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Integrating genomic information and signaling dynamics for efficient cancer therapy

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Abstract

The field of cancer systems biology has made great strides in understanding oncogenic pathway signaling and enumerating mutations involved in oncogenesis. However, application of these datasets to patient stratification, and to the design of personalized therapy, is in its infancy. We review BRAF and BRCA mutant targeted therapy, where patient stratification has had critical, albeit mixed success. We contrast the work on genomic targeted therapy with orthogonal studies on the dynamics of signaling pathways for designing optimal treatment schedules. We suggest that an integrated approach, combining genomic data and the dynamics of signaling pathways, is required for developing pathway specific computational models and for systematic deployment of targeted combination regimes.

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Introduction

Cancer systems biology is the study of how complex homeostatic systems are perverted by alterations to signaling networks leading to uncontrolled growth and proliferation. Two main perspectives have dominated this field: a genomic (or more generally OMIC) perspective focused on the identification of common features of cancer samples to identify likely genomic culprits of unconstrained growth, and a mechanistic focus on how specific mutations alter cellular signaling. However, outside of a few important examples, neither of these approaches alone has been generally efficacious in determining how to tailor treatment regimens to specific tumors with known mutations. The limitations of the OMIC and signaling perspectives are complementary, one provides a broad overview and a 'parts list' of potential alterations and the other the details of each genomic irregularities role.

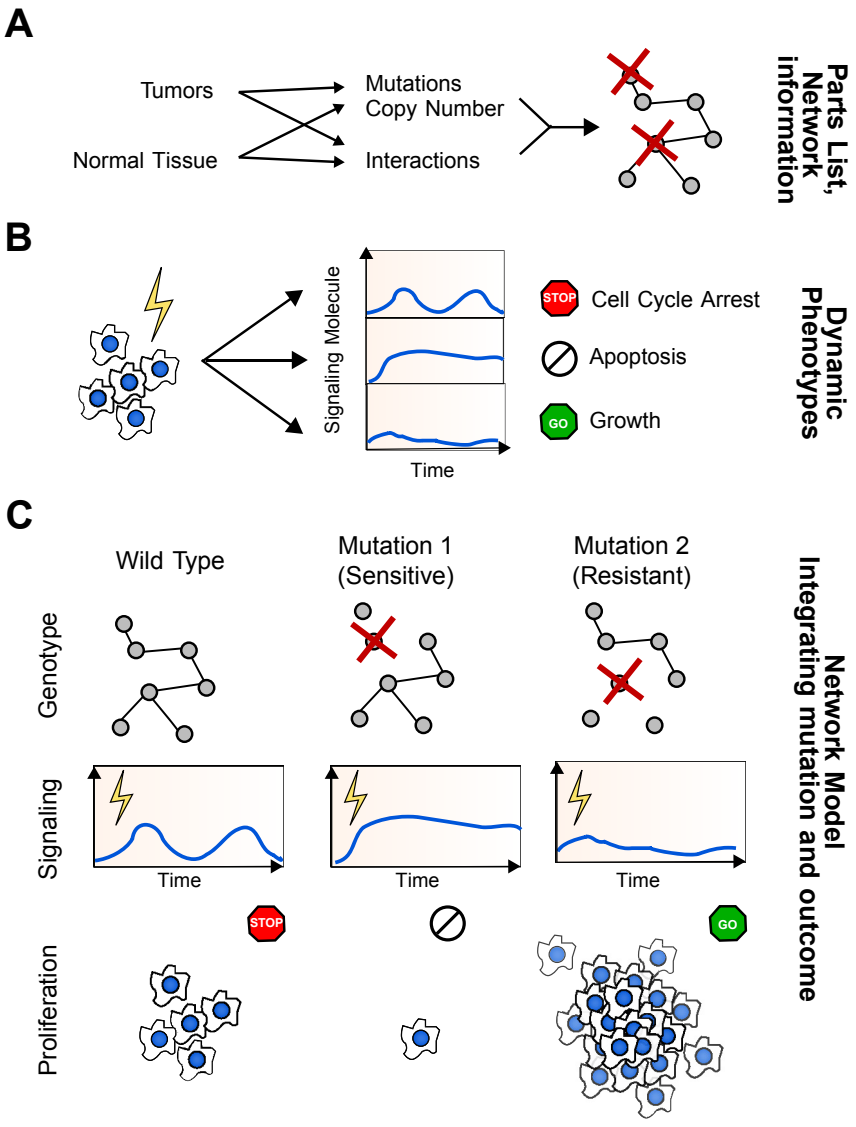
The development of powerful predictive models of disease states and outcomes to therapy require the integration of low and high throughput datasets into genome scale computational and dynamical frameworks. These models will be parameterized with new forms of experimental data, emphasizing the dynamic response of cells to therapy at the level of single cells and population dynamics. Here we will review successes in identifying and characterizing tumor suppressing or oncogenic pathways and suggest ways in which computational and dynamic experimental approaches may make mutation tailored therapy more efficacious.

