The tumor circadian clock: a new target for cancer therapy?

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Cancer is among the most frequent causes of death in humans. To date, established cancer therapies include surgery, radiation, chemo- and immunotherapy as well as hormonal treatments up to stem cell transplantation. In this editorial, we highlight an additional and so far underestimated factor involved in carcinogenesis and prognosis, namely biological clocks, which should be carefully considered to establish future cancer therapies. Disruption of the circadian clock was documented both in host-tissue and in cancer cells, and functional oscillations were associated with cancer prognosis and survival. We summarize recent advances in the field of chronobiology regarding the role of the host's and tumor-intrinsic circadian clock functions in carcinogenesis.

The biological circadian clock controls cancer-related pathways

A central clock in the hypothalamus and peripheral clocks in almost all organs and tissues altogether regulate physiological processes in a time of day dependent (circadian) manner. The molecular bases of circadian clocks are cellular oscillations generated by a number of rhythmically expressed interconnected clock genes [1]. High-throughput experiments on murine tissues showed that in any given cell-type or organ, hundreds or thousands of genes are expressed with a circadian rhythm, and led to the estimation that around 50% of all genes oscillate in at least one organ [2]. As a consequence of this, most cellular and physiological processes show 24 h oscillations. This is the case of many aspects of the immune system, including many immune cells and responses of relevance to cancer [3]. Also, major regulators of the cell cycle and tumor suppressor genes were shown to be regulated by the circadian clock, such as WEE1, c-MYC and p21 [4]. Consistently, cell-cycle progression and proliferation show circadian regulation.

Circadian clock disruption is associated with cancer

Mismatch between the internal clock and the external time disrupts the circadian network, as reported for example in shift workers [5], and has been associated with a wide range of pathologies and disease states including cancer [4]. Mounting evidence from human-based epidemiological studies suggested an effect of circadian disruption during shift work on cancer risk. For example, the risk to develop breast cancer was extensively increased in nurses exposed to long-term rotating night shift work [6]. Similar results were obtained for prostate cancer risk in night shift workers [7]. Out of note, in 2007 an agency of the WHO has classified night shift work leading to circadian disruption as 'probably carcinogenic' to humans [8]. Since then, there has been growing interest in understanding how circadian disruption may play a role in cancer development and progression. In support of human studies, experimental work in rodents documented accelerated tumor growth when mice, engrafted with lung adenocarcinoma or Glasgow osteosarcoma, were exposed to repeated jet lag conditions [9,10]. Similarly, enhanced tumor development has been described in mice with genetic disruption of the circadian clock (although this is not the case for all clock gene mutants) [9,11–13] or surgical lesion of the central clock [14]. Taken together, circadian disruption coincides with increased carcinogenesis and faster tumor growth in a wide range of experimental setups.

Considering circadian rhythms in treatments

Targeting the circadian clock may be a powerful tool to control tumorigenesis, since the literature reports that many cancer-related cellular processes, such as the cell cycle, cell proliferation, apoptosis and DNA damage are controlled by the circadian clock [15]. Interestingly, the targets of about half of the drugs currently on the market are encoded by genes showing circadian regulation [2]. In addition, the circadian regulation of processes such as metabolism or detoxification has severe consequences on the pharmacokinetics and pharmacodynamics of anticancer drugs, and thus, the outcome of anticancer therapies [16]. Therefore, chronotherapy, the principle of judiciously timed administration of therapeutic interventions, has been recognized as an important aspect in anticancer therapy [16]. The goal is to increase the treatment efficacy while minimizing toxicity [16]. For example, timed delivery of a therapeutic for colorectal cancer patients significantly improved the treatment efficacy and prolonged the patient's survival [17].

Targeting the clock in tumors?

Would it be possible to treat cancer by acting on circadian clocks? This is a question worth asking, given the circadian regulation of physiological systems relevant to cancer (e.g., immune responses, cell cycle, DNA repair), and the effects of circadian disruption on tumorigenesis (see the first two sections). Indeed, host circadian rhythms have often been found to be disrupted in cancer patients and mouse cancer models [10,12,15,17]. For example, in mice with lung adenocarcinoma, circadian gene expression was largely affected in the liver, with many genes losing rhythmicity, others becoming rhythmic, and some being shifted earlier or later [15]. In humans, alterations in clock gene expression and consequently clock-controlled gene expression was documented in the healthy gut tissues surrounding the tumor [18,19].

