Navigating the Challenge of Tumor Heterogeneity in Cancer Therapy

Clare Fedele1,2,3, Richard W. Tothill1,3, and Grant A. McArthur1,2,3,4

Summary: The future of cancer treatment lies in personalized strategies designed to specifically target tumorigenic cell populations present in an individual. Although recent advances in directed therapies have greatly improved patient outcomes in some cancers, intuitive drug design is proving more difficult than expected owing largely to the complexity of human cancers. Intratumoral heterogeneity, the presence of multiple genotypically and/or phenotypically distinct cell subpopulations within a single tumor, is a likely cause of drug resistance. Advances in systems biology are helping to unravel the mysteries of cancer progression. In this issue of Cancer Discovery, Zhao and colleagues define a path for functional validation of computational modeling in the context of heterogeneous tumor populations and their potential for drug response and resistance. Cancer Discov; 4(2); 146-8. ©2014 AACR.

see related article by Zhao et al., p. 166 (5).

As first proposed by pioneers of cancer evolution theory, such as Nowell and Vogelstein, cancer results from the sequential acquisition of heritable genetic and epigenetic events, which under selective pressures leads to propagation of dominant cancer cell populations (1, 2). Although we usually think of cancer as having a dominant clone, it is becoming increasingly apparent that the propensity for cancer evolution can also lead to significant genetic heterogeneity in an individual patient’s cancer (3). This is perhaps the greatest challenge to the concept of personalized cancer medicine, as one drug targeting a single genetic driver may not be sufficient for adequate disease control. This concept is not new, and the use of multiagent chemotherapy may prevent the emergence of drug resistance (4), just as multiagent antibiotics prevent the emergence of resistance in tuberculosis. The advent of new technologies such as next-generation sequencing, recently approved by the U.S. Food and Drug Administration (FDA), is now providing the tools to accurately define the genomic landscape of an individual patient’s cancer and measure heterogeneity. This approach offers the potential to rationally target an individual patient’s cancer.

One simple concept of heterogeneity is the existence of subclones of resistant cells, such as BCR-ABL acute myelogenous leukemia (AML) with a subpopulation of cells with T315I mutations, or BRAF-mutant melanoma with a subpopulation of cells with NRAS mutations. In this model, ABL or BRAF inhibition kills the sensitive cells, allowing the outgrowth of resistant cells. The solution, then, is to inhibit the resistant cells upfront with a T315I inhibitor or the combination of a BRAF and MAP-ERK kinase (MEK) inhibitor that kills BRAF/NRAS-mutant cells. However, this is a simple case of oncogene addiction with the straightforward approach of turning off the signaling of an oncogene. In reality, cancer can have complex mechanisms of drug resistance, including antiapoptotic signals and alterations to cell-cycle checkpoints or mechanisms of DNA repair. So, how can we deal with such biologic complexity that overlies the inherently heterogeneous nature of human cancer? The answer may come from providing the road map of an individual patient’s cancers using sophisticated genomic and epigenetic interrogation of a cancer coupled with functional knowledge of how the heterogeneity can be targeted. Is it then simply a case of adding together drugs that each alone or in synergy kill the major subpopulations of cells? The study of Zhao and colleagues (5) in this issue of Cancer Discovery indicates it may not be that simple.

Zhao and colleagues (5) address the impact of intratumoral heterogeneity on treatment response using computational modeling and functional validation in Eμ-Mycp19ε/ε−/− mouse lymphoma cells, a well-characterized model of human MYC-driven lymphoma. This current study builds on previous work by this laboratory, which determined the responses of homogenous short hairpin RNA (shRNA)–expressing Eμ-Mycp19εε−/− cell populations to combination therapies (6, 7). By using this dataset of drug–genotype interactions, Zhao and colleagues (5) devised a mathematical algorithm to predict the response of known mutant-cell populations to two-drug combination therapies. To phenocopy intratumoral heterogeneity, three-component tumor populations were generated by combining two shRNA-expressing subpopulations...
with parental cells. Interestingly, although most preclinical drug development studies use sensitivity as readout of drug efficacy, here, prevention of subclonal resistance was the desired outcome. Computational simulation of different tumor compositions was undertaken and optimal combination therapy was systematically predicted. Through these analyses, Zhao and colleagues (5) discovered a key finding: that the optimal treatment for a heterogenous tumor may not necessarily incorporate drugs that most efficiently kill each population separately.

In one such case, homogenous cell populations expressing either Chk2- or Bok-targeted shRNAs (shChk2 or shBok) were not predicted to have optimal sensitivity to SAHA treatment alone or in combination with vincristine (Vin). However, when resistance was taken into consideration, a heterogeneous tumor composed of both subpopulations was predicted to optimally respond to Vin/SAHA treatment. Therefore, the overall composition of a tumor may dictate the best treatment option, and this may not necessarily follow an intuitive rationale if considering drug sensitivity alone.

