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Unraveling the complex regulatory relationships between metabolism and signal transduction in cancer

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Abstract

Cancer cells exhibit an altered metabolic phenotype, known as the Warburg effect, which is characterized by high rates of glucose uptake and glycolysis, even under aerobic conditions. The Warburg effect appears to be an intrinsic component of most cancers and there is evidence linking cancer progression to mutations, translocations, and alternative splicing of genes that directly code for or have downstream effects on key metabolic enzymes. Many of the same signaling pathways are routinely dysregulated in cancer and a number of important oncogenic signaling pathways play important regulatory roles in central carbon metabolism. Unraveling the complex regulatory relationship between cancer metabolism and signaling requires the application of systems biology approaches. Here we discuss computational approaches for modeling protein signal transduction and metabolism as well as how the regulatory relationship between these two important cellular processes can be combined into hybrid models.

Background

Cancer Systems Biology

Systems biology is the integration of theoretical and experimental methods to build a predictive model of a complex biological system. Tumor environments are extremely complex and encompass a large number of cells interacting with a changing microenvironment across a variety of spatial and temporal scales. Cancer systems biology, then, aims to understand the interactions that occur across microscopic and macroscopic scales in a tumor and, importantly, aims to exploit these interactions in a predictive way. Ideally, cancer models build using systems biology methods will have translational significance and can, for example, be used to predict rational therapeutic targets.

Cancer Signaling and Metabolism

Cancer cells exhibit an altered metabolic phenotype characterized by high rates of glucose uptake and glycolysis, even under aerobic conditions. This altered metabolism, first described by Otto Warburg [1], is referred to as the Warburg effect and is so pervasive among cancers that it is routinely leveraged in the clinic with fluorodeoxyglucose-positron emission tomography (FDG-PET). In general, high tumor glucose uptake observed in FDG-PET scans correlates with poor prognostic outcome [2–3]. There is evidence to suggest that

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reliance on non-oxidative glycolytic metabolism sustains the biosynthetic requirements of rapid proliferation [2].

While the Warburg effect appears to be an intrinsic component of most cancer progressions, a precise etiology remains elusive. Both oncogenic signaling [4–5] and interactions with the tumor microenvironment [6] play important roles in the induction of the malignant metabolic phenotype. For example, the activity of the M2 isoform of pyruvate kinase (PKM2), an important glcyolytic enzyme, has been linked to the induction of the Warburg Effect via tyrosine kinase signaling [7–8].

Despite the enormous amount of genetic diversity found within a single tumor and across different cancers, many of the same signaling pathways are routinely dysregulated in cancer cells [9]. Importantly, many of these pathways have important downstream effects on metabolic behavior. For example, the phosphatidylinositol 3-kinase AKT pathway is commonly dysregulated in many human cancers [10]. AKT, a key component of this pathway, is known to play a critical role in stimulating glycolysis [11–12]. In addition, there is evidence linking cancer progression to mutations, translocations, and alternative splicing of genes that directly code for, or have downstream effects on, key metabolic enzymes [13–14].

It should be noted that there is some debate about whether increased glucose uptake translates into increased glycolytic flux and net glycolytic ATP gain in cancer cells [15]. It is possible that a significant amount of the glucose uptake in cancer cells is shunted to pathways other than glycolysis (e.g., to the pentose phosphate pathway). Metabolic transformation, however, is increasingly recognized as an important hallmark of cancer [2,16].

Modeling Intracellular Biochemical Processes

Because it is not practical to create models that are exact replicas of a complex system, trade-offs must be made between the scope and level of detail included in a model [17]. Complex cellular processes are commonly modeled with systems of continuous ordinary (ODE) or partial (PDE) differential equations. ODE and PDE models are built from underlying biophysical principles and, as a consequence, are inherently predictive. The use of continuous ODE based approximations is justified when the system is assumed to be well mixed and the number of molecules of a given reactant ranges from 100 to 1000 [18].

