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Organelles in focus

Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism

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ABSTRACT

Applying basic biochemical principles, this review analyzes data that contrasts with the Warburg hypothesis that glycolysis is the exclusive ATP provider in cancer cells. Although disregarded for many years, there is increasing experimental evidence demonstrating that oxidative phosphorylation (OxPhos) makes a significant contribution to ATP supply in many cancer cell types and under a variety of conditions. Substrates oxidized by normal mitochondria such as amino acids and fatty acids are also avidly consumed by cancer cells. In this regard, the proposal that cancer cells metabolize glutamine for anabolic purposes without the need for a functional respiratory chain and OxPhos is analyzed considering thermodynamic and kinetic aspects for the reductive carboxylation of 2-oxoglutarate catalyzed by isocitrate dehydrogenase. In addition, metabolic control analysis (MCA) studies applied to energy metabolism of cancer cells are reevaluated. Regardless of the experimental/environmental conditions and the rate of lactate production, the flux-control of cancer glycolysis is robust in the sense that it involves the same steps: glucose transport, hexokinase, hexosephosphate isomerase and glycogen degradation, all at the beginning of the pathway; these steps together with phosphofructokinase 1 also control glycolysis in normal cells. The respiratory chain complexes exert significantly higher flux-control on OxPhos in cancer cells than in normal cells. Thus, determination of the contribution of each pathway to ATP supply and/or the flux-control distribution of both pathways in cancer cells is necessary in order to identify differences from normal cells which may lead to the design of rational alternative therapies that selectively target cancer energy metabolism.

1. Basic biochemical concepts

The supply of ATP in mammalian and human cells is provided by glycolysis and oxidative phosphorylation (OxPhos). There are no other pathways or processes able to synthesize ATP at sufficient rates to meet the energy demands of cells. Acetate thiokinase or acetyl-CoA synthetase (EC: 6.2.1.1), a ubiquitous enzyme catalyzing the synthesis of ATP and acetate from acetyl-CoA, PPi and AMP, might represent an exception under hypoxia in cancer cells, although the flux through this branch is negligible (≤10%) when compared to the glycolytic flux (Yoshii et al., 2009).

Glycolysis in human cells can be defined as the metabolic process that transforms 1 mol of glucose (or other hexoses) into 2 moles of lactate *plus* 2 moles of ATP. These stoichiometric values represent a maximum and due to the several reactions branching off glycolysis, they will be usually lower under physiological conditions, closer to 1.3–1.9 for the lactate/glucose ratio (Travis et al., 1971; Jablonska and Bishop, 1975; Suter and Weidemann, 1975; Hanson and Parsons, 1976; Wu and Davis, 1981; Pick-Kober and Schneider, 1984; Sun et al., 2012).

OxPhos is the metabolic process that oxidizes several substrates through the Krebs cycle to produce reducing equivalents ($NADH$, $FADH_2$), which feed the respiratory chain to generate an H^+

Abbreviations: AT, aspartate transaminase; CS, citrate synthase; DHAP, dihydroxyacetone phosphate; GDH, glutamate dehydrogenase; Glc6P, glucose-6-phosphate; GLUT, glucose transporter; HK, hexokinase; HPI, hexosephosphate isomerase; ICD, isocitrate dehydrogenase; Mal, malate; MDH, malate dehydrogenase; ME, malic enzyme; 2-OGDH, 2-oxoglutarate dehydrogenase; OxPhos, oxidative phosphorylation; PFK-1, phosphofructokinase-1; PYK, puruvate kinase; SDH, succinate dehydrogense; Gln, glutamine; Glu, glutamate; IC, isocitrate; OAA, oxaloacetate; 2-OG, 2-oxoglutarate; 3PG, 3-phosphoglycerate; PPi, pyrophosphate; Pyr, pyruvate; Succ, succinate.

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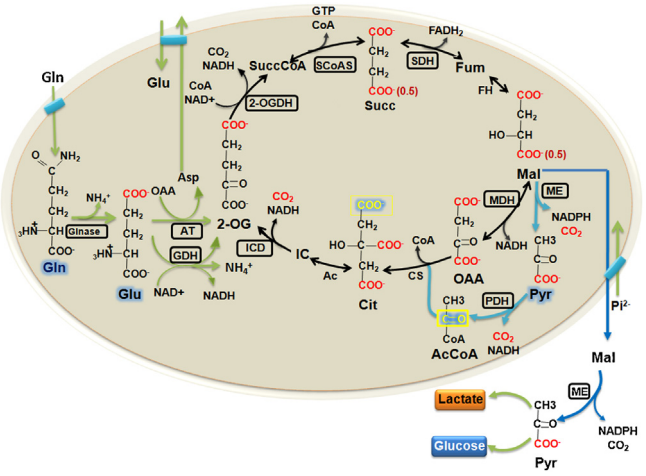

Fig. 1. Labeling patterns of ^{13}C-glutamate or ^{13}C-glutamine mitochondrial metabolism in cancer cells. Red colors indicate the ^{13}C labeled carbons. ^{13}C radioactive isotope labeling distributes equally between the symmetric carbons (0.5) of succinate (Suc), malate (Mal) and oxaloacetate (OAA). Malate is decarboxylated by malic enzyme (ME) to pyruvate + $^{13}CO_2$. Yellow color represents the citrate labeling in C5 (γ-carboxylate) found in the reductive carboxylation of 2-OG by ICD, but which can also be derived from the C1 (α-carboxyate) of acetyl CoA as shown.

electrochemical gradient across the inner mitochondrial membrane that drives ATP synthesis through chemiosmotic coupling catalyzed by ATP synthase. Depending on the oxidized substrate, different stoichiometric values of reducing equivalents are produced. For instance, pyruvate oxidation by the Krebs cycle generates 4 NADH, 1 $FADH_2$, 1 GTP (from substrate-level phosphorylation in the Krebs cycle catalyzed by succinyl-CoA synthetase; GTP is readily transformed into ATP by dinucleotide kinase), and 3 CO_2.

