

Chapter 2

The Warburg Effect and Beyond: Metabolic Dependencies for Cancer Cells

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Abstract Current definitions of cancer are best realized as a list of traits or hallmarks, such as tissue invasion, metastasis, cell-autonomous growth, and resistance to apoptosis. A recent update included deregulated cellular energetics as an emerging hallmark. However, debate about tumor cell metabolism occupied center stage in the pre-oncogene era. Over the last 15 years, direct links of oncogenes and tumor suppressor genes to cell metabolism have brought cancer metabolism to the forefront once again. Current tools provide much greater opportunities for probing metabolic differences between normal and cancer cells, in some cases revealing flux through unexpected metabolic pathways. Metabolic networks may also be truncated, presenting opportunities for selective growth inhibition or death by targeting non-redundant pathways in cancer cells. These “metabolic dependencies” are not likely to be associated with classically defined oncogenes or computationally derived drivers and thus may require novel strategies for discovery.

2.1 Pre-Molecular Biology Research

Two giants of biochemistry, Otto Warburg and Herbert Crabtree, developed novel insights into cancer metabolism in the 1920s. Warburg improved on previous manometric techniques to study respiratory quotients in a variety of cell types and tissue slices. Based on his demonstration of a sixfold increase of oxygen uptake in sea urchin eggs following fertilization, he entertained the notion that tumor growth could be explained by increased bioenergetic metabolism. In opposition to his original predictions, the respiratory rate in Flexner rat carcinomas was similar to normal rat tissues. However, the rate of glycolysis was increased up to 30-fold in the carcinoma compared with rat liver.

Comparing glycolysis in air and nitrogen, Warburg observed that both tissues had higher rates of lactate generation in the absence of oxygen. In normoxic

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samples, glycolytic rates were at or below the limit of detection in most normal tissues. In contrast, glycolysis in normoxic cancer tissues still accounted for the majority of glucose turnover. The ratio of aerobic glycolysis to respiration was substantially elevated in cancers, expressed in various ways as mole percent glucose metabolized to lactate (~90 %), ATP generated by glycolysis (35–50 %), or mass of lactate produced (10 % of tissue weight per hour) [1].

Warburg extended these results to human cancer tissues and also demonstrated that although many adult tissues did not produce lactate even under hypoxic conditions, normal growing tissue (e.g., embryonic samples) had intermediate rates of aerobic glycolysis. According to the Pasteur effect, oxygen should inhibit fermentation (lactate production). Warburg interpreted his findings as demonstrating that respiration was insufficient to suppress glycolysis, either due to limitations in oxygen supply or due to mitochondrial activity. Since his aerobic experiments were conducted in thin tissue slices in which oxygen diffusion was not rate limiting, the problem seemed to Warburg to be intrinsic to mitochondria. Furthermore, if respiration was insufficient for the cellular energy demand, selection of cells with high glycolytic rates for survival was possible. Several normal tissues, including retina, kidney medulla, cartilage, bone marrow, skin, fibroblasts, intestinal mucosa, placenta, and proliferating thymocytes, have been demonstrated to have high rates of aerobic glycolysis. In fact, Warburg argued against using aerobic glycolysis as a specific test for cancer cells [2].

The central observation that cancer cells have high rates of aerobic glycolysis, now known as the Warburg effect, has been repeated numerous times and is the basis for the use of 2-deoxy-2- (^{18}F) fluoro-D-glucose positron emission tomography (PET) scans for tumor staging. However, mitochondrial dysfunction as a basis for the Warburg effect is still debatable. The Warburg effect is inhibited by treatment with mitochondrial uncouplers, indicating a well functioning Krebs cycle and electron transport chain. Anaerobic glycolysis occurs at higher rates in cancer cells than aerobic glycolysis, while the ratio of the decrease in lactic acid production to oxygen consumption in normoxia is similar in cancer and normal tissues. Thus, if energy demand remains constant, the efficiency of oxidative phosphorylation at suppressing glycolysis is similar in tumors and normal tissues.

