COMMENTARY

Metabolic plasticity meets gene regulation

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Tumor metabolism has been investigated as an exploitable avenue for treatment in the last several years. The link between tumor and metabolism traces its origin to a seminal observation by Otto Warburg, almost a century ago, that cancer cells, regardless of oxygen availability, convert most intracellular glucose to lactate—that is, aerobic glycolysis (1). This elevated glycolytic state of cancer cells has been elegantly exploited to detect tumors and their response to drugs by positron emission tomography through the use of fluorodeoxyglucose (2). Originally, tumor cells were thought to have dysfunctional mitochondria, but accumulating evidence suggests that they utilize glycolysis, oxidative phosphorylation, or both, depending on context unrelated to the integrity of mitochondria (3). Additionally, cancer cells can switch their metabolic phenotypes during tumor progression and metastasis and/or in response to external perturbations (4). This metabolic plasticity provides both the energy and the necessary intermediates for biosynthetic processes required for tumor growth. Recently, this "deregulation of cellular energetics" has been recognized as an emerging hallmark of cancer (5).

Although our understanding of metabolic plasticity has increased over the years, the relationship between metabolism and gene regulatory networks (GRNs) remains understudied. In PNAS, using a systems-level approach, Jia et al. (6) explore the links between metabolism and gene regulation. Their key observation is that differential activity of the master regulators AMP-activated protein kinase (AMPK) and HIF-1 give rise to distinct metabolic phenotypes in cancer. Furthermore, based on experimentally validated model predictions, they demonstrate that cancer cells may exhibit additional metabolic states not usually present in normal cells, termed high-high or low-low. This intriguing conclusion challenges the conventional dichotomous classification of tumor metabolism as either glycolysis or oxidative phosphorylation (OXPHOS) and suggests novel avenues of experimentation.

Metabolic pathways are interconnected and flexible, providing tumor cells with the property to reprogram their metabolism and maintain redox balance under changing environments. Such metabolic flexibility in a tumor becomes a clinicians' nightmare, judging from recent therapeutic strategies targeting cancer metabolism that have proved to be largely ineffective. At least in part, these shortcomings may be overcome by considering metabolic pathways and their regulators from a systems perspective. However, the complexity of metabolic network topology can be overwhelming to the systems biologist, due to the lack of experimentally measured kinetic parameters, reactions happening at different timescales, and the convergence of diverse reactions on one metabolite. Furthermore, metabolic network performance may

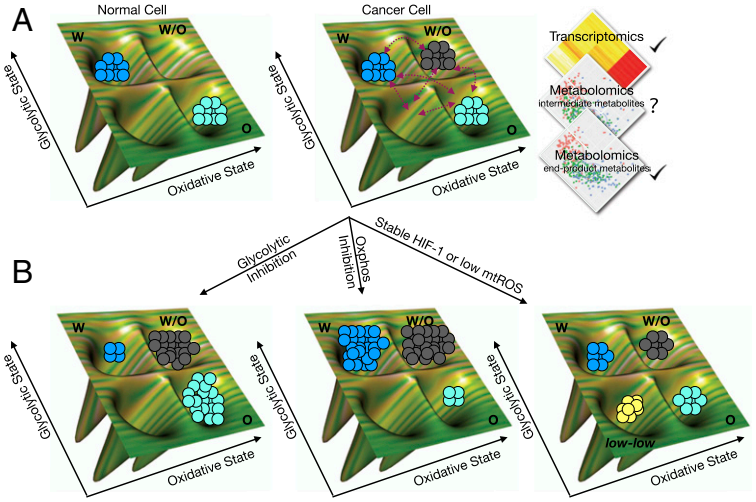

Fig. 1. Metabolic landscape of normal and cancer cells. (*A*) Transcriptomics and metabolomics (end-product metabolites) analyses show the coexistence of three distinct metabolic phenotypes: glycolytic (W), oxidative (O), and high-high, or hybrid (W/O) states in cancer cells, whereas normal cells exhibit only two states. (*B*) External perturbations lead to metabolic reprogramming in cancer cells. (*Left*) glycolytic inhibition increases the O and W/O states. (*Middle*) OXPHOS inhibition increases the W and W/O states. (*Right*) Stable HIF-1 or low mitochondrial ROS (mtROS) induces the metabolic low-low phenotype.

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be heavily biased by GRNs, via differential regulation of enzyme gene expression depending on context.