Genomic identification of frequent mutations and assembly of a 'parts list'

In cancer biology genomic data has largely been treated as observational, with comparisons between normal tissues and cancer derived from these tissues (Fig. 1A). As large numbers of tumors were sequenced in the mid-late 2000s, statistical identification of recurrently mutated genes became possible [1]. One particularly notable success of this approach has been the identification of Isocitrate DeHydrogenase (IDH) mutations as oncogenic in glioma and acute myeloid leukemia (AML). IDH mutations were first flagged as potentially oncogenic due to recurrent active site (H132R predominantly) mutations in the IDH1 gene in glioblastoma [2]. IDH mutations were closely associated with younger patients and better clinical outcomes [3]. Subsequent studies confirmed IDH mutation as oncogenic, with mutations in IDH1 and IDH2 resulting in neomorphic production of the 'onco-metabolite' 2-hydroxyglutarate (2-HG) from alpha-ketoglutarate [4]. Reanalysis of sequencing data from a range of cancers showed that IDH was mutated at low frequency in many tumors and at relatively high frequency in AML [3]. IDH1 inhibitors are undergoing clinical trials for treatment of solid and liquid tumors [5,6].

The discovery of IDH mutation as a common oncogenic alteration illustrates the strengths of unbiased genome wide studies for identifying novel tumorigenic mutations. However, the majority of commonly mutated oncogenes and most oncogenic pathways such as myc, RAS, and PIP3K were identified prior to the era of high

Figure 1



Systems biology approaches to cancer biology. (A) Sequencing data comparing mutations or copy number alterations in normal and tumor samples produce a "parts list" of potentially oncogenic alterations. (B) The dynamics of signaling molecules (middle panel) are measured in single cancer cells in response to DNA damage and correlated with cellular outcomes (right panel). (C) The establishment of new models of cellular signaling networks is required to predict the specific dynamic phenotypes that each mutation may cause, and the phenotypic consequences of such dynamical alterations in response to treatment.

throughput sequencing using older 'genomic' approaches. The first oncogenes were defined by their ability to induce focus (colony) formation in vitro; partially transformed rodent cells were transfected with viruses or cDNA libraries and selected for their ability to aberrantly proliferate [7,8]. These approaches identified the transcription factor cMYC and small GTPase (h) RAS as potent oncogenes, as well as the transforming potential of dominant negative alleles of the tumor suppressor p53, all of which were later confirmed to be frequently mutated in tumor sequencing data [9–11]. Sequencing data is now available for thousands of tumors and analysis of these datasets suggests that relatively few common oncogenes remain to be discovered [12,13].

As OMIC approaches enter a post-discovery era, the goals have subtly shifted towards understanding the implications of identified alterations. Current attempts to use genomic data to inform treatment has had mixed success. One important example is in melanoma, where genomic identification of frequent $BRAF^{v600e}$

mutations, has allowed widespread deployment of small molecule BRAF inhibitor (BRAFi) therapy which show substantial superiority to traditional chemotherapy [14,15]. Other cases have been less clear cut. PARP inhibitors (PARPi) for example, were developed as a synthetic lethal treatment for tumors with a defect in homologous recombination (typically the BRCA1/2 mutations). However, the genomic status of BRCA1/2 or ATM activity is a moderate to poor predictor of drug efficacy [16,17], suggesting that a more complete and complex understanding of how genomic state predicts DNA repair activity of a tumor is required for meaningful stratification of patient populations.

