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ORIGINAL PAPER

Gibbs free energy of protein-protein interactions correlates with ATP production in cancer cells

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Abstract

In this paper, we analyze several cancer cell types from two seemingly independent angles: (a) the over-expression of various proteins participating in protein-protein interaction networks and (b) a metabolic shift from oxidative phosphorylation to glycolysis. We use large data sets to obtain a thermodynamic measure of the protein-protein interaction network, namely the associated Gibbs free energy. We find a strong inverse correlation between the percentage of energy production via oxidative phosphorylation and the Gibbs free energy of the protein networks. The latter is a measure of functional dysregulation within the cell. Our findings corroborate earlier indications that signaling pathway upregulation in cancer cells is linked to the metabolic shift known as the Warburg effect; hence, these two seemingly independent characteristics of cancer phenotype may be interconnected.

Keywords Gibbs free energy · Protein-protein interactions · Cancer

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1 Introduction

It is well known that oxidative phosphorylation takes place within the mitochondria, and for each molecule of glucose consumed, about 30+ ATP molecules are produced. A proton gradient in mitochondria is required to produce most of the ATP. On the other hand, during glycolysis, a process taking place in the cytoplasm, only two ATP molecules are produced for each glucose molecule molecule consumed. Continued growth and proliferation under hypoxic conditions in cancer cells entails significant energy and material demands. Since the publication of Warburg's foundational paper on cancer metabolism in 1925, much research has focused on the role of glycolysis as the primary source of energy in cancer cells. However, recent reviews [1–3] and meta-analyses of studies on cancer metabolism have shown that many cancers rely heavily on ATP produced from oxidative phosphorylation (OXPHOS). These studies identify a wide range of experiments, including ^{13}C- and ^{14}C-labeled fuel sources [4], to corroborate their conclusions. The work of Moreno-Sánchez et al. [3, 5] and Rodriguez-Enriquez et al. [6] demonstrates high levels of oxygen consumption in tumors, and also points out flawed methodologies in several papers seeming to validate the Warburg effect. Zu and Guppy [2], in particular, emphasize that ATP generation should be tracked from O_2 consumption and lactate production at a minimum, and that studies which fail to track either of these molecular species are inherently flawed, in their perspective, on the roles of glycolysis versus oxidative phosphorylation. They further argue that cancer metabolism is glycolytic only insofar as the environment is hypoxic. Additionally, Wu et al. [7] note that lactate buildup causes a shift back to an oxidative phosphorylation phenotype in numerous cancer cell lines. It is clear that beyond merely a shift from oxidative phosphorylation to glycolytic metabolism, ATP generation in cancer is highly dependent on cell lineage and environmental constraints.

The limitless and self-sufficient proliferative potential exhibited by cancer cells entails significant metabolic demands. Many cell activities carried out during mitosis are very energy-intensive and require considerable stores of ATP, particularly the formation and disassembly of the mitotic spindle and unfolding and refolding of the DNA double helix. The sheer quantity of mass required for cell replication also involves considerable material demands; thus, angiogenesis is frequently observed in cancer tumor environments in order to provide additional nutrient supply through the recruitment of blood vessels. The persistently high rates of energy and material consumption in tumors result in fierce competition for resources with surrounding host cells, which is a driving force behind cancer's invasiveness and eventual lethality. Tumors that reproduce most prolifically have optimized metabolic pathways that seize substrates in their micro-environment and hence pose the greatest danger to the host [8].

Jose et al. [1] argue that it is time for a unifying theory describing ATP production in cancer cells. This more comprehensive theory would include bioenergetic profiles of patients' tumors and thus enable a more personalized treatment of tumors based on specific energetic and biochemical pathways [9, 10]. This manuscript takes a step in the direction of a quantitative understanding of cancer metabolism and calculates a thermodynamic measure known as Gibbs free energy for a number of cancer cell types using available literature data. We then show that the Gibbs free energy of the protein-protein interaction (PPI) networks for these cancer cell types is closely correlated (with an estimated value of $R = -0.716$) with the percentage of ATP contribution obtained from OXPHOS. This provides a mechanistic interpretation of the metabolic energy demands correlating with protein expression data. A high percentage of the OXPHOS contribution is associated with a relatively low level of malignancy, while high values of the over-expressed PPI network's Gibbs free energy indicate the opposite, hence an

inverse correlation relationship. The PPI network complexity as measured by degree entropy has earlier been shown to correlate to a similar degree with cancer lethality [11]. Putting this together links protein expression dysregulation to increased metabolic energy demands with a glycolytic shift as its characteristic and eventually to poor survival prognosis.