To render this complexity manageable, a possible approach is to construct a simple framework that reduces the size of an extensive regulatory circuit to essential components, and yet captures its basic principles and overall network behavior. The study by Jia et al. (6) provides a modeling framework which distills complex molecular steps of metabolism into a three-node, coarse-grained network and connects GRN feedback that may regulate each node grouping. They show that a minimum network consisting of the AMPK:HIF-1:reactive oxygen species (ROS) three-node circuit and three metabolic pathways, while greatly reducing chemical reactions to consider, explains key experimental observations and describes the coupling of gene expression with pathway activity. The work builds upon a recent study by Yu et al. (7) that demonstrated the coexistence of three metabolic states (glycolytic, oxidative, and hybrid) in cancer cells, in contrast to normal cells that exhibit only two (glycolytic and oxidative) (Fig. 1*A*). Results show that a hybrid state, specific only to cancer cells, might arise due to cancer cells having different mitochondrial ROS (mtROS) production and HIF-1 degradation rates compared with normal cells. Having identified three clusters from steady-state solutions in cancer cells, Jia et al. (6) proceeded to characterize each metabolic node, and observed unique metabolic pathway dependencies. The glycolytic state, referred to as W state, is characterized by high HIF-1, high glycolysis, low AMPK, and low OXPHOS. The oxidative O state is opposite (low HIF-1, low glycolysis, high AMPK, and high OXPHOS). The hybrid state (W/O) exhibits high activity for all. The W and O steady-state solutions, predicting the association of high HIF-1 with high glycolysis (W) and high AMPK with high OXPHOS (O), are consistent with recent literature (8), providing support for the three-node model. The hybrid state was a novel prediction (7), deserving additional studies that Jia et al. appropriately perform (see below).

The third node in the model, ROS, is not a gene product, but rather by-products of mitochondrial respiration (mtROS) or NADPH oxidase activity. Although understated in their paper, this was an intriguing choice by Jia et al. (6). The cellular response to ROS is thought to be hormetic (9) (i.e., it exhibits biphasic characteristics): at low ROS levels it may elicit sustained network signaling, while at high ROS levels, it induces oxidative stress. Thus, the ROS node in the Jia et al. model likely covers a broad spectrum of cellular processes, quite effectively it would appear, given the realistic predictions from the model. It certainly warrants further, more in-depth consideration.

How stable are the W, O, and hybrid W/O metabolic phenotypes? How do they adapt under perturbations? Given the poor performance of metabolic inhibitors in (pre)clinical studies, there is a growing interest in understanding how cancer cells rewire their metabolism under pressure. For example, recent studies show that a subset of *BRAF*-mutated melanoma cells, insensitive to *BRAF* inhibitors, can activate *MITF*-driven expression of *PGC1a* and hence mitochondrial respiration to evade therapy (10). Others have established that the effects of *BRAF* inhibitors are maximized when melanoma cells are heavily reliant on glycolysis and/or when forced to solely utilize glycolysis by depleting mitochondria (11, 12). Together, these studies suggest that amputating the ability of cancer cells to adapt metabolically might enhance the therapeutic benefits of clinical drugs. To analyze the stability of metabolic phenotypes under external perturbations, Jia et al. (6) utilize their modeling framework and examine changes in phenotypes by varying HIF-1 degradation rate and mtROS production rate. Interestingly, they observe that a more stable HIF-1 (lower degradation rate) gives rise to a higher percentage of the W and W/O states and a lower percentage of the O state (Fig.1*B*, *Left*). In contrast, a high mtROS production rate stabilizes the O and W/O states, while depleting the W state (Fig.1*B*, *Middle*). Both perturbations led to a more stable W/O state, while exhibiting opposite effects on the others. Together, the results reported here could explain initial failures in the use of metabolic inhibitors in (pre)clinical studies and open new research questions into exploring the importance of the W/O state in tumor progression, metastasis, and drug resistance.

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A laudable aspect of Jia et al.'s (6) study is their use of bioinformatics approaches to generate data that inform mechanistic mathematical modeling. In general, one or the other is present in systems biology literature. With the rise in high-throughput "omics" datasets, there is no question that bioinformatics approaches should be the first step in any systems-level project. This coupling will no doubt strengthen our understanding of gene regulation, feedback loops, and networks as a whole. Jia et al. use transcriptomics and metabolomics data from breast cancer (BC) patients to explore activity of the master regulators AMPK and HIF-1 in their model within physiologically relevant conditions.

