in the phase when they tolerate it more poorly."

Into the clinic

The real test of any therapy is what it can do in the clinic, and here too chronotherapy has shown some impressive results, particularly in patients with metastatic colorectal cancer. Indeed Lévi can claim some responsibility for the introduction of oxaliplatin, one of the most common cytotoxics in use today, which his team at the Paul Brousse rehabilitated after its manufacturer had consigned it to the 'failed drug' cupboard on the grounds that it was too toxic.

"We first worked with oxaliplatin in 1987 in experimental mice, long before the drug was approved. In the late 1980s, the company that owned oxaliplatin

THE RATIONALE FOR CHRONOTHERAPY



5-fluorouracil (5-FU) is an antimetabolite that kills dividing cells by interfering with the process of DNA synthesis, which occurs during the 'S-phase' of the cell cycle. In healthy human beings, the proportion of S-phase cells rises and reaches a maximum near 16.00 daily in proliferating tissue such as bone marrow and oral mucosa. In tumour cells, this rhythm is either lost altogether or it can become out of phase with healthy cells, offering a window of opportunity for hitting cancer cells at a time point when healthy cells are least vulnerable

Source: Lévi et al. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. *Phil Trans Royal Soc* 366:3583, © Royal Society 2008

decided it was too toxic to develop further, so the drug was completely stopped. We were the only ones at this time who wanted to work with this drug. We showed that if we gave oxaliplatin in the animals near the middle of the activity that would correspond to around 4.00 pm in humans, the drug was safe and effective.

"Then we did clinical studies that showed the same was true in humans. We compared constant-rate infusion versus chronomodulated infusion of oxaliplatin with peak delivery at 4.00pm. We showed it was much safer than flat infusion. And we were the first to show the efficacy of oxaliplatin with 5FU and leucovorin (LV), in phase I, II and III trials totalling more than 2000 patients with colorectal cancer."

The results in 1992, long before approval of this oxaliplatin, were encouraging. In a first phase II single-institution trial, 93 patients, 46 of whom had received previous chemotherapy, were treated with the chronomodulated combination of 5-FU–LV and oxaliplatin for five days every three weeks. The 5-FU–LV was administered during sleep, with maximum delivery at 4.00 am; the oxaliplatin during the day, maximum delivery at 4.00 pm. This new treatment achieved a 58% response rate, almost four times higher than that produced by the conventional daily bolus of 5-FU–LV (Lévi et al, *Cancer* 1992).

Two subsequent studies involving 278 previously untreated patients showed that administering 5-FU–LV and oxaliplatin using a chronomodulated instead of a flat-rate administration reduced the incidence of severe mucositis fivefold, halved the incidence of functional impairment from peripheral sensory neuropathy and reduced the incidence of grade 4 toxicity requiring hospitalisation by threefold. It also increased the objective response rate to the cancer chemotherapy, from 29% to 51% (Lévi et al, *Adv Drug Deliv Rev* 2007).

INTO THE MOLECULAR ERA

With the progress in molecular imaging techniques, researchers are now able to explore the mechanisms by which the body's circadian timing system regulates the molecular clocks of individual cells. Fifteen 'clock genes' have now been identified, every one of them has been shown to function abnormally in tumours.

Intriguingly, research from Canada is now revealing that, while the 15 genes that are largely responsible for circadian time keeping are very similar in men and women, only 10% of the genes whose transcription is controlled by these molecular clocks are the same in both sexes. This finding helps explain a recent, unexpected, observation, that while the chronomodulated regimens used in metastatic colorectal cancer patients have delivered marked survival benefit for men, women

Using a chronomodulated instead of a flat-rate administration reduced the severe mucositis cases fivefold

have tended not to benefit at all, or even to do worse than on conventional treatments.

The search is now on for other individual differences – genetic polymorphisms, lifestyles, biological rhythms – that may impact on the circadian behaviour of cells, opening up the prospect of personalised chronotherapy. This is the focus of a major project, TEMPO (temporal genomics for tailored chronotherapeutics), which is funded by the EU to the tune of €2.7 million.

A new twist has now been added to the story with the discovery that it may be possible to reset broken molecular time clocks. "We are working with a kinase inhibitor that inhibits the cell cycle, and we have found that this drug can induce a functional molecular clock in a tumour where it was previously defective. And when you induce a functional molecular clock, the tumour grows much more slowly," says Lévi.

One possibility being investigated would be to find a way to reset chaotic tumour cell clocks so that the normal 24-hour cell-cycle is restored, but out of phase with that of normal cells, in order to create the perfect conditions for administering anti-cancer drugs in a way that maximises both efficacy and tolerability.

Into the mainstream?

Despite the growing evidence from clinical and animal studies and molecular biology labs, circadian timing systems and chronotherapy remain on the margins of both clinical research and practice. The Paul Brousse hospital routinely uses chronotherapy to treat patients with metastatic colorectal cancer, but the technique has not yet spread beyond a small number of interested centres. This might have been understandable, says Lévi, in the days when both doctors and patients would have been required to get up in the middle of the night to administer the treatments.

Today, however, combinations of up to four drugs can be delivered by dedicated pumps, in what is called a sinusoidal pattern – starting slow, peaking in the middle and tailing off at specified time points – all in the comfort of the patient's own home. Lack of interest from pharmaceutical companies could be part of the problem, says Lévi – the need for a specific technology could conflict with their aim of maximising their market. When oxaliplatin was finally submitted for marketing approval, he notes, the company chose not to use the chronomodulated administration protocol that had been so important in its development.

But even among clinical researchers, chronotherapy remains a bit of a niche area. An international cooperative group has now been established – the Chronotherapy Group – which involves some 50 centres in 12 countries including Canada, China and the USA. Yet mainstream clinical research continues to largely ignore the potential importance of the time-of-day factor. "I think the major problem is conceptual," says Lévi. "We see things as static. We examine genes at a single timepoint. We measure the mitotic index at a single time point, and we think that by doing that we have a good picture of what is going on. To accept this is wrong is very difficult."

Given that biostatisticians are already struggling with the task of making sense of the massive amount of data now available, for instance, from a microarray, it is perhaps understandable that the prospect of having to deal with four or five microarrays, taken at different times of day, to get the full picture, might put people off. However, a lot of work is now going into developing mathematical modelling that would be needed to make this work.

Signs are now emerging that this is an approach to cancer whose time may have have finally come. "Over the past year there has clearly been renewed interest in the circadian timing approach, partly because of the accumulation of so much basic mechanism data," says Lévi.

The recognition by IARC that circadian timing has a probable connection to breast cancer also gave an important endorsement of their work – as Vincent Cogliano, who leads IARC's work on the evaluation of carcinogenic risks to humans, acknowledges.

"We were struck by how this evidence is accumulating and by the consistency between the animal studies and some of the human studies. I think the IARC monograph really put a spotlight on this area and brought it into the mainstream.

"The scope of my programme is to evaluate hazards. But when you see that the time of day for administering a chemotherapeutic agent affects its effectiveness, that's further evidence that there is some kind of a daily cycle of when carcinogenic events happen, and when things happen to cells, and that's probably part of the mechanism by which shift work is affecting cancer risk."

Lévi would now welcome greater interaction with the clinical research community. "There are many medical oncologists with whom we work, but this issue of circadian timing in cancer therapy has never yet been really debated as I think it should be, and I do regret it."

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