The dynamics of cellular response and its implications for therapy

Increasing the ability of genomic data to predict and improve treatment outcomes requires incorporation of a second strand of cancer systems biology: how cellular systems *dynamically* respond to treatment. DNA damage repair is one of the most highly conserved pathways from bacteria to humans, involving a pause cell cycle progression and the mobilization of cellular resources to repair the damage [18]. In multicellular organisms an additional layer has been added to this regulation, involving the induction of apoptosis when a cell 'perceives' it has received so much DNA damage that a faithful repair is impossible [19]. This combined DNA damage/apoptotic response, and its relative strength and dynamics, determine the degree to which genotoxic therapies are efficacious against tumors and cause side effects in normal tissues [20].

One striking example of the importance of dynamics in the DNA damage response is the oscillatory signaling by the tumor suppressing transcription factor p53 triggered by double strand DNA breaks [21]. These oscillations play a role in fate determination, and manipulation of p53 dynamics results in different cellular outcomes [22,23; Fig. 1B]. Computational models of this pathway have been constructed based on decades of biochemical and genetic data on the p53 system, and can be used to design precise combinations of DNA damage and small molecule inhibitors to modify p53 dynamics and achieve various fate outcomes [22,24]. The response to other apoptotic stimuli, such as Tumor Necrosis Factor, also shows complex dynamic behavior which directly determines the cellular outcome of the stimulus [25].

A more comprehensive understanding of the dynamical response of tumors and tissues to therapy will require a genomic perspective linking treatment to gene expression programs and ultimately to phenotype. Recent works by the Regev and Smale groups on immune cells—dendritic and macrophages, respectively—have shown how high resolution temporal profiling of gene expression after stimulus can reveal both mechanistic insights into gene expression regulation, as well as to the phenotypic response of cells to stimulus [26,27]. The expansion of sequencing for gene expression analysis suggests that these studies will be soon complemented with many others, allowing for a genome scale analysis of the dynamical response of cells to different stimuli and how these expression dynamics relate to cellular phenotypes. Such genome wide measurements of gene expression dynamics will provide new insights and put new stress on the design of computational models.

Towards a model based unification of genomic and dynamic data to design therapy regimes

New approaches are required to use increasingly ubiquitous genomic mutational information to predict tumor specific changes in the dynamics of gene expression and associated phenotypes following therapy. This goal necessitates a quantitative understanding of oncogenic and DNA damage signaling pathways and how they change in the context of cancer with a particular mutational profile. Current approaches mainly aim to link tumor mutations to sensitivity to a specific drug or therapy (in one or multiple cancer types, so called basket trials). For example, PARPi therapy is typically indicated for BRCA1/2 mutated tumors. However, this approach is clearly limited due to the multiplicity of mutations in a single cancer and uncertainly about the interactions between these mutations. Indeed, the sensitivity of BRCA1 mutant cell lines can be suppressed by a second mutation in Rev7 or 53bp1, rendering these double mutant lines resistant to PARPi therapy and demonstrating the need for a more systematic framework [28,29].

To complement PARPi therapy other DNA damage signaling pathways have been explored as potential targets. For example, the blockade of the ATR pathway has been proposed as potentially a potent synergistic complement to PARPi therapy [30,31]. However, these combinations have the risk of greatly enhancing the toxicity of therapy, especially with co-drugging of targets such as ATR that are essential for normal body function [32,33]. This suggests that time dependent therapy, where systemic PARPi or other chemotherapy, is complemented with precisely timed application of DNA damage repair inhibitors such as ATRi or ATMi. The complexities of designing such regimes are formidable, and require quantitative understanding of the kinetics of various DNA damage and repair processes in vivo, as well as the toxicity spectrum of each drug integrated into a model based framework.