However, it has been suggested that cancer cells may have reduced mitochondrial reserve in response to glycolytic inhibition [3]. Isolated mitochondria from cancer cell lines with pronounced Warburg effects have been shown to exhibit specific defects in substrate utilization, respiratory control ratios, and mitochondrial content [4, 5]. Reduced expression of the β subunit of the mitochondrial F_1F_0 ATPase has been linked to breast, lung, and colon adenocarcinomas with poor prognosis [6–8]. Upregulated expression of the ATPase inhibitor IF1 has also been reported in cancers [9, 10]. Aside from intrinsic differences in mitochondrial function, several regulatory mechanisms restricting mitochondrial oxidative phosphorylation in cancer cells have been identified.

The Crabtree effect refers to the ability of glucose to inhibit respiration in cancer cells, as opposed to the stimulation of respiration noted in normal tissues [11]. This dynamic regulation provides another perspective on the glycolytic shift in cancer cells identified by Warburg. Typically observed in cells in which

glucose metabolism by glycolysis equals or exceeds oxidation in the TCA cycle, the Crabtree effect has been proposed to represent competition between glycolysis and oxidative phosphorylation for limiting substrates such as ADP and phosphate, and the regulation of respiration by the energy charge $[ATP]/[ADP][Pi]$ [12, 13]. Although ATP is also a negative feedback regulator of glycolysis at phosphofructokinase-1 (PFK-1), the allosteric activator fructose-2,6-bisphosphate (F-2,6-BP) is overproduced in many cancer cells and lessens the effect of ATP [14]. Thus, high rates of ATP synthesis can suppress oxidative phosphorylation to a greater extent than glycolysis under these circumstances. Like aerobic glycolysis, the Crabtree effect is also observed in normal cell types, such as proliferating thymocytes [15].

2.2 Glycolytic Regulation in Cancer

Current hypotheses rationalize the association of aerobic glycolysis and cancer by the high demand for glycolytic intermediates in rapidly growing cells. Glucose is utilized for the production of nucleosides (the pentose phosphate pathway produces ribose; 3-phosphoglycerate is precursor for glycine; serine and glycine are utilized in one carbon folate metabolism), amino acids (pyruvate is transaminated to produce alanine, 3-phosphoglycerate yields serine, glycine, and cysteine), and reducing power (NADPH from pentose phosphate pathway). Paradoxically, several steps in the glycolytic pathway are regulated in cancer to increase the levels of glycolytic intermediates by slowing downstream enzyme turnover. However, some cancer cells can have similar growth rates on slowly metabolized carbohydrates, such as fructose and galactose, in which case most metabolism is diverted to the pentose phosphate pathway, with minimal lactate production [16]. This suggests that glycolysis in the presence of glucose operates in large excess to the requirements for anabolic reactions.

The final enzyme in glycolysis, lactate dehydrogenase, produces lactic acid, which is transported out of the cell rather than serving as a synthetic intermediate. Two ideas to explain the high rates of lactic acid production have been proposed. The first involves an “overflow” phenomenon, in which the rate of pyruvate generation exceeds the capacity of the mitochondrial TCA cycle metabolism [17]. Alternatively, LDH may fulfill the requirement for regeneration of NAD^+ for glycolysis to proceed, in the setting of low activity of malate–aspartate and glycerol-3-phosphate mitochondrial shuttles for reducing equivalents [18].

In vivo, poor perfusion due to the disordered tumor microvasculature may evoke a hypoxic response associated with expression of the hypoxia-inducible transcription factors (HIFs), upregulation of glycolysis, and diversion of substrate fuels away from mitochondrial metabolism. However, unless there is some mechanism for “fixing” this response, it seems unlikely to account for aerobic glycolysis measured under normoxic conditions.

