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The effects of near optimal growth solutions in genome-scale human cancer metabolic model

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Abstract— **Cancer cells inefficiently produce energy through glycolysis even in ample oxygen, a phenomenon known as "aerobic glycolysis". A characteristic of the rapid and incomplete catabolism of glucose is the secretion of lactate. Genome-scale metabolic models have been recently employed to describe the glycolytic phenotype of highly proliferating human cancer cells. Genome-scale models describe genotype-phenotype relations revealing the full extent of metabolic capabilities of genotypes under various environmental conditions. The importance of these approaches in understanding some aspects of cancer complexity, as well as in cancer diagnostics and individualized therapeutic schemes related to metabolism is evident. Based on previous metabolic models, we explore the metabolic capabilities and rerouting that occur in cancer metabolism when we apply a strategy that allows near optimal growth solution while maximizing lactate secretion. The simulations show that slight deviations around the optimal growth are sufficient for adequate lactate release and that glucose uptake and lactate secretion are correlated at high proliferation rates as it has been observed. Inhibition of lactate dehydrogenase-A, an enzyme involved in the conversion of pyruvate to lactate, substantially reduces lactate release. We also observe that activating specific reactions associated with the migration-related PLCγ enzyme, the proliferation rate decreases. Furthermore, we incorporate flux constraints related to differentially expressed genes in Glioblastoma Multiforme in an attempt to construct a Glioblastoma-specific metabolic model and investigate its metabolic capabilities across different glucose uptake bounds.**

Keywords- cancer metabolism; optimal growth; genome-scale network; in-silico modeling; Glioblastoma Multiforme

I. INTRODUCTION

Otto Warburg first observed in 1924 that cancer cells, as opposed to normal cells, produce a substantial amount of energy inefficiently metabolizing glucose via glycolysis, even in the presence of sufficient oxygen [1]. Normally, in mammalian cells glycolysis is inhibited by the presence of oxygen. The metabolic shift of cancer cells to aerobic glycolysis is characterized by significantly increased glucose uptake and elevated secretion of lactate [2].

Interest in the metabolism of cancer cells has been recently revived [2-6]. Imaging techniques have been developed to detect the increased glucose uptake among other characteristics observed in tumors. These measurements have also been used clinically in diagnosis [7, 8]. R. J. De Berardinis et al. [3] have used 13C–nuclear magnetic resonance spectroscopy measurements to show that glioblastoma cells in culture convert as much as 90% of glucose and 60% of glutamine they acquire into lactate or alanine [2]. Furthermore, Jain et al. [6] recently investigated cellular consumption and release (CORE) profiles of several metabolites across NCI-60 cancer cell lines and showed that highly proliferating cancer cells exhibit important alterations in their metabolism with the common characteristic of incomplete catabolism of nutrients accompanied by secretion of by-products. Analysis of all monitored metabolites showed that total measured carbon consumption was correlated with total measured carbon release.

In order to computationally describe the glycolytic phenotype of cancer cells, Shlomi et al. [4] utilized the genome-scale modeling approaches that have been successfully used in the past to predict the metabolic state of fast-growing microorganisms [9] assuming that cancer cells are under a selective pressure to increase their proliferation rate. In their work, Shlomi et al. [4] used a genome-scale human metabolic network reconstruction [10, 11]. A biomass reaction was introduced in order to describe the metabolic demands for biomass synthesis required for high proliferation rates. By accounting for cellular capacity for metabolic enzymes, their model captures several metabolic phenotypes observed experimentally during cancer development. The model has also been used to predict metabolic-related drug targets for cancer therapy [12].