However, the literature is much more abundant regarding circadian disruption within cancer cell lines and tumor tissues [13]. Notably, recent evidence indicates a significant reduction of circadian rhythmicity restricted to murine colon tumor tissue, whereas rhythms were unchanged in healthy colon tissue surrounding the tumor [19]. In fact, the intratumoral circadian disruption correlates with the patient's prognosis and survival [17,18]. Consequently, tumor cell-autonomous clock mechanisms may be contributing to the increased carcinogenesis rather than circadian rhythms in the host.

Is the tumor-intrinsic circadian clock involved in carcinogenesis? Daytime rather than nighttime administration of seliciclib, a cyclin-dependent kinase inhibitor, reduced Glasgow osteosarcoma progression, and concurrently improved the amplitude of clock gene expression rhythms within the tumor tissue [20]. Similarly, timed feeding, a major synchronizer of peripheral clocks, stimulated clock gene rhythmic expression in pancreatic adenocarcinoma, while reducing its growth speed [21]. Although these interesting animal studies were consistent with the idea that tumor-intrinsic circadian rhythms may impact on tumor growth, direct evidence was not provided.

In recent studies, two opposite approaches have been used to directly test a causative link between the tumor clock and its growth. Papagiannakopoulos and colleagues addressed the question by genetically 'switching off' the clock specifically in cancer cells [9]. Deleting *Bmal1* or *Per2* in a genetically-engineered mouse models of lung carcinoma led to increased proliferation *in vitro* and tumor formation *in vivo*. This is in keeping with the many reports showing faster proliferation in cultured cancer cells [13].

We have approached the question from a different angle. Instead of inactivating the tumor clock, we reasoned that since most cancer cell lines and various tumor tissues harbor a dysfunctional circadian clock, then rescuing the circadian clock in tumor cells might inhibit or slow down their growth [22]. We found suppressed clock gene expression in B16 mouse melanoma cells, and were able to restore rhythmic clock gene expression by various treatments such as dexamethasone (DEX), forskolin or heat shock. Each of these clock-inducing treatments significantly slowed down B16 cell proliferation. When used to generate subcutaneous solid tumors in mice, the B16 cells did not show clock gene rhythms *in vivo* unless they were injected with DEX. This treatment both restored clock function and dramatically slowed down tumor growth (about two-third reduction after only 1 week) [22]. This was not due to an effect on the host-immune response, and moreover, inhibition of the tumor clock by shRNA-mediated knockdown completely prevented the effects of DEX, implying that they were happening via the tumor clock. Similar results were found in another cancer cell-type, the human colon carcinoma HCT-116 cells, pointing to the broader applicability of the concept [22].

What is the molecular mechanism underlying the clock's control over tumor growth? In contrast to healthy cells with a cell-division rate of about 24 h, cancer cells are characterized by uncontrolled fast cell division. In fact, the literature reports that cell-cycle gene expression is altered in cancer cells [11,15] and that increased levels of the

proto-oncogene Myc, upon mutation of clock genes in tumor cells, correlate with the cell-division speed [9]. Since the cell cycle is under circadian control, cancer cells might enhance their proliferation through manipulation of the circadian control over the cell cycle. Notably, our evidence indicates that the reduced proliferation and tumor growth due to activation of the tumor clock is achieved by inducing oscillations of cell-cycle genes important for the G1-to-S transition, such as *c-Myc*, *Cyclin E* and *p21*, which subsequently decreases the entry in S phase [22]. Taken together, current literature supports the strategy of acting on the tumor clock in order to control tumor progression.

Conclusion & future perspective

Slowing down tumor growth by manipulating the tumor circadian clock may critically grant more time for established anticancer approaches, such as chemotherapy and surgical tumor resection. Thus, this new idea of acting on the tumor clock might represent an innovative strategy to improve the outcome of the treatments and potentially the patient's survival. Additionally, the development of long-term strategies to strengthen circadian rhythms in populations at risk, such as shift workers, may prevent the incidence of cancer.

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