From previously defined signatures of AMPK and HIF-1 activity, the authors show that key metabolic features of multiple types of tumors could be captured. In particular, the comparison of BC samples with corresponding benign tissue indicates that there is an elevated glycolytic activity in BC samples. Furthermore, there is a significant heterogeneity in both AMPK and HIF-1 activity in BC samples compared with the normal tissue samples. Together, these results suggest that cancer cells exhibit heterogeneity in their metabolic activity, which may form the basis for metabolic adaptation under harsh conditions such as drug exposure.

From the metabolomics screen, Jia et al. (6), however, did not observe specific metabolic states, except that BC samples exhibit a higher abundance of most metabolites. This clear lack of association between metabolite abundance and metabolic activity could be due to the highly unstable nature of many intermediate metabolites and the cross talk between metabolic pathways. The authors show instead that end-product metabolites such as lactate classify BC samples into three distinct metabolic states: W, O, and W/O. They further evaluated the expression of key enzymes to classify metabolic pathway activities and show that three metabolic clusters emerge, with each cluster exhibiting distinct patterns of enzyme expression and a strong association with AMPK/HIF-1 activities, consistent with their model predictions. These findings were consistent even at the single-cell level, which further corroborates the coexistence of distinct metabolic states in cancer cells. To move beyond statistical association, the authors show commitment to validating their model predictions with experiments. Experimentally, they show that cancer cells can switch their metabolism when specific inhibitors are used. For example, the use of mitochondrial inhibitors such as oligomycin induces an increase in glycolytic phenotype, and glycolytic inhibitor

enhances the activity of AMPK and hence the oxidative phenotype. This metabolic plasticity could be thwarted with dual inhibition of both glycolytic and mitochondrial respiration. These results are consistent with the model predictions and underscore the importance of metabolic plasticity in cancer cell survival. Albeit performed in a limited number of cell lines and experimental systems, the experiments are sufficiently convincing so as to consider the model results as biologically plausible. Furthermore, given the widespread interest in targeting metabolism in cancer, such experiments could lay the groundwork for rational design of therapeutic strategies not only for effective drug combination, but also for realizing the ultimate goal of personalized medicine.

Although one can always question the utility of mathematical models, work like this provides a refreshing reminder that novel biological insights and new testable hypotheses could be derived from modeling approaches. Here, the insight is that the W/O hybrid metabolic phenotype, because of the capability of tumor cells to utilize various kinds of nutrients, enables tumors cells to maintain redox homeostasis and support their survival and proliferation, even under unfavorable conditions. Whether the proposed W/O metabolic state applies to multiple cancer types remains to be explored. It would also be interesting to compare whether the W/O hybrid state defines a specific cancer subpopulation such as cancer stem cells.

Another intriguing result is the emergence of the metabolic low-low phenotype, especially when the HIF-1 degradation rate is high or the mtROS production is low (Fig.1 *B*, *Right)*. This metabolic state may be a new state that is drug induced and could describe cancer cell subpopulations that withstand an initial and continued drug challenge, a phenomenon commonly termed drug tolerance. Mostly, drug tolerance is thought to be due to quiescence (13) or senescence (14). More recently, entry of cancer cells into a nonquiescent idling state of balanced division and death was reported (15). It is tempting to speculate that these idling cancer cells may exhibit repressed metabolism (i.e., low-low phenotype), which can be experimentally tested by measuring their levels of glycolysis and oxidative phosphorylation. Several reports point to the nonmutational nature of drug tolerance, and metabolic adaption like the emergence of the metabolic low-low phenotype may provide a mechanistic basis. Whether the metabolic low-low phenotype describes most of the drug-tolerant cancer cells remains to be examined, and given that drug-tolerant populations act as a reservoir from which acquired-resistance genetic mutations arise, functionally characterizing such a phenotype might provide a rationale for therapeutic combinations to eradicate them.

Cancer systems biology is rapidly coming of age. Jia et al. (6) address an important unexplored avenue to enable complex network modeling: a simplified coarse-grained approach to modeling complex metabolic networks, informed by bioinformatics approaches, and validated by experiments. Its utility is supported by novel biological insights that guide additional experimentation. Indeed the work by Jia et al. could have not been a better endorsement for the adage that "all models are wrong but some are useful" (16).

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