Based on the metabolic model introduced by Shlomi et al. [4] and by also taking into account the observed correlation between glucose uptake and lactate release, we investigate the metabolic strategy, which maximizes cellular proliferation followed by lactate secretion. Specifically, we investigate the metabolic rerouting that takes place in the generic human metabolic network when we attempt to maximize lactate secretion by allowing near optimal or suboptimal growth

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solutions. Additionally, we explore how oxygen and glucose uptake, lactate secretion and cellular proliferation rate depend on glucose availability. In addition to the generic human cancer model, we also incorporate flux constraints arising from differentially expressed metabolic genes in Glioblastoma Multiform (GBM) [2, 5, 13] in order to generate a glioblastoma-specific metabolic model. The metabolic characteristics of the GBM-specific metabolic model are also explored across increasing growth rates. GBMs comprise the most common and deadly brain tumors; they are aggressive, highly glycoloytic and accumulate the highest levels of lactate in their microenvironment.

There is a wealth of evidence supporting the interdependence of regulatory mechanisms interacting with the extracellular microenvironment and signaling pathways involving known oncogenes and tumor suppressors with metabolism and cellular proliferation [14]. Additionally, these cellular and sub-cellular characteristics of cancer cells both shape and are affected by the host tissue microenvironment playing an important role in tumor morphology, invasion and metastasis [15]. Incorporating microscopic, genotype-phenotype characteristics into well-developed tissue-level, spatiotemporal models [16, 17], comprises an important next step and a challenge for *in silico* multi-scale approaches.

II. METHODS

A. Constraint-based modeling for cancer metabolism

Utilizing a genome-scale metabolic network reconstruction of an organism, constraint-based metabolic approaches model the relation between the genomic information and metabolic activity at flux level (v) avoiding detailed enzymatic kinetics and reveal properties that cannot be predicted by descriptions of individual components [18, 19]. The core assumption of constraint-based models is that the system, constrained by its stoichiometry, S, reaches a steady state (intracellular flux balancing) that satisfies the physiochemical constraints under a given environmental condition (2). Flux Balance Analysis (FBA) further assumes that a cell follows an optimization strategy in order to accomplish cellular tasks, such as maximizing its growth rate (1). FBA requires an upper and a lower bound for the fluxes of some metabolic reactions to be known (3); constraints, which correspond to maximum and minimum rates of the reactions. Exchange reactions drive the uptake of compounds to the cell such as glucose and oxygen or release compounds from the cell such as lactate. Bounds to these reactions reflect substrate availability and are usually set to experimentally measured values. Thermodynamic constraints that determine the reversibility of the metabolic reactions and enzymatic capacity constraints are also usually included to place limits on the range of possible fluxes [20, 21].

$$\text{maximize} \quad v_{growth} \tag{1}$$

$$\text{subject to} \quad Sv = 0 \tag{2}$$

$$v_{\min} \le v \le v_{\max} \tag{3}$$

In order to describe the metabolic capabilities of cancer cells, Shlomi et al. [4] utilized a genome-scale human metabolic network accounting for 1496 open reading frames (ORFs), 3742 reactions and 2766 metabolites [10, 11]. Under the assumption that cancer cells operate towards maximization of their proliferation rate, a biomass reaction that describes the metabolic demands for biomass synthesis was introduced in the metabolic model. They showed that standard FBA method based on stoichiometry alone was insufficient to predict the metabolic characteristics of cancer, but when accounting for cellular capacity for metabolic enzymes, several metabolic phenotypes observed experimentally during cancer development were obtained. The solvent capacity constraint plays a particularly important role when nutrients are in abundance and a global reorganization of the metabolic fluxes has been observed [21].

The flux v_i through a metabolic reaction is proportional to the enzyme concentration E_i and the coefficient of proportionality can be estimated by the enzyme's turnover number, k_{cat_i} [21, 22] as shown in (4). The mass of enzyme i (per mg dry weight (DW) of cells) is given by the product of its molecular weight MW_i and the corresponding flux v_i (expressed in mmol/mgDW*h) divided by the enzyme's turnover number k_{cat_i}. The solvent capacity constraint limits the enzymes allocated in the cell and results in metabolic flux constraints (5). The estimated limit on the total enzyme mass, C, equals to 0.078mg/mgDW [4].

