Tailoring combinatorial cancer therapies to target the origins of adaptive resistance

Aaron Goldman*

Department of Medicine; Harvard Medical School; Brigham and Women's Hospital; Division of Biomedical Engineering; Cambridge, MA USA

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The development of resistance to chemotherapy is a critical yet poorly understood phenomenon driving cancer-related deaths. We recently reported a study that explored the origins of adaptive chemotherapy resistance. During this investigation we unexpectedly discovered that inherent vulnerabilities were unmasked, creating temporal targets for rational combinations of established drugs.

Background

The development of resistance to chemotherapy is a key driver of breast cancer locoregional recurrence, a manifestation that leads to metastasis and mortality.¹ There are several models that attempt to explain how drug resistance develops. Older belief systems rely on Darwinian dynamics and stochastic evolution of resistance-conferring mutations.² Newer models, however, take into consideration the subclonal heterogeneity of tumors with an increasing appreciation for the inherent phenotypic plasticity of cancer cells.^{3,4} This latest model, built from a cell hierarchy, incorporates a role for subpopulations inherently resistant to chemotherapy that also harbor tumor initiating potential. These subpopulations are often defined by high expression of the cell surface protein biomarker cluster of differentiation 44 (best known as CD44) and classically referred to as cancer stem cells (CSCs).^{5,6} Nonetheless, a clear understanding of the cell dynamics and cellular origins of drug resistance remains elusive, leading to a series of unanswered questions: How does subclonal heterogeneity contribute to chemotherapy resistance? Can we develop translational strategies to target the origins of drug resistance? What are the tools that we can use to explore adaptive resistance in such a dynamic and heterogeneous disease?

Our Study

We recently attempted to address these questions by incorporating mathematical modeling and primary human tumors as tools to understand biological behaviors in the context of adaptive drug resistance to standard-of-care cytotoxic chemotherapies.^{7,8} We first developed a primary human tumor explant model that was generated from patients who demonstrated refractoriness in the clinic.⁹ This model allowed us to capture the heterogeneity of tumors in 3 dimensions that preserved their integrity *ex vivo*. Histopathology indicated that chemotherapy induced the emergence of a new phenotype in resistant tumors, which was defined by increased expression of CD44 and CD24 cell surface glycoproteins.⁷ Rather than chemotherapy-driven selection of subclones, these studies revealed that phenotypic plasticity was instructed by exposure to the chemotherapy agents. In an effort to determine the cells of origin that acquired the CD44^{Hi}CD24^{Hi} drug resistant state, we developed a mathematical approach. We based these computational models on experimental analysis and flow cytometry data, allowing us to predict subclonal state transition rates driving adaptive resistance. These studies led us to the conclusion that chemotherapy informs a subpopulation of CD44^{Lo} cells to adapt into a CD44^{Hi}CD24^{Hi} drug-resistant phenotype with a capacity to reinitiate tumor development following cessation of treatment.⁷

This phenotype was found to recalibrate over time to a heterogeneous state, revealing the reversible nature of the cell state transition.⁷

In the course of this work we made some unexpected observations. While the classical definition of a breast CSC is based largely on the mesenchymal-like CD44^{Hi}CD24^{Lo} phenotype,⁶ we determined that cells were reorganizing their heterogeneity following exposure to chemotherapy by inducing *both* CD44 and CD24. Excitingly, this suggested a phenotypic switch in which cells were shifting into a state that took on some features that were suggestively 'stem-like', yet doing so imperfectly or at least incompletely. These studies have led to new appreciations and insights of the power of cellular plasticity, which can allow reconstruction of survival instincts in cells. Rather than conforming to a classic hierarchical model, this behavior was more consistent with a continuum of phenotypes in which cells can transition imperfectly, incompletely, or with varying requisite features of "CSC-ness" to adapt to and overcome stress. Could there be a new role for non-CSCs in chemotherapy relapse?

Having pinpointed how resistance arises, we sought to unravel our second question: Can we develop translational strategies to target the origins of drug resistance? Through functional studies and analyses of molecular mechanisms, we

*Correspondence to: Aaron Goldman; Email: goldman1@mit.edu

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Figure 1. Harnessing the phenotypic plasticity of malignant cells for anticancer therapy with temporally sequenced drug schedules. Intratumoral heterogeneity can be defined by varying expression profiles of CD44 in tumors. The application of a primary therapy (i.e., docetaxel) shows efficacy in most cancer cells of a single population, leading to cell death. However, small subsets of cells are instructed through phenotypic plasticity to transition into a temporally altered cell state by expression of cell surface proteins cluster of differentiation 44 and 24 (CD44 and CD24, respectively) as well as phosphorylated (active forms) of proteins in the SRC family of kinases (SFKs). The acquisition of this phenotype is time dependent, and results in addiction to SFKs. This temporal alteration in phenotype creates a brief window following docetaxel treatment in which attacking these persisting cells with SFK-targeted drugs such as dasatinib is most effective.

determined that cells that adapted into the CD44^{Hi}CD24^{Hi} drug resistant phenotype developed a rewired cortex scaffold of kinases leading to a prosurvival response.⁷ This scaffold was built from membrane lipid rafts between caveolin-1, CD44, CD24, and a SRC family kinase (SFK) protein known as hemopoietic cell kinase (HCK).⁷ We discovered that HCK was suppressing apoptosis through nuclear translocation, resulting in a “signaling addiction” to this survival pathway and unmasking a vulnerability to SFK-targeted

inhibitors.⁷ We soon discovered that timing the sequence of therapies could be leveraged to transition cells into the temporally vulnerable phase by the application of cytotoxic chemotherapy, thus offering a window of opportunity to then target HCK with established pharmacologic agents. A summary of this phenotypic transition and the clinical impact of a temporally sequenced drug schedule is detailed in **Figure 1**. By obeying the time-dependent nature of phenotypic plasticity and unmasking signaling addictions,

temporally-constrained sequential drug therapies significantly increased the effect of combination therapy and extended the survival of mice bearing mammary tumors.⁷

Computational Biology as an Emerging Tool to Understand Chemotherapy Relapse

To perform these studies we utilized computational and mathematical modeling, a tool that is often overlooked in biology, in order to understand cell behavior. In our recent work this approach enabled a clear understanding of how intratumoral heterogeneity contributes to adaptive chemotherapy resistance—events that lead to therapy relapse. We discovered that transition rates between phenotypes are instructed by the application of chemotherapy, thus uncovering a role for subclonal populations in chemotherapy resistance. Indeed, experimental analysis alone cannot elucidate the behavior of cancer cells under therapeutic pressure, and computational modeling therefore becomes a valuable asset to provide new insights into rational drug scheduling. The use of mathematical modeling extends beyond these studies and we are now using this resource to develop treatment schedules for clinical implementation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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