Circadian-tumor suppressor crosstalk: Emerging opportunities in cancer chronotherapy

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Previously, cancer treatment modalities relied primarily on chemotherapeutic agents; nowadays, advances in rationally-designed drugs and targeted therapies have enabled the manipulation of cancer-specific regulatory molecules that are frequently mutated and globally identified in various cancers. Regardless the approach, the objective for controlling cancer progression has always been to attenuate, eliminate, or control the neomorphic activity of target driver mutations in tumors by maintaining steady levels of therapeutic agents. As precision medicine gains momentum, so does the possibility of customizing individual patients' treatments to the "time-of-day" when tumor cells exhibit the highest susceptibility to therapeutics (1). However, a gap exists in our knowledge regarding the times at which therapeutically-targeted molecules are likely to be most susceptible to drugs and yield the greatest cellular effect. As a result, there is a need to unveil "*when*" and "*where*" druggable targets are in the cell and "*to what extent*" the tumor's time-keeping system differs from normal tissue (Fig. 1A). Defining priorities that address those needs across the hierarchical system of organization will allow researchers to find the best time-windows where delivery of treatment modalities can be most effective.

Emerging connections among components of the circadian time-keeping mechanism and key regulators of cell cycle progression advocate for the application of chronobiology to the treatment of proliferative diseases (2). This is particularly relevant when considering disorders like cancer, as circadian factors are now known to directly bind to both tumor suppressors and oncogenes that are mutated or amplified in more than 70% of cancer cases. An example of this approach comes from our recent studies on the molecular interaction and spatial-temporal distribution of the circadian component PERIOD 2 (PER2) and p53, a commonly mutated tumor suppressor associated with aggressive forms of cancer, which is therapeutically exploited for regulation at multiple levels.

Gotoh *et al.* findings show that PER2, a core clock component directly involved in the generation and maintenance of circadian rhythms, directly interacts with the tumor suppressor p53 and its negative regulator, the proto-oncogene mouse double minute 2 homolog (MDM2) to modulate p53 stability in unstressed cells and transcriptional activity in response to genotoxic stress (3-4). The existence of this crosstalk mechanism at the p53-node reshapes the current landscape of the checkpoint signal by identifying additional points of control that can intersect with other cellular pathways (*e.g.*, metabolic networks) to which circadian components are intimately linked; thus, integrating checkpoint signaling more broadly in the cell. Furthermore, the importance of such a connection to human health is apparent since the proper timing of cell division and its response to genotoxic stress are major factors contributing to the regulation of normal growth and emerges as a fundamental process in the development of most cancers. Because of the multi-dimensional nature of the cellular response, our findings open potential avenues for research and development of new therapeutic agents that target unconventional cellular players and include treatment regimens based on the dynamics of the circadian control system.

As is usually the case in science, our most recent work, *PNAS* 2016, began with the inconspicuous and puzzling finding that

seemed, *a priori*, in conflict with the proposed model for how PER2 and p53 proteins interact and signal (3-4). In short, the initial assumption was simple in concept - if PER2 stabilizes p53, the levels of both proteins should rise and fall together. However, we found this not to be the case as the phase distribution of these proteins over time were, surprisingly, out-of-phase when analyzed in lysates from circadian synchronized cells. Thus, the initial question with which we were confronted was simple in nature: "*How can discordant Per2 and p53 phases coexist in a model in which Per2 enhances p53's stability?*" Most intriguing was our later finding that Per2 and p53 phases were in sync in the nucleus but out-of-phase in the cytoplasm.

Addressing this puzzle required a combination of mathematical and experimental approaches and outstanding collaborators. First, we transformed our preliminary experimental findings in a series of equations that helped predict biological scenarios and regulation types for which the Per2-p53 phase relationship was maintained. Second, the common characteristics of the successful scenarios suggested testable predictions that provided the initial framework for our experimental work. Predictions spanned a unique variety of biochemical modifications (*e.g.*, the importance of ubiquitination for Per2 and p53 binding) and processes (*e.g.*, p53 stability and spatio-temporal distribution of Per2 and p53) that were experimentally confirmed. As a result, our work contribute to: *i*) establishing the use of a model-driven experimental approach to reveal new networks of interactions, *ii*) unveiling the mechanism by which the circadian oscillations in p53 are generated, and *iii*) reconciling previous and new experimental findings in a new unifying model in which the spatio-temporal dynamics of each component plays a central role. Importantly, the relevance of the underlying dynamics behind the spatio-temporal location of Per2 and p53 extend beyond the control of their distributions, because both molecules act as sensors of extracellular stimuli and intracellular conditions and are versatile modulators and integrators of cellular networks; all of which can help us understand how their associations affects the cell's physiology and its relationship with the environment.

The clinical relevance of these findings is evident when one considers the numerous rationally-designed drugs currently in preclinical or clinical trials that are aimed at attenuating, eliminating, or controlling the activity of either p53 or MDM2. We know now that the application of therapies that directly target p53 should be delivered at times in which PER2 concentration is at a low level or absent in tumor cells (Fig. 1B). In accord with this statement, we predict that small-molecule stabilizing agents meant to reactivate mutant forms of p53 and restore its wild-type conformation would likely fail to do so when the tumor suppressor is found to be part of the PER2:p53 complex (Fig. 1C). As a result, our laboratory advocates for a more comprehensive study of treatment modalities that take into consideration the direct role of circadian components on the modulation of the tumor suppressor's function.

References

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Figure 1. A. Schematic representation of possible scenarios (I-III) by which administration of therapeutics based on circadian timing would be beneficial. As shown, treatment modalities should consider whether the tumor's clock is in phase (or not) with its surrounding tissue or, instead, is simply absent. Furthermore, therapeutic protocols should also recognize the pharmacokinetics and pharmacodynamics of a specific drug in the context of the patient circadian physiology. **B.** Schematic representation for the distribution of PER2 (pink) and p53 (blue) in the nucleus and cytosolic compartments based on Gotoh *et al.*, Proc. Natl. Acad. Sci. 20126. Arrows indicate times at which the delivery of a therapeutic targeting p53 would be optimal. **C.** Diagram of an hypothetical scenario in which the spacio-temporal distribution of mutant p53 protein should be consider for effective interventions.