The alternative explanation that increased glycolysis is matched to an increased ATP demand is less favored in the face of evidence that tumor cells exhibit

adaptations that diminish ATP generation. Pyruvate kinase catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP, generating pyruvate and ATP. Originally described by Sato in 1978 [19], the pyruvate kinase splicing isoform M2 (PK-M2) is highly expressed in cancers, embryonic tissues, and several adult tissues, including adipocytes, retina, and lung [20]. PK-M2 is active as a tetramer, but mostly occurs as inactive dimer in cancer cells. Tetramer formation is induced allosterically by the proximal glycolytic intermediate, fructose-1,6-bisphosphate (F-1,6-BP), but opposed by tyrosine phosphorylation of PK-M2 or interactions with phosphotyrosine-bearing proteins at the F-1,6-BP-binding site [21–24]. Reduced activity of PK-M2 increases the diversion of upstream glycolytic intermediates into anabolic pathways, including the pentose phosphate pathway [25], as well as slowing ATP generation. Interestingly, substitution of the PK-M1 splicing isoform increases glucose oxidation and reduces lactate production by an unexplained mechanism [23].

Reduction in the PK-M2 activity in cancer cells raises questions as to the source of pyruvate utilized for lactate generation in aerobic glycolysis. Analogous to bacterial pathways, the substrate for PK-M2, PEP can be used as a phosphate donor in protein kinase reactions [26, 27]. One of the protein substrates for PEP-dependent phosphorylation is the glycolytic enzyme phosphoglycerate mutase (PGAM1), which is phosphorylated on the catalytic histidine (H11) involved in transferring phosphate to the C-2 position of 3-phosphoglycerate. This reaction produces pyruvate as a product in the absence of ATP generation. Thus, cancer cells can switch to an alternative glycolytic pathway that does not produce net ATP.

Finally, the concept that the predominant function of glycolysis is ATP production can be questioned by a study of glucose deprivation in IL-3-dependent cells [28]. Glucose is necessary for cell growth and proliferation in many cell types. In this example, glucose was shown to be required for N-linked glycosylation of the IL-3 receptor, required for its cell surface localization and in turn, uptake of an alternative substrate fuel, glutamine. Glucose could be replaced by the specific product of the hexosamine biosynthetic pathway, N-acetylglucosamine, enabling glutamine uptake and cell growth.

Three major pathways are involved in deregulating glycolysis in cancer, the transcription factors c-Myc and HIF-1, and the serine/threonine kinase, Akt. The c-Myc oncoprotein is overexpressed in more than 50 % of human cancers and binds to 15 % of all gene promoters in the genome. Among the pathways controlled by c-Myc is glycolysis, with 9/10 glycolytic enzymes and glucose transporters type 1, 2, and 4 identified as Myc targets. Myc-dependent cell cycle entry and transformation are associated with increased lactic acid production [29, 30]. Stable isotope labeling studies indicate that Myc directs glucose carbons to multiple anabolic pathways, including nucleotide, amino acid, and lipid biosynthesis [31, 32]. N-Myc appears to share similar targets in glycolysis [33].

The hypoxia-inducible transcription factor, HIF-1 α , is degraded by the ubiquitin–proteasome system following oxygen-dependent proline hydroxylation. HIF-1 α is overexpressed in 13/19 common tumor types [34]. HIF-1 targets include glycolytic enzymes, glucose transporters and the pyruvate dehydrogenase kinase

(PDK1) that inactivates pyruvate dehydrogenase, required for pyruvate oxidation in the TCA cycle. HIF-1 can also be stabilized in normoxia by mutations in succinate dehydrogenase and fumarate hydratase that lead to product inhibition of proline hydroxylases [35], increased concentrations of pyruvate and lactate [36], and mTOR -dependent translation [37].

Although HIF-1 and c-Myc may have antagonistic functions [38], studies in a Burkitt's lymphoma model demonstrated a cooperative effect on glycolysis [39].