ODE based systems, which are commonly applied to models of protein signal transduction and metabolism, are generally based on mass action and Michaelis-Menten (MM) kinetics [19,17,20–21]. MM kinetics depends on the quasi-steady-state approximation, which assumes that the formation of the complex occurs on a much faster timescale than that of the other reactants. It is important, therefore, to recognize when these assumptions are invalid [22–23].

An alternative to ODE based kinetic models are stoichiometric models where the known structure of a chemical pathway is used to understand the state of the system under a set of specific conditions. Stoichiometric models have demonstrated predictive power using data from prokaryotes. The methods assume an optimization function (e.g., the goal of bacteria is continual production of biomass). Because these methods do not include any regulatory or kinetic information in the model formulation [24], they lack predictive power for multifunctional mammalian cells [25]. In our view, it would be extremely difficult to define an optimization function that adequately captures the complexity of a mammalian cell. Kinetic ODE models will, therefore, tend to be more predictive than stoichiometric methods because they can describe temporal dynamics. Kinetic ODE models require more knowledge

a priori [24] than stoichiometric models, however, and this information is not always readily available.

At the other extreme are discrete logic-based Boolean models which provide a good approximation of the qualitative behavior of a biochemical system [26]. The motivation behind these models comes from the sigmoidal or hyperbolic dependence between regulatory molecules and the compounds they affect that can be thought of as having two states: saturated ("on") and non-saturated ("off"), approximating a Boolean switch. In their simplest form, Boolean models are interaction networks where each biochemical species is represented as a node in one of two possible states: expressed ("on" or 1) or non-expressed ("off" or 0). Transfer functions between states are derived from biochemical interactions using logical operators (e.g., *AND, OR*, and *NOT*). In the transfer functions, there is no notion of reaction rate and, hence, no need to estimate kinetic parameters. Despite this advantage, Boolean models have a major limitation: time is unrelated to physiological time and can provide only, a qualitative chronology of molecular activations [27]. None the less, Boolean models can be important predictive tools in the absence of reliable kinetic data.

Modeling Metabolism

Many ODE based models of glucose metabolism exist in the literature [28–31]. In general, metabolism is considered to be the set of chemical reactions catalyzed by enzymes operating in a living cell that are involved in catabolism or anabolism [19]. Enzymes regulate metabolism by catalyzing reactions [32]. Specifically, an enzyme reacts selectively with a substrate and transforms it into a product. In experimental studies of metabolism, enzyme concentrations are generally assumed to be constant during the catalyzed transformation of substrates into products [33–34]. The majority of ODE based metabolic models have focused on the dynamical behavior of subsets of central carbon metabolism (e.g., glycolysis or the pentose phosphate pathway). In our view, predictive models (especially in the context of cancer) should also consider the nature of the control mechanisms that regulate metabolism.

The most widely used theories of metabolic regulation are biochemical systems theory [35–37], metabolic control theory [38–40] and flux-oriented theory [41–43]. All three of these theories are in essence applications of sensitivity analysis applied to biochemical reaction models. The models consist of coupled ODEs based on the law of mass action. Sensitivity analysis is used to investigate the effects of parameter value changes on model behavior [44]. It is not surprising, then, that the primary difference between these theories is the choice of which parameters to vary when evaluating model sensitivity [45,44].

In biochemical systems theory, the rate constants for the synthesis and degradation of metabolites are usually the parameters chosen for the sensitivity analysis. The metabolites are decomposed into dependent (substrate concentrations) and independent (enzyme concentration) variables where enzyme concentrations generally take constant values [46]. In metabolic control theory, the parameters for the sensitivity analysis are the enzyme activities. The sensitivity analysis gives rise to control coefficients, which are global pathway properties quantifying the control of overall metabolic flux by a single enzyme [45]. Enzyme concentrations are assumed to be constant and reactions rates are treated as constant parameters. Finally, in flux-oriented theory, sensitivities are calculated as the ratio of the relative change of the reaction rate (or flux) in response to a small internal or external stimulus. Enzyme concentrations are generally treated as constants in flux-oriented theory.