2 Methods

The homeostasis of cells is maintained by a complex, dynamic network of interacting molecules ranging in size from a few dozen Daltons to hundreds of thousands of Daltons. A change in the concentration of any one of these may result in alternations of the overall chemical balance. This alteration in chemical balance is usually expressed in thermodynamic terms as Gibbs free energy, *G*. It is one of the fundamental thermodynamic functions of state, which describe the state of a macroscopic molecular system independent of the path or process that has taken the system to this state. This thermodynamic quantity is especially relevant in the context of systems kept at a constant temperature and able to exchange both energy and molecules with the surrounding environment. Gibbs free energy is most readily described in terms of chemical potentials defined for each molecular species represented in the system. If one protein, labeled A, is in high abundance and another protein, labeled B, needed for a specific biochemical reaction involving that first protein is in low abundance, then there is said to be a chemical potential difference between A and B. Chemical potential is defined in terms of the effect it has on the total Gibbs function of a system of arbitrary composition and is given as a molar difference. Its mathematical definition is:

$$\mu_i = \left(\frac{\delta \mathrm{G}}{\delta n_i}\right)_{P,T,j,k,\mathrm{etc.}}$$

The chemical potential is the partial molar free energy of every component in a particular reaction. In the relation above, μ_i is the chemical potential of component i in a system of reactants. In the equation above, it is assumed the molar concentrations of the other components are held constant. In the present context, $_i$ refers to a set of individual proteins $\{i_1, i_2 \ldots i_N\}$ within the PPI network.

Of course, in a biopsy sample, there are a huge number of cells and when the mRNA is extracted, it is considered to represent a well-mixed ensemble. Hence, when one protein, say p53, which is well known to interact with hundreds of neighbor proteins in the PPI network, it is important to keep in mind that we are dealing with an *ideal mixture*. Consequently, no individual p53 is interacting with all its neighbors at the same time. These are statistical properties characteristic of ensemble- or time-averaging outcomes.

In our calculations, we used mRNA transcriptome data as a surrogate for protein concentration. This assumption is largely valid. Kim et al. [12] and Wilhelm et al. [13] have shown an 83% correlation between mass spectrometry proteomic information and transcriptomic information for multiple tissue types. Further, Guo et al. [14] found a Spearman correlation of 0.8 in comparing RNAseq and mRNA transcriptome from TCGA human cancer data (https://cancergenome.nih.gov/).

The values of Gibbs free energy for a cell can be calculated from mRNA expression, a surrogate for protein concentrations, and overlaid on an expression network [10]. The Gibbs values used in this paper were calculated using PPIs from BioGRID, a curated bioinformatics

database (https://thebiogrid.org/). Gene expression data for several cancers were obtained from TCGA (https://cancergenome.nih.gov/).

Given a set of transcriptome data, a representative of protein concentration, we overlay that on the human PPI network from BioGRID. This means we assign to each protein in the network the scaled (between 0 and 1), transcriptome value. From that, we compute the Gibbs free energy of each PPI using the relation:

$$G_i = c_i \ln \frac{c_i}{\sum_j c_j} \tag{1}$$

where c_i is the "concentration" of the protein i, normalized, or rescaled, to be between 0 and 1. The sum in the denominator is taken over all protein neighbors of i, and including i. Therefore, the denominator can be considered a degree entropy. Carrying out this mathematical operation essentially transforms the "concentration" value assigned to each protein to a Gibbs free energy. Thus, we replace the scalar value of transcriptome to a scalar function — the Gibbs free energy. Gibbs free energy of a protein will be some $G_i \leq 0$; the magnitude of which is associated with the amount of that protein bound to interaction partners. Equation (2) below is the formula for obtaining the Gibbs energy of the whole network.

$$G = \sum_i G_i \tag{2}$$

These formulae were implemented in a Python 2.7 script running NetworkX, SciPy, and NumPy packages.

3 Results and discussion

The OXPHOS–ATP production data for this study came from the work of Moreno-Sánchez et al. [6]. Though those researchers cite many types of solid- and cell-line tumors from multiple species, in the interest of consistency, we selected only the human solid tumors for our calculations. These also corresponded to specific tumors from the TCGA transcription data (https://cancergenome.nih.gov/) used for our analysis of the Gibbs free energy values. The paper by Moreno-Sánchez et al. [5] states that the metabolic data were from the center of the solid tumor and the periphery of the same tumor, and the method they used is described at length in Vaupel et al. [15].

