To Sleep or Die: Cell Fate after Chemotherapy

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***Short Abstract* — DNA damage induces cancer cells to arrest their cell cycle and then either repair the damage and proliferate, enter a state of permanent arrest (senescence) or apoptose. Using a combination of flow cytometry and high-content microscopy we have quantitatively monitored cellular signaling and phenotypic outcomes in response to DNA damage. We have quantified signaling within the DNA damage signaling pathways, the cell cycle machinery, the apoptotic machinery and the mitogen/stress activated kinase pathways. Partial Least Squares Regression of these data demonstrate a role for the mitogen activated protein kinase, Erk, in modulating the cellular response to DNA damage.**

***Keywords* — DNA damage, Signal Transduction, Senescence, Apoptosis, Partial Least Square Regression**

I. BACKGROUND

The DNA damage signaling network can be thought of as a computational device in which the input is the type and extent of the DNA damage and the output is cell cycle arrest and repair, permanent cell cycle arrest or cell death. A signaling network consisting of protein kinases makes these cellular decisions, but exactly how the cell commits to these very different outcomes is unclear. To understand how these cell fates are determined we have generated a quantitative, network level understanding of how this cellular decision process works through the synergistic application of experimental and computational methods. This project builds upon the work of Lauffenburger, Yaffe and co-workers who successfully monitored and modeled the activity of pro-death and pro-survival signal transduction pathways in response to EGF, insulin and TNFα [1]. As was the case for the cytokine induced apoptotic decision, cellular signaling space has been probed using a combination of cues. In this case, Doxorubicin (Dox) induced DNA damage in combination with TNFα to drive populations of cells to different levels of senescence and apoptosis.

II. Results

In order to understand the population dynamics and to be able to explore heterogeneous sub-populations of cells we have monitored our cellular results at the level of individual cells. Through the use of single cell methods we have been able to capture the cellular information processing by the DNA damage response, cell cycle machinery, apoptosis machinery and the stress/mitogen activated protein kinases. Using flow cytometry we have monitored the cellular response of the U2-OS osteosarcoma cell line to doxorubicin treatment in combination with TNFα treatment. We quantitatively measured the proliferation, cell cycle arrest kinetics and apoptosis levels. Concomitantly, we have used high-content, fixed cell immuno-fluorescence of protein level, protein localization and phosphorylation state of 30 signaling proteins within the cell. We monitored the dynamics of signaling by taking multiple timepoints over the course of 96 hours. The intracellular signaling measurements and the cellular outcomes were selected because they provide a broad overview of the multiple regulatory pathways that are likely to be affected by the DNA damage response, including progression through various stages of the cell cycle, cell cycle arrest, initiation of DNA repair, survival and stress responses, chromatin remodeling and transcriptional regulation.

A 4 hour pulse of 2 μM Dox induces a pulse of apoptosis with the surviving cells entering DNA damage induced cellular senescence. A 4 hour pulse of 10 μM Dox induces apoptosis. Adding saturating TNFα to these treatment increases the amount of apoptosis, but the 2 μM Dox + TNFα treatment still leads to senescence. Our use of single cell assays has allowed us to capture the dynamics of the emergence of these various cell states. Principle Component Analysis of the response measurements has identified latent variables that correspond to proliferation, apoptosis and senescence. The ability of these unsupervised methods to identify biological relevant axes in cellular outcome space is striking.

Partial Least Squares Regression of the signaling data against the response data identifies the anticipated components of the DNA damage response signaling pathways (e.g. γH2AX, p53), cell cycle machinery (e.g. Cyclin B, p21), apoptosis machinery (e.g. Bcl-XL) that regulate the cellular decision to apoptose or senesce. Interestingly, the regression analysis also identifies roles for the stress/mitogen activated protein kinase signaling pathways in modulating the cellular response to DNA damage. In particular, we have found that the activities of Erk, p38, Jnk and Akt contribute to the cell fate decision. Armed with this network understanding of how intra-cellular signaling defines phenotypic outcome we are capable of engineering the fate of heterogeneous cell populations. Such controlled modulation of cell fate could prove invaluable in a clinical or engineering situation.

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