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Beyond genetics in personalized cancer treatment: assessing dynamics and heterogeneity of tumor responses

Darren R. Tyson* and Vito Quaranta

Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Cancer has been considered a genetic disease for over a century – ever since von Hansemann first described abnormal chromosomes in cancer cells [1] and Rous demonstrated the ability of transmissible agents (viruses) to cause cancer in chickens [2]. By linking genetic alterations with disrupted biochemical processes, Tatum and Beadle paved the way for others to identify individual genes responsible for oncogenic transformation [101]. Major breakthroughs came in the 1970s when Martin described that a viral gene (v-src) is required to maintain the transformed state of virally infected cells [3] and Varmus, Bishop and colleagues discovered that viral oncogenes have mammalian cell orthologs [4–6]. Subsequent studies determined that many cellular proto-oncogenes are kinases [7–9]. This realization paved the way for the development of targeted cancer therapies that specifically inhibit these oncogenic kinases.

With the advent of high-throughput sequencing and the ability to screen thousands of genes from individual tumor samples, many tumors that were once evaluated solely by their histopathological characteristics are now being genetically characterized in astounding detail. With sufficient numbers of molecularly characterized tumors, statistical correlations have suggested links between specific alterations of oncogenic kinases within distinct tumor types or subtypes [10,11]. Some of these molecular alterations are the basis of successful personalized cancer therapy, including HER2 amplification in breast cancer [12], BCR–ABL translocations in chronic myelogenous leukemia [13], mutations within the kinase domain of the EGF receptor (EGFR) in adenocarcinoma of the lung [14,15] and activating BRAF mutations in melanoma [16]. In each of these cases, specifically targeting the genetically altered kinase has provided significant clinical benefit to patients by prolonging overall survival and decreasing treatment-associated morbidity when compared with conventional chemotherapy. It is thought that the success of these drugs is due to the tumor cells being ‘addicted’ to the oncogenic signaling such that when the oncogenic signal is abrogated, the tumor cells stop dividing or die [17,18]. However, the targeted treatments are not curative and the tumors ultimately progress even in the continued presence of the targeted agents, clearly indicating that not all tumor cells die in response to treatment and setting the stage for resistance. Progression of initially responsive tumors is a major challenge to personalized cancer therapy since the clinical benefit of treatment is generally reported in months rather than years, and many questions remain about how and when tumors recur. Answers to these questions would provide enormous clinical benefit, providing physicians with the information necessary to select optimal patient treatment options.

*To whom correspondence should be addressed: darren.tyson@vanderbilt.edu.

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Genetic variation is a major source of resistance

Tumor progression in the presence of targeted agents has been associated with mutations directly within the targeted kinases that confer resistance [19], as well as alterations in nontargeted genes that are either downstream (e.g., MEK1 mutations [20]) or in parallel pathways (e.g., MET amplification [21]). It is generally assumed that these mutations are the direct resistance-conferring mechanisms and that these mutations are either pre-existing or rapidly develop in response to treatment. Within lung adenocarcinomas harboring activating EGFR mutations, the various resistance-conferring mutations are often represented as a pie chart showing the relative frequency of each specific genetic alteration conferring resistance [14,22].

The most widely accepted view is that genetic variants are the source of the resistant phenotypes, that is, the mutations pre-exist within the tumor or rapidly occur over the course of treatment. These resistance-conferring mutations are the subject of intense investigation since it is thought that progression-free and overall survival will be prolonged by simultaneously targeting oncogenic and resistance-conferring mutations.

It should be emphasized that the paradigm of personalized targeted therapy is a fundamental advancement that is steadily revolutionizing cancer treatment. Although over the last decade this paradigm has developed around tumor genotypes, it is unlikely that a single genetic mutation could ever fully explain tumor progression and response to treatment, especially in light of the remarkable heterogeneity of mutations found within the same tumor [23,24]. Even under favorable circumstances when a genetic mutation predicts response to treatment, such as the presence of activating EGFR mutations in lung adenocarcinoma, one should remember that cancer is a disease of competing clonal lineages derived from single cells. Due to biological noise, the effects of a particular mutation probably diverge from single cell to single cell, ultimately contributing to the overall variability of tumor response to treatment.

Individual cells may respond differently to the same treatment

It can easily be appreciated that, owing to tumor heterogeneity at the level of single cells and their progeny, a change in tumor volume (or tumor cell number) in response to targeted therapies is probably a composite of heterogeneous single-cell responses that include induction of cell death, entry into quiescence (or other nondividing states) and altered cell division rates. To properly account for drug action, these fractional responses within a cancer cell population should be quantified, ideally at multiple time points. Yet our understanding of how cancer cells respond to anti-oncogene treatment remains relatively rudimentary, often defaulting to the average response of a population of cells at single or few time points [25]. Alternative approaches to simultaneously examine multiple cell fates by direct single-cell visualization in the presence of drugs require time-lapse video microscopy, but have long been thwarted by low-throughput and labor-intensive analysis. These challenges have now been largely overcome owing to the development of commercially available instruments for high-throughput microscopy, major improvements in computational power and the development of analytical approaches that facilitate the interpretation of time-lapse imaging data in terms of the rates of various cellular fates (cell division, death and entry into quiescence) [26,27]. Several studies have demonstrated significant variability of single-cell responses to treatment within a genetically constrained cell population [26,28–31], highlighting the importance of studying the nongenetic basis of response variability.

