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Two is better than one; toward a rational design of combinatorial therapy

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Drug combination is an appealing strategy for combating the heterogeneity of tumors and evolution of drug resistance. However, the rationale underlying combinatorial therapy is often not well established due to lack of understandings of the specific pathways responding to the drugs, and their temporal dynamics following each treatment. Here we present several emerging trends in harnessing properties of biological systems for the optimal design of drug combinations, including the type of drugs, specific concentration, sequence of addition and the temporal schedule of treatments. We highlight recent studies showing different approaches for efficient design of drug combinations including single-cell signaling dynamics, adaption and pathway crosstalk. Finally, we discuss novel and feasible approaches that can facilitate the optimal design of combinatorial therapy.

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This review comes from a themed issue on **Multi-protein assemblies in signaling**

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Introduction

Tumorgenesis is an evolutionary process during which a series of mutations rise and accumulate in cells, allowing them to grow beyond physiological limitations. Depending on their history, different cancer clones can use different strategies to escape growth controls. Even in a single tumor multiple regulatory pathways can be defective including apoptosis, migration, cell cycle arrest or suppression of the immune response [1]. Such heterogeneity among individual cancer cells often limits the efficacy of a single anticancer drug to fully eliminate all cells in a tumor.

One approach for overcoming therapy resistance is by combining multiple drugs. The main rationale behind combinatorial therapy is to suppress more than one pathway and therefore to synergistically eradicate the various clones that emerge in a tumor [2••]. Such approaches have been proven successful in culture cells and in the clinic [3,4,5,6,7]. However, despite the great interest in, and potential of combinatorial therapies, the design of drug combination (*e.g.* specific concentration of each drug, sequence of addition, time interval between treatments) mostly relies on the knowledge from administrating each drug alone, and in many cases on trials and errors.

The proper design of combinatorial treatments is critical for its success. Administration of one drug can lead to a dynamic response, which may increase or decrease the sensitivity of the cells to the second treatment. Such interactions may depend on the time interval between treatments, the state of the cells or the concentration of each drug. In most cases we lack the knowledge and understanding of how each drug impact cellular states that may interact epistatically with the second drug. In this review, we present recent discoveries about the dynamics of, and crosstalk between multiple signaling pathways that may have impacts on cellular states and their vulnerability, which can help rationalize the design of drug combination.

Signaling dynamics guide the design of combinatorial therapy

In response to external and internal inputs, signaling molecules act collectively to generate temporal changes in their level, localization or activity, here defined as 'signaling dynamics'. Recently an increasing amount of evidence showed that quantitative features of signaling dynamics, such as the duration of the signal, its amplitude or accumulation rate, can carry biological information that is critical for cellular outcomes [8••]. Specifically, several transcription factors have been shown to exhibit signal- and stimulus-specific dynamics that govern the transcriptional programs for differential cell fates. These include the transcription factor Msn2 in *Saccharomyces cerevisiae* [9], and p53 [10,11] and NF-kB [12] in human cells. The idea that signaling dynamics play an important role for cells was further strengthened by the fact that modulation of the dynamics of p53 levels and of ERK activity result in cell fate switch [11,13].

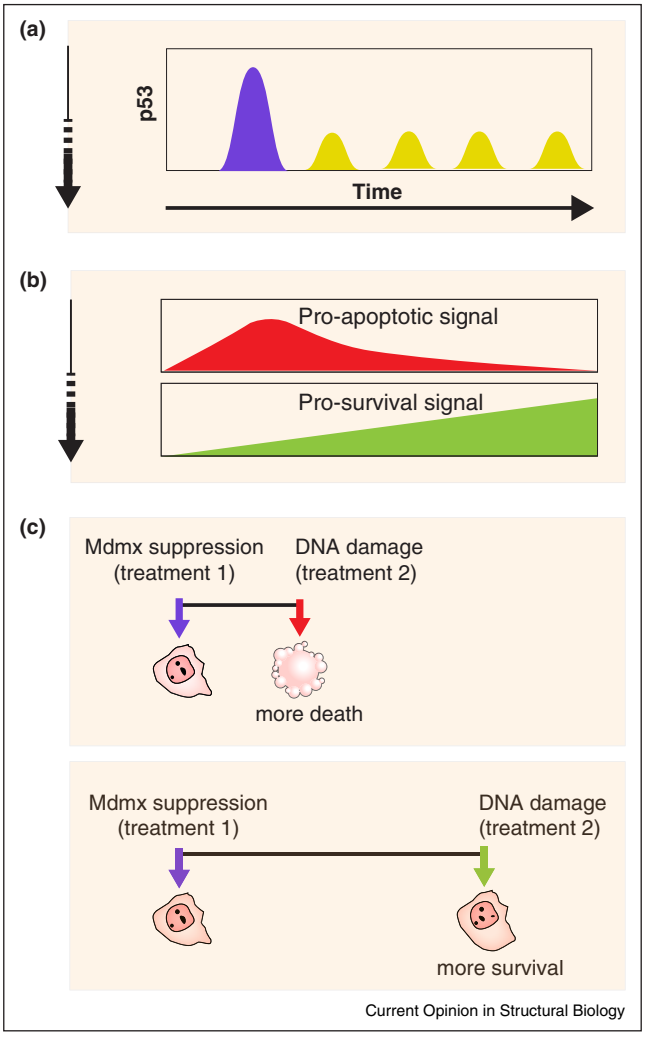
The processing of cellular information can vary dramatically between cells, and even genetically and developmentally equivalent cells may show different behaviors in response to the same stimulus. As a result, the average