$$v_i = k_{cat_i} E_i \tag{4}$$

$$\sum_i \frac{v_i MW_i}{k_{cat_i}} \le C \tag{5}$$

B. Lactate secretion metabolic strategy

Based on the previously described metabolic model for cancer, we apply a strategy that allows near optimal growth solution while maximizing lactate secretion. The lactate secretion strategy is approached as a two-step optimization problem, similarly to the Flux Variability Analysis (FVA) method [23], which has been used to identify alternate optimal and sub-optimal metabolic states. In the first step, the method solves the previously described optimization problem. Specifically, this step maximizes the cellular growth rate (1) subject to flux balancing constraints (2), uptake bounds for the fluxes of the substrate reactions (3) and the solvent capacity constraint (5). The second step determines the maximal lactate production (6) subject to flux balancing (7), uptake bounds for substrate reactions (8), the solvent capacity constraint (5) and the constraint that the growth rate is not less than a given percentage, k, of the optimal growth rate (9) calculated in the first step. k takes values in [0, 1]. As long as lactate secretion rate is less than a value of tolerance, the second step is repeated for smaller k until a solution is found. As lactate rate is conversely related with cellular growth rate (data not shown), varying k from maximum to lower values, the model provides a solution that is closer to optimal growth. The value of tolerance is set to 0.01umol/mgDW/h.

$$\text{maximize} \quad v_{lactate} \tag{6}$$

$$\text{subject to} \quad Sv = 0 \tag{7}$$

$$v_{\min} \le v \le v_{\max} \tag{8}$$

$$v_{growth} \ge k\, v_{growth}^{optimal} \tag{9}$$

C. GBM-specific metabolic model

In order to construct a glioblastoma-specific metabolic model, we included constraints in the metabolic reactions of the model, which are associated with bibliographically reported differentially expressed metabolic genes in GBM [2, 5, 13]. In general, mRNA levels cannot accurately determine enzyme concentrations as inaccuracies in experiments, post-translational modifications and other effects might occur. However, they can determine an upper bound on the amount of available enzyme [20]. Enzyme levels in turn bound the fluxes of the corresponding metabolic reactions through (4).

In this work, metabolic reactions, which correspond to up-regulated metabolic genes, are constrained to carry non-zero fluxes via a lower bound, whereas down-regulated genes constrain the corresponding reactions via an upper bound. In simulation the lower bound for the reactions associated to the up-regulated genes is set to 0.01umol/mgDW/h, while the upper bound for the down-regulated genes is set to 0. Similar results have been obtained when only the up-regulated genes are taken into account.

III. Results

In this work, glucose serves as the single carbon source in cancer cells. Oxygen and other inorganic compounds essential for human cell growth including sodium, potassium, calcium, iron, chlorine, phosphate, sulfate and ammonia (based on the RPMI-1640 medium definition) are assumed to be in excess in the growth medium. Simulations constraining the upper bound of oxygen uptake show that the metabolic capabilities of cancer cells are not affected by oxygen availability (data not shown). Oxygen and glucose uptake rate, lactate secretion, growth rate and growth yield (growth rate divided by glucose uptake rate) are explored for various bounds in glucose uptake.

Simulations are performed using the COBRA toolbox [24]. The glpk solver [25] is used for solving the linear programming problems.

A. Near optimal metabolic predictions

The first step of the lactate optimization strategy (see Methods) predicts an optimal growth rate for the cell under the specified constraints, which include glucose availability. Considering the trade-off between lactate production and growth rate, the method then determines the maximal lactate production rate subject to the constraint that the growth rate must be as close as possible to the optimal value. Fig. 1 shows that a slight deviation (99%) around the optimal growth rate is sufficient for adequate lactate release when glucose is abundant and drops up to 90% when glucose is scarce.