On the basis of these findings, functional validation was performed in vitro. As predicted computationally, treatment of heterogeneous shChk2/shBok cell populations with Vin/SAHA resulted in efficient killing of shBok cells while preventing shChk2 population outgrowth. In contrast, treatment with an alternate strategy systematically predicted to be least effective [irinotecan/chlorambucil (IRT/CBL)] resulted in strong selection and outgrowth of a resistant shChk2 population. The efficacy of Vin/SAHA treatment was maintained even when an additional level of genetic complexity was introduced into the system through inclusion of a cell population expressing both shChk2 and shBok. As expected, drug combinations predicted to optimally target each cell subpopulation individually were not as efficient in the context of population heterogeneity.

The authors further validated their model in vivo. shChk2 and shBok Eμ-Myc:p19Arf-/- lymphoma cells were cotransplanted into immunocompetent mice, followed by systemic treatment with different drug combinations. As predicted, the combination of Vin/SAHA successfully prevented selective outgrowth of any subpopulation. Significantly, tumor-free survival was also improved compared with IRT/CBL treatment. By experimentally determining the responses of homogeneous tumors to drug combinations in vivo, the authors then expanded their analyses to computationally predict the in vivo response of three-component heterogeneous tumors with all possible subpopulation proportions (i.e., 0%–100%). Interestingly, systematic modeling predicted that even the presence of a low-abundance cell variant (0.1% of the overall population) is sufficient to affect the overall tumor response to treatments.

These studies highlight the potential power of computational approaches in modeling and predicting biologic behavior. Over the last decade, advances in systems biology have greatly enhanced our understanding of complex biologic systems, in particular human cancers. Intratumoral heterogeneity presents a significant challenge to the development of effective anticancer therapies. In the endeavor to develop better treatments, systems approaches are likely to play a fundamental role. Toward this end, large-scale molecular analysis and drug screening of human cancer cell lines have been undertaken, such as the human Cancer Cell Line Encyclopedia and Genomics of Drug Sensitivity studies (8, 9). Zhao and colleagues (5) provide functional validation of such systematic approaches, providing a strong foundation for future predictive computing in preclinical drug development and clinical decision-making. In particular, this study highlights the importance of considering resistance as a significant contributing factor in overall drug efficacy, especially in the context of intratumoral heterogeneity. Currently, very few drugs that show promise in preclinical testing, mostly on homogenous populations of cancer cell lines, actually translate to clinical efficacy (10).

However, the application of systems biology to cancer treatment remains in its relative infancy, and there are major hurdles still to be overcome. Computational modeling requires intimate knowledge of subpopulations present within a heterogeneous tumor. We are only beginning to understand the complexity of some human tumors and the level of genetic and epigenetic heterogeneity present therein, which are extremely difficult to model experimentally. One particularly challenging concept is that cancer is not a static entity and that many tumors can potentially undergo continual genetic evolution, allowing adaptation to new selective pressures such as anticancer treatment. It is unclear how systematic modeling will be able to cope with genomic instability and seemingly random genetic alterations occurring within cancer cells, not to mention the added complication contributed by non-genetic factors affecting reversible tumor cell behavior, including microenvironmental influences such asstromal components and even exosomes.

Notwithstanding the biologic complexities of modeling cancer overall, an additional technical challenge in clinical translation is considering to what extent a single biopsy will be sensitive enough to identify all subpopulations present within a tumor. This may be particularly difficult in solid cancers. Liquid biopsies, including circulating tumor cells and cell-free tumor DNA, could be part of the answer as they will allow continued monitoring of cancer progression without the need for more-invasive tissue biopsy. As we build large compendia and knowledge on drug-to-genotype relationships, focus must therefore also be given to better detection strategies.

But even in the face of such challenges, the potential power of computational modeling cannot be underestimated when considering the future of personalized cancer treatment. Knowledge of a patient’s tumor composition may allow for systematic prediction of therapy response, based on preestablished drug-genotype matrices, that permits clinicians to make rational decisions on treatment strategies (Fig. 1). This may even extend to multiple rounds of treatment to sequentially target individual subpopulations that may arise de novo. Currently, next-generation sequencing provides the tool to form a detailed picture of the genetic composition of a patient’s cancer. Although it does not directly give information of the host microenvironment or subpopulations of cancer-initiating cells or cancer stem cells, it does allow description of heterogeneity and the frequency of genetic subpopulations. If these data are integrated with a broad and deep knowledge base of the ability of drugs to synergize to target heterogeneous cell populations, then the goal of a personalized cancer medicine in a significant proportion of cancers will be within our grasp. To reach this goal, we...
must continue to invest in preclinical and clinical functional datasets of combination drug therapies powered by computational biologic models to provide the data systems needed for personalized cancer medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES

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