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# News & views

Medical research

# Cancer cells spread aggressively during sleep

Harrison Ball & Sunitha Nagrath

The deadly spread of cancer occurs predominantly during sleep, as revealed by an analysis of migrating human tumour cells in the bloodstream. What are the implications of this finding for the treatment of cancer?

Over the years, cancer has remained one of the greatest threats to human health. Despite many discoveries and much progress in the areas of clinical care and of cancer biology, key gaps in our knowledge of this disease remain to be addressed. Among the many phenomena that occur during the development of cancer, none might be more important to understand than the process, termed metastasis, by which cancer spreads from its tissue of origin to grow elsewhere in the body. Writing in *Nature*, Diamantopoulou *et al.*[1] present results that show how the aggressiveness of cancer spread can change during sleep.

Metastasis is linked to up to 90% of all cancer deaths[2]. One main area of clinical research over the past decade has been the study of how tumour cells can circulate throughout the body. In people with cancer, the cellular progenitors of metastasis are theorized to be cancerous cells found in the blood, termed circulating tumour cells (CTCs)[3]. CTCs that have 'broken off' from the tumour at its original site (the primary tumour) can retain their ability to proliferate and survive even in the harsh circulatory environment[4].

CTCs can circulate in the bloodstream (Fig. 1) either as single cells or as aggregates of two or more cells, known as CTC clusters. These clusters are often observed together with other, non-cancerous cells, such as immune cells, which might increase the aggressiveness of the clusters in terms of their capacity to metastasize and the ability of the cells they contain to survive[5].

The dynamics of CTC generation from a primary tumour are poorly understood. The prevailing theory suggests that these cells are released continuously into the bloodstream. Diamantopoulou and colleagues provide evidence indicating instead that CTC release is connected to our sleep cycle. Throughout a 24-hour period, our bodies undergo a series of mental and physical changes that correlate with our daily sleep–wake cycle, which is termed a circadian rhythm. The regulation of hormones such as melatonin (which acts to govern sleep) and cortisol (which helps to control blood-sugar levels) is a key function of this cycle.

Disruption of the circadian rhythm is linked to a series of chronic diseases, including cancer[6]. Previous epidemiological and experimental studies indicate that the circadian rhythm can influence cancer development[7], and Diamantopoulou *et al.* now provide a perspective on how circadian rhythms can directly affect how cancer spreads.

The authors' work suggests that circadian rhythms might be connected to CTC release from the primary tumour. Diamantopoulou and colleagues collected blood samples from 30 people with breast cancer, taking samples at 4 a.m. and 10 a.m., times representing the body's 'resting' and 'active' phases, respectively. Astoundingly, they found that more than 78% of all the CTCs obtained were from the samples taken during the resting phase.

To test whether results consistent with these data could be obtained using another system, the authors undertook a similar type of experiment using a series of representative mouse models. The level of CTCs in the animals oscillated, peaking while the mice were at rest. The authors monitored CTC levels when the circadian rhythms of the mice were disrupted through different means, including by treatment with the hormone melatonin and by alterations in light cycles; they also monitored levels in mice that had been genetically engineered to have disrupted circadian rhythms. In each model, the findings were consistent with the authors' previous results: CTC levels were highest while the mice were resting. For the genetically engineered mice, levels were lower than those of non-engineered control animals.

A rise in CTCs does not necessarily mean that a person's cancer is going to progress and successfully spread to secondary locations. Most CTCs die in the circulation[8], suggesting that only a particularly aggressive subset of these