The assumption of constant enzyme concentration has been questioned for some time, however [47]. Enzymes are not indefinitely stable; they are metabolites like their substrates and products [19]. The synthesis of enzymes is an essential part of metabolism and is

catalyzed by other enzymes. This phenomenon is known as metabolic closure [48]: all catalysts essential for the survival of an organism must be synthesized internally. While the theory of metabolism-replacement has presented an abstract model of metabolic closure [49–51], it has limited practical applicability for the investigation of metabolic regulation [48] in the biomedical sciences. A theory to investigate metabolic regulation in cancer cells that takes into account enzyme production and depletion is critically needed in medicine.

Modeling Signal Transduction

A number of ODE based models of signal transduction can be found in the literature [20,52,21,53–54]. In contrast to central carbon metabolism, however, significant information about the structure of signal transduction networks is often not known *a priori*. Alternative methods for modeling signal transduction include Bayesian network analysis, Markov models, and Boolean logic based models [55].

As previously mentioned, a number of Boolean network models of gene regulation and signal transduction have generated experimentally valid predictions [26,56,55,57–58]. In its simplest form a Boolean model updates all nodes in a network at the same time, forcing all processes in the network to operate on identical timescales. This assumption results in a deterministic outcome similar to that of cellular automata. Boolean networks can be extended to utilize more biologically realistic variable timescales by performing asynchronous updates where nodes are selected at random and updated instantaneously [26]. Any given Boolean model will have one or more attractors or steady states each associated with a unique set of initial conditions (called its basin of attraction) that converge into that attractor [26,57]. It is, therefore, possible to study the qualitative dynamical behavior of Boolean networks.

We would like to note that it is essential to carefully characterize the interactions included in any logic based model. This is because the signaling dynamics of a network can be very different if an *OR* is used when an *AND* is needed. A detailed survey of the literature is required to build a reliable and robust logic based model. For an example of the level of detail needed to justify each rule in a Boolean model, refer to the appendix in Albert and Othmer [56].

Linking Metabolic and Signal Transduction Models of Cancer

Metabolism and protein signaling do no operate in isolation. Gene expression and protein signal transduction have important downstream effects on metabolism, especially on metabolic enzyme synthesis. It also likely that metabolite levels play a role in the regulation of gene transcription and protein translation.

How can we investigate and analyze the complex regulatory relationships between metabolic pathways and protein signaling in cancer? One possibility is to use large ODE based models of protein signaling and metabolism without any of the simplifying assumptions made in the standard theories of metabolic regulation discussed above. Although this is theoretically possible, it would be a task for Laplace's Demon[1] because it would require a detailed knowledge of every chemical species, every interaction, and all associated rate constants involved in the reactions included in the model.

[1]The idea of a Laplace Demon came from a though experiment proposed by Pierre-Simon Laplace of a perfect entity who would know the precise location of each atom and of all forces in nature at any given moment. This entity (or demon, as it later came to be called) would have incredible predictive power because it could infer the past and determine the future from any set of initial conditions.

In practical terms, if the interactions are known, the law of mass action can be applied to derive an ODE system describing the pathways under consideration. Biochemical reaction dynamics are strongly dependent of parameter values. Central carbon metabolism, which is an essential part of tumor metabolism, has fortunately been well studied and characterized in mammalian cells. As a result, while experimentally and/or computationally intensive, methodologies exist for estimating kinetic parameters for metabolic networks [45]. This is less true of protein signaling networks largely due to their extreme complexity. While a tremendous amount of experimental work has identified a large number of protein interactions involved in both normal and malignant protein signaling, the kinetic details of these interactions are generally not known nor easily obtained. How, then, can we build predictive models that link cancer metabolism and protein signaling? One possibility is the use of hybrid models.