behavior of a population often represents a distorted version of individual patterns. For example, our studies on the p53 signaling pathway in single cells revealed a series of p53 pulses in response to DNA damage [14,15] and spontaneous p53 pulses in non-stressed conditions [16], which were masked by population averaging assays [17]. These newly identified behaviors of p53 led us to develop new models for the signaling circuits controlling p53 dynamics [10,15], and to identify a new information-transfer mechanism in this network [11]. These examples underscore the importance of tracking cellular and molecular responses at the single-cell level.

Can the dynamics of signaling molecules be used to guide the design of combinatorial therapies? In our recent work we found that when the oncogenic inhibitor of p53, MDMX, is suppressed, p53 shows two phases of dynamics in individual cells: an initial post mitotic high-amplitude pulse followed by small amplitude oscillations (Figure 1a) and [18••]. We further showed that these two phases of p53 dynamics are associated with activation of distinct p53 transcriptional programs: the post-mitotic pulse led to a universal p53 response activating the transcription of genes involved in multiple programs including apoptosis and other pro-death signals. The second phase of small amplitude p53 oscillations led to a specific transcriptional activation of p21, a p53 target that regulates cell cycle progression and other pro-survival signals. These results suggest that MDMX suppression results in a transcriptional program switch regulated by biphasic p53 dynamics (Figure 1b). Importantly, these observations prompted us to examine the temporal effects in combining MDMX suppression with DNA damage.

MDMX is overexpressed in multiple cancers including malignant melanoma, glioma and breast cancer, where p53 activity is mostly suppressed [19,20,21]. As a result, MDMX suppression has been suggested as a therapeutic intervention for such cancer patients [22,23]. Since most patients with MDMX overexpression bear wild type p53, we designed a combinatorial therapy composed of MDMX suppression and chemotherapy that activates p53-dependent apoptotic function. The two distinct phases of p53 dynamics (Figure 1a) and its transcriptional program (Figure 1b) after MDMX suppression led to a switch in the interaction between chemotherapy and MDMX inhibition depending on the time interval between the two treatments (Figure 1c). Specifically, when DNA damage was induced during the first phase of p53 dynamics, it synergized with MDMX suppression and led to more killing of the cancer cells. However, when DNA damage was given during the second phase of p53 dynamics (p53 oscillations), MDMX suppression antagonized it and made the cells more resistance (Figure 1c). In another recent study of p53 dynamics in response to the chemotherapy drug, Cisplatin, we revealed that the rate of p53 accumulation determined the likelihood of cell death [24]. This was caused by induction of the anti-apoptotic IAP pathway that antagonizes with p53-mediated apoptosis, leading to a combinatorial treatment where p53 is activated and the IAP pathway is inhibited, increasing the efficacy of Cisplatin. These studies showed that quantifying the dynamics of signaling molecules in single cells provide valuable

Figure 1



Schedule dependent interaction between MDMX suppression and DNA damage.

(a) Mdmx suppression leads to two phases of p53 dynamics in single cells; post-mitotic pulse (blue) followed by low amplitude oscillations (yellow).
(b) The first phase of p53 activates pro-apoptotic signals, and the second phase activates pro-survival signals.
(c) The time interval between Mdmx suppression and DNA damage determines their interaction. A short interval leads to a synergistic effect and to more cell death. A long interval leads to an antagonistic effect and protect cells from death.