

Circadian function in cancer: Regulating the DNA damage response

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Proper circadian clock function is essential for the coordination of cellular functions within an organism in response to light and dark cycles. Although epidemiological studies have linked altered circadian rhythms to cancer susceptibility (1, 2), the molecular mechanisms tying the circadian clock to cancer are poorly understood. In PNAS, Lee and Sancar (3) demonstrate that loss of the core circadian clock proteins *Cry1* and *Cry2* can sensitize tumor cells to DNA damage-induced apoptosis. This effect is shown to be particularly relevant in cells that have lost *p53* (*TP53*), a key tumor suppressor and mediator of chemosensitivity, which is mutated or deleted in the majority of human cancers. These findings imply unique roles for circadian rhythm in regulating the DNA damage response and point to a potential avenue for treating refractory *p53*-deficient cancers.

The central oscillator of the circadian clock is made up of the transcriptional activators *Clock* and *Bmal1*, which heterodimerize to form an active complex. This induces transcription of a large suite of genes controlling multiple physiological processes, such as the cell cycle and metabolism (4). In addition, the *Clock/Bmal1* complex activates the cryptochrome (*Cry1* and *Cry2*) and period (*Per1*, *Per2*, and *Per3*) genes, which form a negative feedback loop capable of suppressing *Clock/Bmal1*-mediated transcription. This so-called transcription–translation feedback loop results in cyclical expression over a 24-h period, with the *Clock/Bmal1* complex activity generally highest during daylight hours and *Cry-Per* inhibitory activity peaking during the night.

A previous report by Sancar and colleagues (5) demonstrated that germline disruption of cryptochrome in *p53*-deficient mice, which are highly tumor prone, increased their tumor-free survival. Notably, this effect was associated with an increase in DNA damage sensitivity of the *Cry1/Cry2/p53*-deficient tumor cells compared with those lacking *p53* alone. This was a provocative finding given the crucial role of *p53* as a cellular executioner after DNA damage. When cells undergo irreparable DNA damage due to exposure to genotoxins such as UV radiation, the *p53* protein activates transcription of proapoptotic target genes, effectively removing the damaged cells from the or-

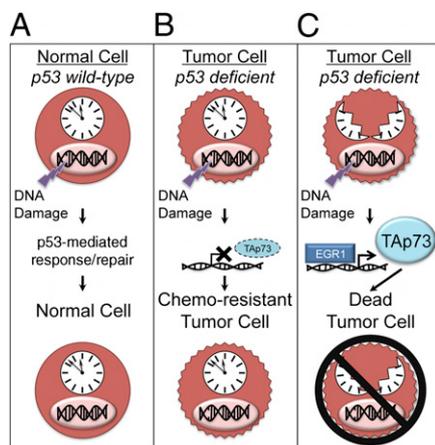


Fig. 1. Circadian clock-dependent sensitization of *p53*-deficient cells to DNA damage. (A) In normal cells with functional *p53*, the DNA damage response is mediated primarily by *p53*. (B) Tumor cells that have lost *p53* are resistant to DNA damage-inducing chemotherapy. (C) Tumor cells lacking both *p53* and circadian clock proteins *Cry1* and *Cry2* have high *Egr1* levels. *Egr1* bound to the *TAp73* promoter allows for increased activation of *TAp73* after DNA damage, resulting in tumor cell death.

ganism. Consequently, loss of *p53* in both humans and mice allows cells to persist and acquire new mutations necessary for cancer progression, yielding tumors that are refractory to our most common therapies, including ionizing radiation and chemotherapy. This previous report therefore suggested that loss of cryptochrome could reverse the DNA damage resistance induced by loss of *p53*. Killing such *p53*-deficient tumor cells is clearly one of the major challenges in cancer therapy.

In their new article, Lee and Sancar offer insight into the mechanism by which cryptochrome deficiency sensitizes cells lacking *p53* to apoptosis. Remarkably, they demonstrate that cryptochromes are involved in the regulation of the *p53*-related gene *p73* (*TP73*), which like *p53* functions as a tumor suppressor and helps maintain genomic integrity (6–8). Transcription of *TAp73*, a *p73* isoform with strong structural and functional similarity to *p53*, is enhanced in *Cry1/Cry2*-deficient cells in response to DNA damage. Loss of *Cry1/Cry2* relieves repression of *Clock/Bmal1*, thereby leading to *Egr1* up-regulation and recruitment to the *TAp73* promoter. Activation of *TAp73* after *Cry1/*

Cry2 loss also requires the DNA damage-induced removal of the repressor C-EBP α from the *TAp73* promoter. Thus, *TAp73* is both temporally regulated by the circadian clock and acutely regulated in response to DNA damage (Fig. 1).