Glutamine (Gln) oxidation (Fig. 1) generates 2 NADH, 1 $FADH_2$, 2 NH_4^+, 1 CO_2, 1 GTP and 1 malate (when glutamate is oxidized) or 1 NADH, 1 $FADH_2$, 1 NH_4^+, 1 CO_2, 1 GTP and 1 aspartate (when glutamate is transaminated); these two reactions catalyzed, respectively, by glutamate dehydrogenase (GDH) and aspartate transaminase (AT) produce 2-OG (Fig. 1) and are functional in normal and cancer mitochondria, prompting significant CO_2 production through the forward Krebs cycle reactions (Kovacevic, 1971; Watford et al., 1980). Malate and aspartate may exit the mitochondrial matrix toward the cytosol in direct exchange with other dicarboxylates or Pi, and glutamate, respectively. Malate may continue its oxidation through the malic enzyme reaction in both the mitochondrial matrix and cytosol generating pyruvate, CO_2 and one extra NADPH. In the cytosol, pyruvate may in turn serve for formation of lactate (*i.e.* glutaminolysis) and glucose (*i.e.* gluconeogenesis); in the mitochondrial matrix, pyruvate is transformed to AcCoA for further condensation with OAA for citrate formation.

It should be noted that to maintain steady Gln transformation into glutamate (Glu), 2-OG, Mal and OAA, re-oxidation of the reducing equivalents produced by GDH, 2-OG dehydrogenase (2-OGDH) and malate dehydrogenase (MDH) is required (Fig. 1), *i.e.* the respiratory chain functioning is essential for efficient mitochondrial Gln metabolism which in turn leads to net ATP synthesis in cancer cells (Lazo, 1981; Kovacevic et al., 1987; Guppy et al., 2002). In this regard, it has been documented that increased activities exist for tumor mitochondrial AT (1.4 times; Sheid et al., 1965), malic enzyme (10–20 times; Moreadith and Lehninger, 1984), 2-OGDH and MDH (1.4–1.6 times; Dietzen and Davis, 1993), and the c-Myc upregulated Gln transporter (4–10 times) and glutaminase (10–20 times; Molina et al., 1995; Matsuno and Goto, 1992), all much higher than those of their original normal tissue counterparts. These changes readily explain the increased mitochondrial Gln metabolism in cancer cells (Reitzer et al., 1979; Rodríguez-Enríquez et al., 2006; DeBerardinis et al., 2007).

1.1. The problems of cancer cell metabolic analyses made only using molecular biology approaches

We are aware that the Biochemistry text book descriptions and explanations of the previous paragraphs may be judged by some as not useful and representing irrelevant knowledge. It is most unfortunate that in many recent studies on cancer biology, there has been a reliance predominantly on the analysis of gene expression or protein content levels in order to reach conclusions about biological functions and processes in tumor cells. It is becoming increasingly apparent that it is not valid to make inferences about biological functions based on determinations at only one level of regulation, *i.e.* gene expression (mRNA content or transcriptome). Very often variation in mRNA content is not proportional to changes in enzyme/transporter protein content (proteome) and activity (kinetome), and much less to changes in metabolic flux (fluxome).

Analyses based only on transcriptional/proteomic changes, without making the actual assessment of their impact on the pathway or cellular function of interest may be informative, but they do not provide complete understanding of the molecular mechanisms regulating these biological processes. Regrettably, in the field of cancer biology such generalizations are commonplace (for example, see Penta et al., 2001; Brandon et al., 2006; Chatterjee et al., 2006; Hu et al., 2013). Furthermore, variations in activity of a single enzyme/transporter most of the time does not elicit a proportional change in pathway fluxes (fluxome) or metabolite concentrations (metabolome), unless the affected protein has a significant controlling role on these metabolic variables (Moreno-Sánchez et al., 2008).

Until recently biochemical (including metabolic and kinetic) experimentation in cancer biology was considered "old-fashioned", "laborious" and "technically difficult", and only a few studies have properly analyzed enzyme/transporter activities and pathway flux rates, *i.e.* actual biological functions. However, with the advent of improved technologies on respirometry and gas/liquid phase chromatography coupled to mass spectroscopy, biochemical analyses and approaches are now becoming fashionable again. This resurgence is also possibly as a consequence of the poor outcomes in clinical cancer treatment that have developed based solely on genetic approaches and the lack of understanding of the molecular mechanisms involved in cancer development. Thus, cancer metabolism and bioenergetics are currently being considered as alternative and more suitable targets which require solid comprehension at several levels of cellular regulation: mRNA, protein content, protein activity, and pathway/cellular process function. Moreover, a survey of the cancer literature reveals misuse and misunderstanding of some fundamental concepts of basic biochemistry. Therefore, it appears necessary to clearly restate the biochemistry of, and alterations that occur in, cancer cells.

2. Cancer energy metabolism and the Warburg hypothesis

There is no doubt that many of the more long term established cancer cell lines as well as solid tumors and blood cancers do exhibit enhanced glycolysis as compared to normal cells (reviewed in Moreno-Sánchez et al., 2007, 2009; Marín-Hernández et al., 2009). In tumor cells, 60–90% of produced lactate by glycolysis from external glucose is actively secreted to the extracellular *milieu*; a smaller fraction of 10–25% of the glycolytic flux is oxidized by OxPhos as pyruvate (Portais et al., 1993; Metallo et al., 2009). Then, both anaerobic (glucose to lactate) and aerobic (glucose to pyruvate)