Hybrid Models

Hybrid models link discrete and continuous models across timescales and are widely used in the engineering and computational sciences. In models of tumor growth, cells can be modeled as discrete entities that respond to intracellular and extracellular signals which are modeled continuously [59–63]. For example, Ribba et al. [61] developed a multiscale model that linked a set of discrete models with continuous models of colorectal cancer growth. The model accounted for the cellular, genetic, and environmental factors regulating tumor growth. Key oncogenes involved in colorectal cancer evolution were integrated into a Boolean gene network regulated by a discrete cell cycle model. The response to signals from the intracellular gene network determined whether each cell proliferated or died and, therefore, directly influenced the cellular and the extracellular tissue scales. The spatial distribution of cells was computed using a continuous macroscopic tissue model based on Darcy's law. Finally, the number and spatial configuration of cells were used to activate antigrowth signals, which in turn were input into the Boolean model. This combination of discrete and continuous modeling was used to predict the qualitative effect of therapeutic protocols on colorectal cancer and demonstrated that the efficacy of irradiation protocols depends on the type of anti-growth signals to which tumors are exposed. Thus, a primary conclusion of this work was that the efficacy of irradiation therapy could be improved (without increasing radiation doses) by devising therapeutic schedules that take into account features of tumor growth through cell cycle regulation.

In a recent paper by Singhania et al. [64], a continuous model of the cell cycle was linked to a Boolean gene network model that regulated critical substrates involved in the progression of the cell cycle. By combining a continuous ODE model with a discrete Boolean model, the authors effectively obtained a piecewise ODE model system. In the model, each state was composed of a set of ODEs where specific species or parameters were null (or effectively "off") based on node values in the Boolean network.

In a similar manner, we propose that it is possible to combine ODE based models of metabolism with discrete signaling models. While discrete and continuous hybrid models have been used in cancer research for more than 10 years, we are not aware of any that have directly linked metabolism and signal transduction. To successfully implement a hybrid model of this type, timescale separation will need to be carefully considered.

Comparing average protein half-life with average turnover in the number of enzyme molecules can provide insight into the separation of timescales needed in such a model. An assay of 100 proteins in living human cancer cells showed protein half-life range between 45 minutes to 22.5 hours [65]. The turnover numbers of most enzymes with their physiological substrates range from 1 to 10^5 substrate molecules converted into product molecules per second [66]. Using these numbers, we estimate that enzymes convert between 3.9×10^3 to

1.1×10^{10} substrate molecules into product molecules during their mean lifetime. Thus, due to the large difference in timescales, metabolic enzyme catalyzed reactions can be assumed to effectively operate under a steady-state kinetics. If an enzyme concentration decreases, the steady state kinetics will change from a state of high enzyme steady-state kinetics to a low enzyme steady-state kinetics. Changes between these kinetic states will be driven by signal transduction pathways approximated in the discrete Boolean model.

Conclusion

Over the last 30 years much of cancer research has shifted to focus on molecular features of cancer and away from cancer metabolism and the Warburg effect. As a result, a wealth of experimental data now exists related to the role of gene and protein expression in cancer. Glucose uptake and metabolism are essential features of cancer that, in our view, should be included in system level models of intracellular regulation (and dysregulation) in cancer. Developing theories that integrate this wealth of molecular information with experimental evidence related to cancer metabolism is the domain of cancer systems biology.

Of course, it is not practical to create models that exactly replicate the complexity of a tumor cell. Trade-offs, therefore, must be made between the scope and level of detail included in any model of cancer. Continuous ODE models are useful when kinetic information is available. When kinetic information is not available, logic based Boolean models can be used to understand regulatory dynamics of known interactions from any set of initial conditions. A large number of regulatory interactions have been characterized in human cancers but the kinetic parameters governing the interactions are typically not known. As a result, Boolean models are useful tools for understanding the dynamics of these regulatory networks.

A theory to investigate the regulation of the malignant metabolic phenotype is critically needed. We suggest that hybrid models can be leveraged to integrate discrete Boolean signaling models with continuous metabolic models of cancer. The ultimate goal of such models will be to predict rational therapeutic targets that can be further experimentally validated.

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