These findings may have particular implications for the field of cancer chemotherapy, which seeks to determine whether the effectiveness and tolerability of chemotherapy can be linked to the time of day treatment is given. Indeed, it has been found that treatment schedule can impact both long-term survival and non-specific toxicity (9, 10). However, the widespread application of such observations to standard clinical practice has been hampered by a lack of insight into how the circadian cycle influences the response to specific chemotherapeutic agents. The new study demonstrates that *p53*-deficient tumor cells with compromised circadian function exhibit increased apoptosis and slower tumor growth in vivo after treatment with oxaliplatin (3). The finding that this effect is linked to *p73* supports previous studies showing that platinum agents induce apoptosis at least in part through phosphorylation-dependent activation of *TAp73* (11, 12) and that *TAp73* levels are a key determinant of chemosensitivity (6, 13). It will therefore be of interest to determine whether the cryptochrome- and *TAp73*-dependent response observed by Lee and colleagues is specific to treatment with platinum or will be seen with other common chemotherapeutic agents.

In addition to enhancing tumor chemosensitivity, these findings might also provide hope for approaches to limit the toxicity of chemotherapy for normal tissues. The model proposed herein suggests that in normal cells, the circadian cycling may result in a window when the amount of *Egr1* bound to the *TAp73* promoter is low, potentially blunting the apoptotic response of normal cells to chemotherapy. Interestingly, it has been found that tumor cells in patients do not cycle with the same kinetics as their

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normal cells (14). Thus, an optimal treatment schedule might involve administration of DNA-damaging agents when TAp73 levels are lowest in normal cells to reduce toxicity but higher in tumor cells to increase sensitivity. On the other hand, it is possible that additional circadian clock-regulated genes may also contribute to the response of tumor and normal cells to DNA damage. Interestingly, the DNA repair protein XPA is elevated in *Cry1/2*-deficient primary cells (which express wild-type p53), and as a result cells undergo more efficient nucleotide excision repair in response to cisplatin treatment than wild-type cells (15). Thus, although loss of cryptochrome proteins makes p53-deficient tumor cells more sensitive to chemotherapy, the opposite may be true in normal cells. This difference may be explained in part by the fact that in some cellular contexts, p53 can mediate cell cycle arrest and DNA repair as opposed to inducing apoptosis. Thus, therapeutic targeting of cryptochrome function may allow for a coordinate increase in chemosensitivity of tumor cells and decrease in toxicity for normal cells. In any case, this possibility highlights the complex inter-

play between the circadian clock machinery and the DNA damage response, which will have to be considered to translate these mechanistic findings into a clinical regimen.

An additional question provoked by this study is why TAp73 would be regulated in a circadian fashion in normal cells. The Clock/Bmal1 complex activates target

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genes in response to light, and thus *Egr1* is likely to be highest during daylight hours, when it could contribute to activation of TAp73 in response to UV radiation. Circadian regulation of TAp73 may therefore work together with p53 to add a second layer of DNA damage monitoring and protection to skin cells exposed to such

environmental insults. It is worth noting that circadian clock-mediated regulation of the DNA damage response is tissue-specific: hepatocytes exhibit oscillation in nucleotide excision repair efficiency, which is not seen in the testis (15). Collectively, these findings suggest that tissue-specific context will add an additional layer of complexity to understanding the link between the circadian clock and DNA damage response.

The widespread application of cancer chronotherapy faces many hurdles, both conceptual and practical. Nevertheless, the appeal of enhancing therapeutic efficacy while minimizing toxicity through chronobiology is clear. For this promise to become a reality, it will be essential to understand which treatments are affected by circadian cycles and which are not, and how the timing of treatment might have to be altered in a tumor- and patient-specific fashion. There is no doubt, however, about the unmet need for more tailored and effective treatment approaches for p53-deficient cancers, which are both common and commonly treatment-refractory.

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