Circadian function in cancer: Regulating the DNA damage response

Matthew R. Ramsey and Leif W. Ellisen[1]

Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA 02114

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 organism. 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.

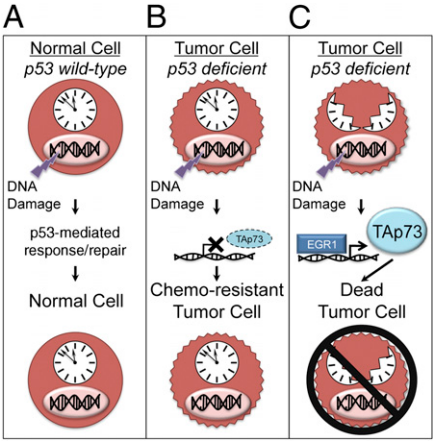

Fig. 1. Circadian clock-dependent sensitization of p53-deficient cells to DNA damage. (*A*) In normal cells with functional p53, the DNA damage response is mediated primarily by p53. (*B*) Tumor cells that have lost p53 are resistant to DNA damage-inducing chemotherapy. (*C*) Tumor cells lacking both 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.

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-EBPα 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 chronotherapy, 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 nonspecific 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

Author contributions: M.R.R. and L.W.E. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 10668.

[1]To whom correspondence should be addressed. E-mail: ellisen@helix.mgh.harvard.edu.

Downloaded at Palestinian Territory, occupied on December 21, 2021

www.manaraa.com

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 interplay 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 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.

Loss of the core circadian clock proteins Cry1 and Cry2 can sensitize tumor cells to DNA damage-induced apoptosis.

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.

1. Hoffman AE, et al. (2009) Clock-cancer connection in non-Hodgkin's lymphoma: A genetic association study and pathway analysis of the circadian gene cryptochrome 2. *Cancer Res* 69:3605–3613.
2. Schernhammer ES, et al. (2001) Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. *J Natl Cancer Inst* 93:1563–1568.
3. Lee JH, Sancar A (2011) Circadian clock disruption improves the efficacy of chemotherapy through p73-mediated apoptosis. *Proc Natl Acad Sci USA* 108: 10668–10672.
4. Sahar S, Sassone-Corsi P (2009) Metabolism and cancer: The circadian clock connection. *Nat Rev Cancer* 9: 886–896.
5. Ozturk N, Lee JH, Gaddameedhi S, Sancar A (2009) Loss of cryptochrome reduces cancer risk in p53 mutant mice. *Proc Natl Acad Sci USA* 106:2841–2846.
6. Irwin MS, et al. (2003) Chemosensitivity linked to p73 function. *Cancer Cell* 3:403–410.
7. Wang W, Kim SH, El-Deiry WS (2006) Small-molecule modulators of p53 family signaling and antitumor effects in p53-deficient human colon tumor xenografts. *Proc Natl Acad Sci USA* 103:11003–11008.
8. Tomasini R, et al. (2008) TAp73 knockout shows genomic instability with infertility and tumor suppressor functions. *Genes Dev* 22:2677–2691.
9. Hrushesky WJ (1985) Circadian timing of cancer chemotherapy. *Science* 228:73–75.
10. Lévi F, et al. (1990) Chemotherapy of advanced ovarian cancer with 4′-O-tetrahydropyranyl doxorubicin and cisplatin: A randomized phase II trial with an evaluation of circadian timing and dose-intensity. *J Clin Oncol* 8:705–714.
11. Gong JG, et al. (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* 399:806–809.
12. Leong CO, Vidnovic N, DeYoung MP, Sgroi D, Ellisen LW (2007) The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. *J Clin Invest* 117:1370–1380.
13. Ibrahim N, et al. (2010) BRCA1-associated epigenetic regulation of p73 mediates an effector pathway for chemosensitivity in ovarian carcinoma. *Cancer Res* 70: 7155–7165.
14. Klevecz RR, Shymko RM, Blumenfeld D, Braly PS (1987) Circadian gating of S phase in human ovarian cancer. *Cancer Res* 47:6267–6271.
15. Kang TH, Lindsey-Boltz LA, Reardon JT, Sancar A (2010) Circadian control of XPA and excision repair of cisplatin-DNA damage by cryptochrome and HERC2 ubiquitin ligase. *Proc Natl Acad Sci USA* 107:4890–4895.

Downloaded at Palestinian Territory, occupied on December 21, 2021
www.manaraa.com