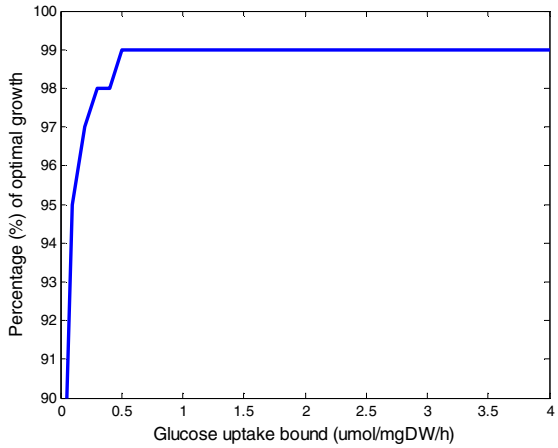

Figure 1. The maximal percentage of optimal growth rate achieved allowing the cell to by-produce sufficient lactate.

The metabolic rerouting that is necessary for lactate production involves the activation of reactions related to mitochondrial and extracellular lactate transport as well as reactions related to the enzyme lactate dehydrogenase-A (LDHA), which mediates the conversion of pyruvate to lactate in the final step of glycolysis. We also observed inactivation of reactions related to NADH transport, HCl/NaHCO3 exchange and reactions implicated in aspartate metabolism. However, the importance of these deactivations must be further explored.

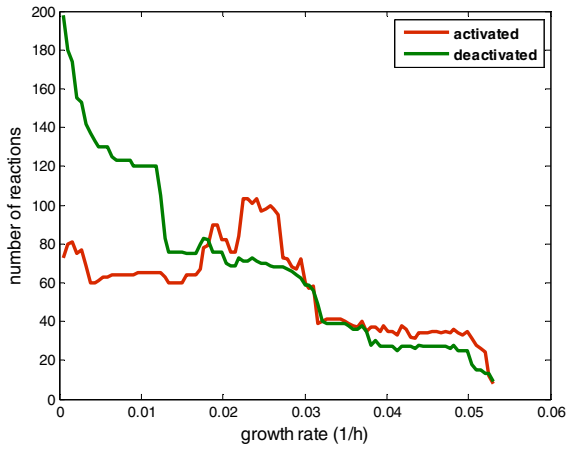

Figure 2. The metabolic rerouting. The number of differentially activated (red line) and deactivated (green line) reactions increases with the demand for higher lactate production permitting lower growth rates.

As expected the number of the differentially activated metabolic reactions increases substantially when switching the optimization strategy from maximizing growth to maximizing lactate by allowing less optimal growth solutions and demanding for higher lactate production (Fig. 2). Flux Variability Analysis [23] has been also performed for these reactions to identify the range of their flux variation.