The Akt serine/threonine kinases are activated downstream of phosphoinositide 3-kinase (PI3K) and direct both glucose uptake and metabolism. Glucose transporters Glut 1 and 4 recycle between insulin-responsive storage compartments and the plasma membrane via Akt phosphorylation of the Rab GTPase activity TBC1D proteins [40, 41]. Akt also activates 6-phosphofructo-2-kinase (PFK-2), the enzyme responsible for synthesis of fructose-2,6-bisphosphate, by phosphorylation [42]. Finally, Akt promotes association of hexokinases I and II with mitochondria [43], increasing glucose phosphorylation, by preventing GSK3 β phosphorylation of the voltage-dependent anion channel (VDAC) [44]. Overexpression of c-Myc, HIF-1 α , and constitutively active Akt stimulates aerobic glycolysis [29, 45–47].

2.3 Therapeutic Strategies Based on Glycolysis Inhibition

Inhibition of glycolysis is often lethal in tumor cell lines in vitro, prompting both early and renewed interest in this strategy for cancer treatment. The glucose analog, 2-deoxy-D-glucose (2-DG), is phosphorylated by hexokinase, but in the absence of further glycolytic transformations, 2-DG-6-P accumulates in cells and inhibits hexokinase. 2-DG was first administered to human cancer patients [48] with a variety of cancers and has since been studied in early stage clinical trials in patients with glioblastomas [49] and advanced solid malignancies [50]. Novel LDH-A inhibitors have been reported [51, 52]. A peptide inhibitor of PK-M2 is in clinical trials for melanoma and renal cell carcinoma (www.thallion.com). Several classes of inhibitors of the PI3K–Akt signaling pathway are in clinical trials, including PI3K inhibitors, Akt inhibitors, and dual PI3K–mTOR inhibitors [53]. A common theme in cell death following glucose deprivation or glycolytic inhibition is an increase in mitochondrial generation of reactive oxygen species, with decreased generation of NADPH reducing equivalents in the pentose phosphate pathway [54, 55].

2.4 Alternative Mitochondrial Fuels

Mitochondrial substrate fuels are not restricted to pyruvate-derived acetyl-CoA. The role of glutamine as a major fuel for cancer cell mitochondria was recognized more than 50 years ago [16]. Glutamine is initially metabolized to glutamate by

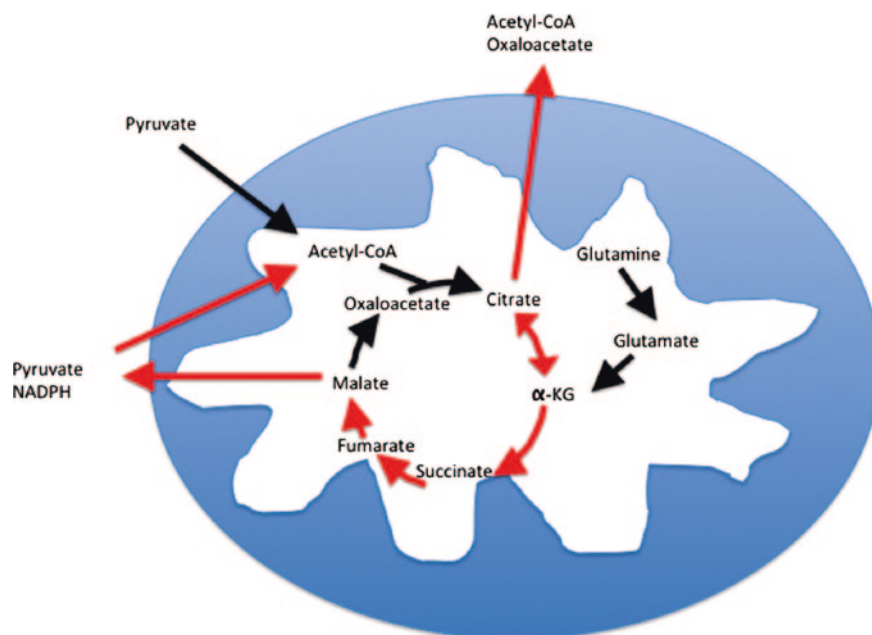


Fig. 2.1 Truncated and reversed TCA cycles (red) with glutaminolysis, involving malic enzyme producing pyruvate and NADPH, and reductive carboxylation of α -ketoglutarate to citrate