Figure 1 shows a negative correlation ($R = -0.716$) between Gibbs free energy and the percentage of ATP derived from oxidative phosphorylation. The graph suggests that certain cancers obtain their ATP predominately from OXPHOS and others from glycolysis.

The extended metabolic pathway used in OXPHOS and the high number of subunits found in the enzymes of this pathway are likely to be significant contributing factors to the observed results in Fig. 1. The networks (from KEGG, https://www.genome.jp/kegg/) for the metabolic OXPHOS machinery are shown in Fig. 2a. By contrast, the glycolysis network is shown in Fig. 2b. The difference between the highly symmetric OXPHOS network geometry and the much less regular glycolytic network architecture is striking.

In the following, we describe several network statistical measures; details of their algorithms can be found in Newman [16]. The first thing to notice about the two networks is the large difference in connectivity. The OXPHOS network has three key nodes: LHPP, PPA1, and

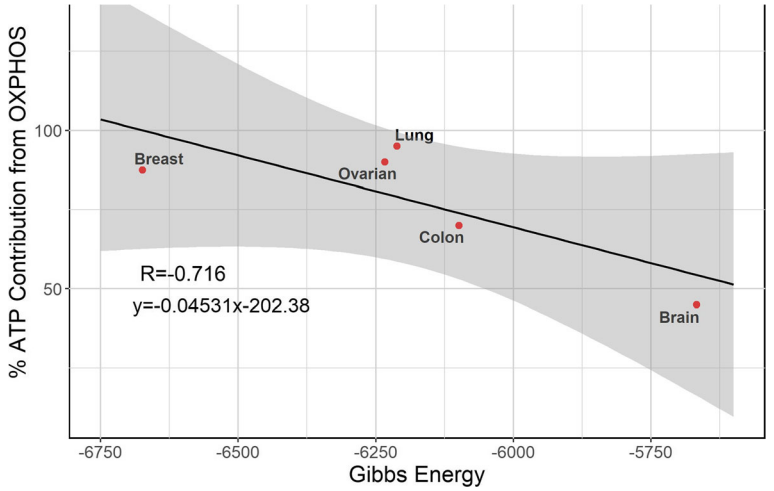


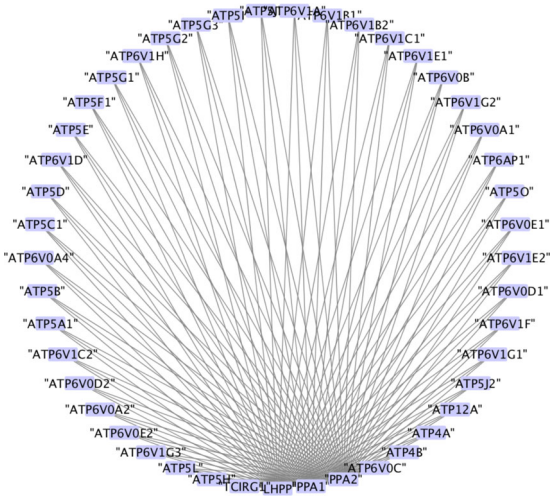
Fig. 1 Cancer metabolism by type. *Y*-axis data refer to the overall contribution to ATP generation from oxidative phosphorylation and were determined from a number of metabolic flux analyses whose results are tabulated in Moreno-Sánchez et al. [5]. The percentages may be contrasted with % ATP obtained from glycolysis. "Lung" refers to lung squamous cell adenoid cancer, and "brain" refers to glioblastoma. The Gibbs energy estimates are in arbitrary units. The gray region represents the 95% confidence interval on the linear regression

PPA2. These three proteins each connect to 42 other proteins to form large molecular machine parts and then these three machines interact for the purpose of ATP production. In contrast, the glycolytic network has many hubs with degrees ranging from 16 to 4. The same network has 66 nodes and 252 edges. The shortest path in the OXPHOS network is 1.05 for the three hub nodes and the remaining nodes have a shortest path of 1.9. On the other hand, the shortest path in the glycolytic network is 2.8 and increasing to 5.8. The clustering coefficient is 0.0 for the OXPHOS and 0.66 for the glycolytic network. Lastly, the betweenness centrality is found to be 0.303 for the three hub nodes in the OXPHOS network and $< 10^{-5}$ for the remaining nodes. In the glycolytic network, the betweenness centrality is in the range from 0.46 to 0.0017 (largest to smallest). These network measures imply that there is a greater amount of free energy utilized in the OXPHOS network relative to the glycolytic network.

