Toggling Cell Fate by Kinase Control

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***Short Abstract* — How do cells robustly process information to consistently generate biologically mandated cell fates? For example, DNA damage can drive cells into a range of outcomes including temporary cell cycle arrest, permanent cell cycle arrest, which is also known as DNA damage induced senescence (DDIS), and cell death via apoptosis. DNA damage induced apoptosis and senescence create roadblocks on the path to tumorigenesis by preventing cells with damaged genomes from proliferating. It is not understood how cells integrate signals from the DNA damage response (DDR) with other signaling pathways, which reflect their internal state and/or their environment, so as to commit to these very different outcomes. Through a combined experimental-computational approach we found that the Jnk and Erk signaling pathways regulate initiation of DDIS. In addition, Jnk and Erk activity regulate the Senescence Associated Secretory Phenotype (SASP) that is a hallmark of DDIS. Furthermore, Jnk signaling is required for oncogene-induced senescence in a mouse model of prostate tumorigenesis. These results establish roles for signaling pathways other than the DNA damage response in regulating the cell decision to senesce after DNA damage and point to cellular information processing strategies for robustly achieving essential cellular outcomes.**

***Keywords* — Cell fate, Computational Cell Biology, Signal Transduction, Senescence, Apoptosis, Partial Least Square Regression**

I. BACKGROUND

In response to discrete levels of genotoxic stress mammalian cells either die (typically by apoptosis), enter a state of permanent cell cycle arrest that is known as DNA damage induced senescence, or temporarily arrest the cell cycle, presumably to provide a period of time for the DNA damage to be repaired. The work of numerous groups identified the core DNA damage signaling machinery and showed that the canonical DDR signaling molecules including among others ATM, Chk2, H2AX, Nbs1, 53BP1 and p53 regulate all three of these outcomes. Given that all DNA lesions activate this machinery it is not clear how high levels of DNA damage induce apoptosis, while low levels induce only a temporary cell cycle arrest. In other words, we do not understand how cells evaluate the extent of DNA damage and couple this information to the appropriate cell fate. We hypothesized that additional signaling pathways contribute to this "cell fate decision process" and used data-driven modeling to identify the Jnk and Erk pathways, as candidate regulators of DNA damage induced senescence (DDIS). Our computational model of the relationship between signal-response relationship after DNA damage motivated experiments that showed Jnk and Erk contribute to both the decision to senesce and to the regulation of the Senescence-Associated Secretory Phenotype (SASP).

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II. RESULTS

In order to maximize our understanding of how signaling events drive distinct cellular responses we used experimental methods that generate data at single cell resolution. Our single cell data show that the dose response of U2OS cells to DNA damage is complicated and that genotoxic stress can induce complex mixtures of cellular states. Increasing the dose from very low to intermediate sees the cell population respond with an increasing fraction of cells undergoing DDIS. Increasing the dose further sees a fraction of the cells apoptosing. These complicated *in vitro* mixtures of cell fates are likely relevant to the clinical use of standard DNA damaging chemotherapeutics. Treating solid tumors with chemotherapeutic agents such as doxorubicin creates gradients of dose as a function of the structural complexity and vascularization of the tumor. Because cells in a tumor experience the full range of low to high levels of DNA damage, canonical chemotherapy likely generates a complex mixture of transiently arrested, senescent and dying cells. The complicated interplay of this heterogeneous population of cells could significantly impact clinical outcomes.

Apparently, at potentially repairable levels of DNA damage Jnk and Erk activity are needed to insure the robustness of the senescence decision. Why would cells require an additional signaling pathway to drive cell fate at these transitional levels of DNA damage? Even if a mammalian cell could repair all or at least most the DNA damage, it is conceivable that the fate the organism is better served by sequestering the cell in the senescent state. We propose that Jnk and Erk signaling pathways integrate other aspects of the cell state, such as its microenvironment, with DDR signaling to generate the cell fate decision. It is likely that this need to process multiple inputs is responsible for the 2-4 day timeframe necessary for the cell to commit to senescence. Understanding the cell fate machinery allowed us to toggle cells between the cellular outcomes of DDIS and proliferation through the addition of small-molecule kinase inhibitors and through genetic methods *in vivo*. The ability to toggle between cell fates by modifying distinct signaling pathways raises the intriguing possibility of the rational design of combination therapies. Of course, there will be numerous challenges to orchestrating cell fate, but we believe that improved data-driven models of cellular outcome can facilitate the rational design of combination therapies.