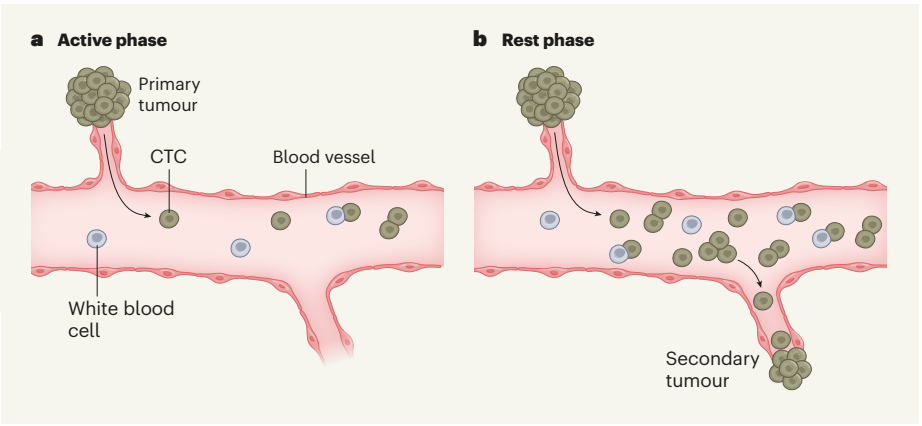


**Figure 1 | The spread of cancer at different phases of the day.** Diamantopoulou *et al.*[1] assessed the spread of cancer in the body by monitoring tumour cells in blood samples from people with breast cancer and from mice. The authors examined samples from the active phase of the day (when awake) and from the rest phase (associated with sleep). **a**, The bloodstream contains 'break-away' tumour cells, called circulating tumour cells (CTCs), that have migrated there from the initial site of tumour growth (the primary tumour). Some CTCs migrate in cellular clusters, either with other CTCs or with immune cells called white blood cells. This clustering might boost cancer spread[5]. **b**, The authors found substantially higher levels of CTCs in the blood during the rest phase compared with the active phase. Moreover, compared with active-phase CTCs, rest-phase CTCs had a greater capacity for proliferation and were more likely to form tumours at a secondary site.



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cells has the propensity to form tumours at secondary sites (metastases). Diamantopoulou *et al*. analysed the differences between rest- and active-phase CTCs and quantified their ability to metastasize. Using their mouse models, the authors took rest- and active-phase CTCs and injected them into healthy, tumour-free mice during different phases of the animals' circadian cycle. Strikingly, the authors found that CTCs from the rest phase not only form tumours more aggressively than do those from the active phase, but are also more likely to form tumours when injected into resting mice than into active mice.

Diamantopoulou and colleagues assessed gene expression in the cells using a technique called single-cell RNA sequencing. This helped them to understand the molecular mechanisms driving the aggressive properties of CTCs from the rest phase. Single-cell RNA sequencing provides a way of tracking RNA at a single-cell level, which is useful for attempting to understand nuanced differences in a population of cancer cells. Such differences are known to contribute to driving advanced cancers[9].

In CTCs from the rest phase, the authors identified a distinct rise in the expression of genes associated with cell proliferation. In CTCs from the active phase, by contrast, they found an increase in the expression of genes associated with protein synthesis. Diamantopoulou *et al*. also identified the upregulated expression of genes associated with proliferation in cells taken from primary tumours during the rest phase; the pattern of upregulation matched that which the authors identified in the CTCs. Finally, the authors found that in mouse models that had been treated to modify their circadian rhythms by controlling hormones related to this daily cycle, the patterns of cellular proliferation in CTCs matched what would be expected from the imposed circadian cycle. Together, these results paint a remarkable picture that demonstrates how CTC biology changes over a 24-hour period in response to the body's rhythms.

Diamantopoulou and colleagues' work has striking implications for the field of CTC studies and for cancer treatment in the clinic. The authors assessed human breast cancer, and it will be interesting to see whether these results hold true for other types of tumour.

The time-dependent nature of CTC dynamics might transform how doctors assess and treat patients. The data pointing to CTC proliferation and release during the rest phase suggest that doctors might need to become more conscious of when to administer specific treatments. However, most of the research conducted in this study was validated using mouse models. The findings will therefore need to be tested through large-scale clinical trials before any consideration of circadian rhythms is incorporated into standard practice.

The evidence presented suggests that a more holistic approach to studying CTCs, including harnessing technologies for continuous *in vivo* monitoring[10], might be necessary to fully understand the dynamics of cancer metastasis. A new chapter in blood-based biomarker studies can now start — taking into account how various regulators, such as hormones, affect the proliferation and spread of cancer.

**Harrison Ball** and **Sunitha Nagrath** are in the Department of Chemical Engineering, Rogel Cancer Center, Bio-interfaces Institute, University of Michigan, Ann Arbor, Michigan 48105, USA.
e-mails: hmball@umich.edu; snagrath@umich.edu

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