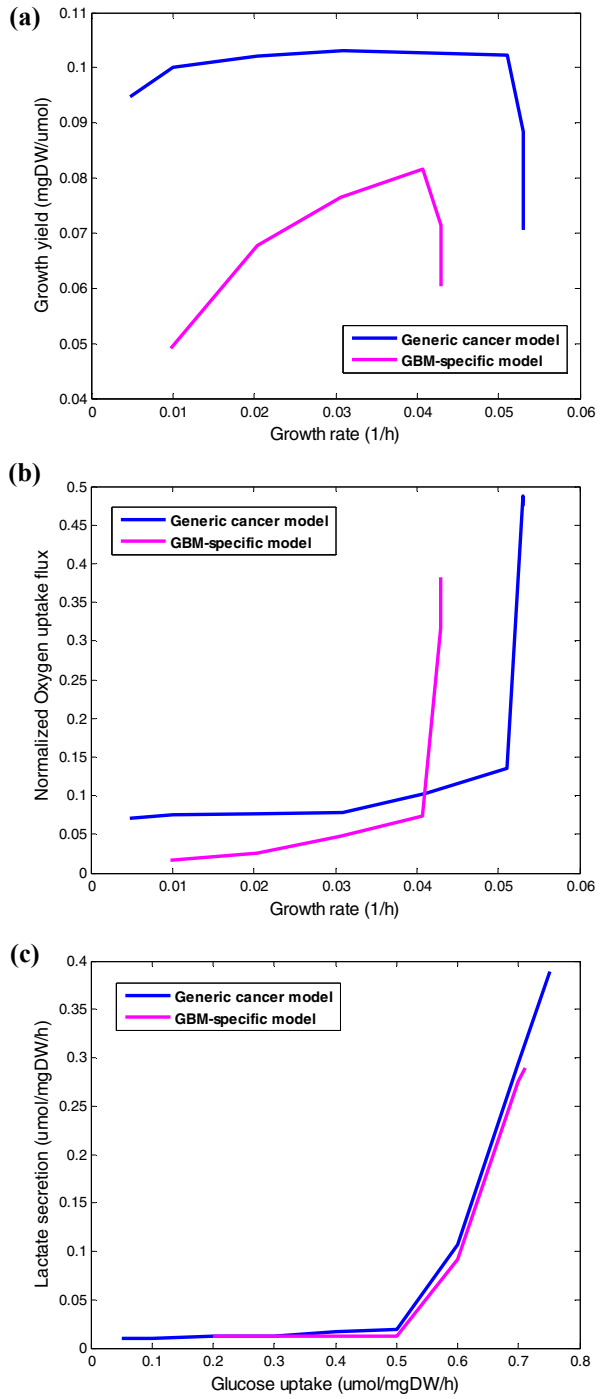

Figure 3. Metabolic predictions across increasing glucose availability of a generic (blue line) and a GBM-specific cancer model (purple line), when incorporating the lactate strategy. (a) Predicted growth yield shows a decline at high growth rates. (b) Predicted oxygen consumption rate (normalized dividing by glucose uptake rate) is increased at high growth rates. (c) Lactate secretion rate versus glucose uptake rate.

As shown in Fig. 3, the GBM-specific metabolic model and the generic cancer model exhibit similar metabolic characteristics with respect to growth yield, oxygen uptake and lactate secretion across increasing growth rates. Specifically, as the cells are shifted from lower to higher growth rates a decline in growth yield is observed (Fig. 3a), the oxygen consumption is increased (Fig. 3b) and lactate secretion is elevated (Fig. 3c) at high growth rates in accordance to the main characteristics of aerobic glycolysis [14]. Furthermore, glucose uptake rate and lactate secretion rate are correlated at high proliferation rates in consistence with the experimental observations [6].

As can be seen in Fig. 3a, the GBM-specific model achieves maximal growth rate, which is less than the maximal growth rate of the generic model. This is explained because the flux constraints that we applied in the generic model in order to construct a GBM-specific metabolic model restrict further the solution space of the allowable fluxes. By definition, the optimal growth rate of any model derived from the generic through the application of additional constraints cannot be higher than that obtained in the generic model.

B. LDHA inhibition affects lactate production predictions

In normal cells, lactate dehydrogenase mediates the conversion of pyruvate to lactate in the final step of anaerobic glycolysis. The increased expression of LDHA in many cancers indicates the metabolic reprogramming of cancer cells and explains the observed lactate accumulation [14, 26]. LDHA is also a direct target of oncogenes such as c-Myc [27] and it has been shown that its inhibition inhibits tumor progression [28]. The simulations show (Fig. 4) that inhibiting LDHA related reactions, reduces lactate secretion in fast growing cancer cells where glucose is in abundance. The proliferation rate of cancer cells remains unaffected (data not shown), which is in accordance to experimental observations under normoxic conditions [26].

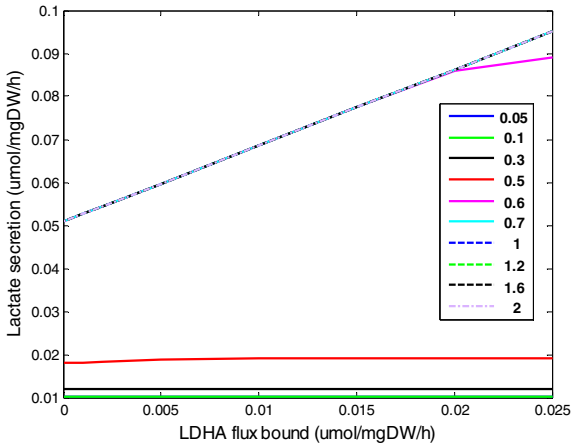

Figure 4. Predicted lactate secretion rates across various bounds on LDHA related reactions for different glucose uptake bounds as shown in the legend. The predictions are based on the generic cancer model.