Drug responses are dynamic and different cell fates can occur at different rates

Although it may be obvious that the induction of multiple cell fates can contribute to the overall response of a tumor to targeted therapy, the rates at which these cell fates are induced can vary dramatically to different agents, even for drugs that appear to have similar overall effects on a population of cells. This is particularly relevant in the context of determining the concentration of drugs that reduce tumor cell proliferation in vitro (e.g., the concentration that inhibits the growth of a tumor cell population to 50% of untreated cells [GI50]) and linking these effects to genetic variation. Numerous studies aimed at determining the link between genotype and response to drugs using the GI50 value as the relevant metric of drug efficacy toward a target tumor cell population are underway. A major assumption of these studies is that GI50 values directly correlate with the effectiveness of a drug to inhibit (or activate) its target – essentially that the GI50 values of a particular drug in different cellular contexts represent the concentrations of a drug that produce the same overall effect in each cell type. This is a tenuous assumption, since similar effects on the overall size of a tumor cell population can be mediated by very different effects on individual cells within the population. This has been shown to be true both theoretically and experimentally by assays in which the same reduction in tumor cell number can be achieved by reducing cell division rates, increasing the rate of cell death, increasing the rate of entry into quiescence, or a mixture of all three. The newly described quiescence-growth model of cell proliferation incorporates all three of these rates to predict how cell populations will change in response to drugs that affect any or all of these rates simultaneously [26]. Importantly, endless combinations of these three rates can produce a nearly identical effect on the cell population size [26]. Thus, without directly quantifying the true cellular effects of a drug (e.g., amount of cell death, quiescence and division) comparisons of the effects of drugs across cell lines (or patient tumors) will be extremely difficult to interpret. Fortunately, several tools are now at our disposal to quantify these parameters in a high-throughput manner [26,27].

Relationship between ex vivo & in vivo studies remains a challenge

An important challenge toward optimizing personalized cancer therapies is linking response variability quantified at the cellular level ex vivo to the dynamics of tumor responses in patients. Increasingly, treatment responses in patients are examined longitudinally using advanced imaging techniques [32] to obtain the dynamics of response [33]. These studies, although suffering from relatively low resolution (single-cell resolution is probably years away) are nonetheless producing accurate predictions of overall response at the tumor level. However, very little is known about the cellular changes that associate with noninvasive in vivo imaging, which does not have sufficient spatial resolution to distinguish individual cells. Clinical management of cancer patients would be greatly facilitated by an ability to predict how long a particular targeted therapy would remain effective and what alternative treatment options could be applied. In this regard, mathematical models would be particularly useful.

Mathematical modeling & cancer systems biology can be applied to patient care

Mathematical models – essential tools in cancer systems biology [34–38] – have been developed to bridge the cellular and imaging scales [32,39]. Mathematical models provide discrete quantitative hypotheses about relationships, in this case, between cellular behavior and in vivo imaging data, and can be used to make specific quantitative predictions about

responses to treatment across temporal and physical scales (e.g., from genes to cells, tissues to organisms). By iterating between refining the mathematical models and performing experimental studies to test the hypotheses generated from the models, our understanding of multiscale relationships will continually improve, which in turn will allow more accurate predictions of effective treatment and outcomes. Models describing treatment outcomes in terms of response dynamics (both linear and nonlinear) should be the most relevant since the principal metrics for treatment comparison are themselves dynamic, for example, progression-free and overall survival time.

Conclusion

Correlating the tumor phenotypes with specific genetic alterations has paved the way for major success with targeted cancer therapies. However, these are the early days and patients are not cured by targeted drugs. An enormous amount of resources are being invested toward investigating the genetic diversity across and within patient tumors in hope to garner insight on the most efficacious personalized treatment. However, the distance in temporal and physical scale between genetic alterations and tumor drug responses are daunting. We suggest that genetics and tumor outcomes could be more effectively bridged by a better understanding of an intermediate scale – the dynamics and variability of responses occurring at the single-cell level. Since individual cell behavior is the ultimate arbiter of overall tumor response to treatment, we further suggest that accurate predictions of patient outcomes can be provided by mathematical models populated with data from assays that measure both the heterogeneity and the dynamics of drug responses in a cancer cell population. With these dynamic assays of heterogeneous drug response, we envision using genetic screening to provide a patient-tailored list of candidate targeted therapies that could then be directly ranked for efficaciousness on patient-derived tumor cells – essentially a personalized clinical trial.

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