The earliest approaches to model designed therapy regimes were focused on radiation therapy and applied quantitative models of the differential response of normal and cancerous tissue to design dose-fractionation

schedules for optimal tumor control [34–36]. Analogously, early efforts in chemotherapy dosage design used phenomenological models of tumor growth to compute the minimal therapy regime to maintain some (low) tumor mass [37,38]. More recently, the design of phase one trials and dose escalation protocols to identify maximum tolerable doses of novel drugs or drug combination have begun to incorporate model based regimes to better estimate these values [39]. These models seek to minimize or eliminate tumor populations, but do not generally take into account response heterogeneity or the emergence of resistance to treatment.

More mechanistic models incorporating biological features of certain tumors, such as heterogeneity of population states and the emergence of resistance, have also been developed. For example, elegant work on optimal dosing strategy incorporating different cellular populations has been done by the Michor group in the context of radiotherapy [40] and on the emergence of resistance to EGFR inhibitor treatment [41]. These models design dosing regimens constrained by the toxicity and feasibility of schedule, and return an optimized and potentially personalized schedule, taking into account, for example, starting tumor burden and patient health status. However, these approaches are not yet flexible enough to predict or integrate drug–drug interactions and generally rely on simplified assumptions about cell killing as the major mechanism of the treatment action.

Models of tumor response to chemotherapy have typically focused on genotoxic compounds where the relatively well understood phenomena of DNA repair and proliferation are the major determinants of efficacy. Beyond genotoxic therapy, the incorporation of molecular details into therapy response models becomes more complicated as many of the targeted therapies hit pathways rich in feedback regulation such as the MAP kinase/ERK pathway [42]. This pathway is commonly mutated in melanoma with the $BRAF^{v600e}$ mutation present in roughly 50% of melanomas [43]. Though often effective initially, resistance inevitably develops to BRAF inhibitor therapy (BRAFi) and interest has therefore grown in combining BRAFi with additional targeted therapy to prevent or slow the emergence of resistance.

Further blockade of the MAPK pathway by inhibition of MEK or ERK [44], and targeting an orthogonal pathway such as Yap [45] have been shown to act synergistically with BRAFi, but both of these combinations were identified with ad hock methods. To develop a rigorous approach for identifying promising drug combinations the Sander's group has used a combination of experimental data and computational modeling to predict active combination therapy regimes [46]. This approach uses a relatively simple interaction based model to predict cell killing and arrest in response to inhibition of various nodes in a model corresponding to proteins or interactions. Critically, this approach is amenable to simulating the effect of multiple simultaneous treatments allowing combinatorial therapies to be modeled. Like other analogous signaling models for TNF response [25,47] and NFKB signaling [48], the Sander's model provides a biologically validated complex model that is amenable to predicting how cellular signaling behaves in the presence of mutations or various stimuli.

We suggest that combining mechanistic models, such as the MAPK model constructed by the Sander's group, with population based time dependent dosing models, such as those devised by the Michor group, has the potential to unify the genomic mutation, dynamic signaling data and therapeutic dosing strategies into a coherent whole (Fig. 1C). In the case of DNA damage and p53 dynamics, for example, it may be possible to combine MDM2 inhibitors, which prevent the degradation of p53, and chemotherapy agents or radiation to synergistically activate p53 signaling. As both MDM2 inhibitors [49] and chemotherapy agents often have substantial myelotoxicity, dosing schemes aimed to increase tolerability will likely be required. Radiotherapy, in combination with MDM2 inhibitors, offers an opportunity to *spatially* segregate therapy aiming strong local activation of p53 at the tumor by the intersection between targeted radiation and systemic MDM2 inhibition. A model based understanding of how p53 dynamics and cellular fate are modulated by DNA damage and MDM2 inhibitors or DNA damage related inhibitors such as ATMi or ATRi would be required to optimally design a treatment scheme. Further, to determine if a given patient would respond, the genotypic state of p53 and its core regulators ATM and CHK2 would need to be incorporated. Targeted therapy against p53 is only one possibility, and more generally we suggests that a mature personalized therapy protocol will integrate genomic information on mutational status with computational models of the dynamics of signaling pathways to identify both optional drug combinations and the relative timing of each drug.

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