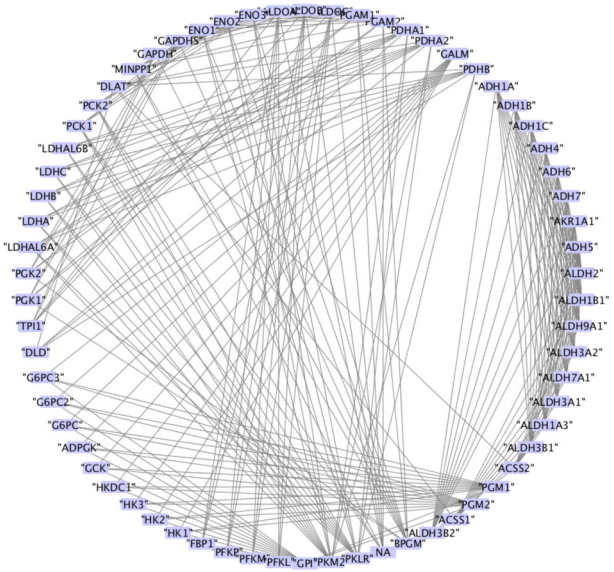
The high number of subunits in OXPHOS enzymes speaks to the intricacy of their operations relative to glycolysis, while glycolysis generates ATP from the group transfer potential of phosphate groups on the substrate. OXPHOS complexes use electron tunneling and proton pumps to establish an H^+ concentration gradient across the mitochondrial membrane. The chemical potential of each individual participant in these complexes may be higher than those in glycolytic monomers and dimers due to their co-expression alongside many interaction partners.

4 Conclusion

According to the Warburg hypothesis, the aggressiveness of a tumor derives from the shift of metabolic energy production from OXPHOS to glycolysis. This shift is considered to be due to the competition between cells preferentially utilizing the glycolytic mode, and cells adopting the OXPHOS mode of energy production. In OXPHOS, the coupling between the redox



a) OXPHOS network



b) Glycolytic network

Fig. 2 Kyoto Encyclopedia of Genes and Genomes (KEGG) networks converted to protein-protein interactions (PPI) networks with KEGGgraph and plotted in Cytoscape. The Appendix includes two tables listing the proteins and the number of connections to each protein, for each network

reactions taking place in the mitochondria and ADP phosphorylation is electrical through the electron transfer mechanism in the mitochondrial membrane protein complexes. Hence, the

metabolic rate is determined by bioenergetic parameters such as the proton conductance and proton potential of the metabolic network. In glycolysis on the other hand, ADP phosphorylation is linked chemically to individual enzyme-catalyzed reactions, so the rate of ATP production is a function of the flux through the pathway as a whole.

However, it is well known that ATP generation through glycolysis is much less efficient than through mitochondrial OXPHOS processes in terms of the number of ATP molecules produced per mole of glucose, but not in terms of free energy produced in a given period of time. Hence, a long-standing paradox is how cancer cells with their metabolic disadvantage can outcompete normal cells. In terms of biochemical reactions [17], mitochondrial respiration defects activate the Akt survival pathway through a mechanism mediated by NADH. Respiration-deficient cells harboring mitochondrial defects exhibit strong dependence on glycolysis, increased NADH, and activation of Akt, conferring a survival advantage [17]. This inter-relation between metabolic and signaling pathways in cancer cells has been indirectly shown in this paper via an inverse correlation between the percentage of OXPHOS utilization and the Gibbs free energy of the corresponding PPI network.

Gibbs free energy is a topological measure on protein-protein interaction networks, which remains to be fully explored in its usefulness for identifying targets for cancer treatment. By targeting proteins based on their participation in multi-subunit complexes, it may be possible to diminish the functionality of entire complexes or pathways using a single customized drug. Drugs targeting proteins with many highly expressed interaction partners should affect the functionality of these partners by proxy, producing a cascade of diminished functionality that may significantly increase the likelihood of cell death. In vivo or in vitro demonstration of this predicted outcome would strongly validate the conclusions of the present paper in the case of cancer metabolism playing a major role in cancer initiation and progression.

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Author contributions EAR and JAT conceived and designed the study. SMG and ANN carried out the computations. All authors contributed to the analysis and to the writing of the manuscript.

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