C. PLCγ activation affects proliferation rate

The model has also been used to show the effect of the enzyme PLCγ on the proliferation rate of the cell. PLCγ is a molecule that lies downstream of EGFR signaling and has been implicated in cell motility and metastasis in several cancers

including glioma [29] and breast cancer [30]. Setting various lower bounds to the fluxes of the reactions that the enzyme PLCγ catalyzes, we show (Fig. 5) that when glucose in not limited, the growth rate decreases as the corresponding fluxes increase, in accordance to experimental results and the general observation that the rate of cell proliferation is decreased during cell migration [30, 31]. Cell signaling pathways interacting with the extracellular microenvironment regulate metabolism [14], while cellular metabolism in turn plays an active role in coordinating signal transduction [32] indicating that attempts to link signaling with metabolism are very important.

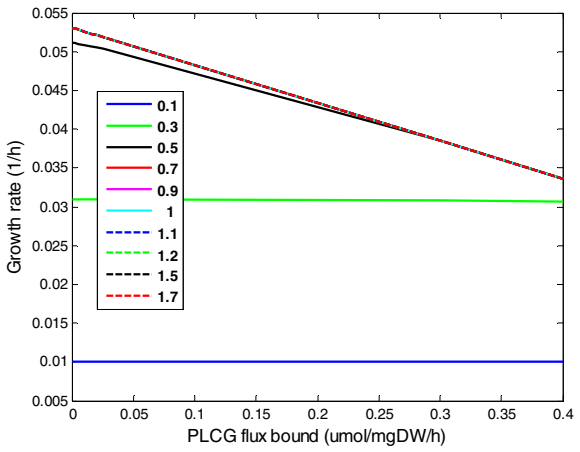

Figure 5. Predicted growth rates across various bounds on PLCγ related reactions for different glucose uptake bounds as shown in the legend. The predictions are based on the generic cancer model.

IV. DISCUSSION

In this work, we study the metabolic capabilities of generic human cancer and glioblastoma-specific cells when we attempt to maximize lactate secretion by allowing near optimal or suboptimal growth solutions. Sub-optimal solutions have been observed to describe the metabolic capabilities of microorganisms under environmental stress and in the absence of sufficient evolutionary pressure [33] indicating that it is not unexpected for biological systems including cancer to show variability around optimal growth solutions. The simulations show that slight deviations (90-99%) around the optimal growth are sufficient for adequate lactate release and that glucose uptake and lactate secretion are correlated at high proliferation rates. The metabolic reactions differentially activated in lactate metabolic strategy include mitochondrial and extracellular lactate transport associated reactions as well as reactions related to lactate dehydrogenase-A enzyme (LDHA). The model shows that inhibiting LDHA related reactions reduces lactate secretion, but the proliferation rate of cancer cells remains unaffected. Furthermore, setting various lower bounds to the fluxes of the reactions that the enzyme PLCγ catalyzes, we show that when the corresponding fluxes increase, the growth rate decreases in accordance to experimental results and the general observation that the rate of cell proliferation is decreased during cell migration [30, 31].

Genome-scale models link metabolism with cellular proliferation, while describing genotype-phenotype relations revealing the full extent of metabolic capabilities of genotypes under various environmental conditions. The potential importance of these computational approaches in understanding the response and adaptation of cancer cells to tumor microenvironment, their metabolic reprogramming and its dependence on signaling and gene regulation, but also their potential role in predicting drugs that target cancer metabolism, is evident. This work demonstrates a few examples towards this direction.

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