phosphate-dependent glutaminase, which can be converted to α -ketoglutarate using either glutamate dehydrogenase or transaminase enzymes. Glutamine entry into the TCA cycle can replenish intermediates (anaplerosis) or serve as a source of acetyl-CoA from pyruvate generated by malic enzyme from malate (Fig. 2.1). The latter reaction is one of the major sources of NADPH generation in cancer cells, used for fatty acid and cholesterol biosynthesis, ribonucleotide reduction, and reduction of GSSG to GSH, in addition to the pentose phosphate pathway. Glutamine can also be utilized for lipid biosynthesis, with conversion of mitochondrial citrate to cytosolic acetyl-CoA by ATP citrate lyase. Two paths for citrate production from glutamate are available, a truncated TCA cycle (α -ketoglutarate to citrate) or reductive carboxylation to isocitrate. ATP citrate lyase activity is activated by Akt -dependent phosphorylation. The energy yield from glutamine metabolism is 6-12 ATP/glutamine, depending on the inclusion of NADP⁺ -dependent glutamate and malate dehydrogenases in the reaction pathway. Glutamine metabolism accounts for 65 % or higher of ATP production in many cancer cells. Chemical inhibition of glutaminase slows growth or kills out-right cells dependent on glutamine, especially hypoxic cells or cells that lack other pathways to generate α -ketoglutarate [56, 57].

Several nonessential amino acids can be derived from glutamate (proline, arginine, aspartate, and asparagine). Glutamine contributes its γ -nitrogen to nucleotide

and hexosamine synthesis and its α -nitrogen to alanine and aspartate in transamination reactions. Glutamate is utilized in glutathione synthesis, and glutamine exchange with leucine and other essential amino acids is essential for mTOR activity [58]. However, a significant portion of glutamine nitrogen and carbon is transported extracellularly, as glutamate, alanine, and lactate, indicating that, like glycolysis, glutaminolysis is an inefficient process in cancer cells [59].

c-Myc has emerged as a major driver of glutamine metabolism. Glutamine deprivation is sufficient to kill Myc-overexpressing cells [60, 61]. Surprisingly, glutamine-deprivation-induced cell death involves depletion of TCA cycle intermediates, but not ATP deficiency, glutathione depletion, or DNA damage, and can be partially rescued with antioxidants [60, 62]. The anaplerotic requirement for glutamine is highlighted by the glutamine-independent growth of tumor cells expressing pyruvate carboxylase, an anaplerotic enzyme associated with glucose metabolism [63]. Glutaminase translation is suppressed by two microRNAs, miR-23a and miR-23b, that are in turn targets for Myc transcriptional repression [62]. Myc also increases the expression of glutamine transporters [61].

Although there are differences in the regulation of glucose and glutamine metabolism, one mechanism for dual control is the basic helix-loop-helix leucine zipper transcription factor, MondoA (also known as MLXIP), related to Myc [64]. In glucose-containing growth media, MondoA functions as a transcriptional activator for TXNIP, which suppresses glucose uptake and aerobic glycolysis. However, the addition of glutamine converts MondoA to a transcriptional repressor, increasing glucose uptake. The ability of a cell-permeable analog of α -ketoglutarate to similarly affect MondoA transcriptional activity suggests that the levels of TCA cycle intermediates may be monitored to adjust glucose uptake and metabolism. Recent data also indicate that Nrf2, a transcription factor responding to oxidative stress, directs both glucose and glutamine utilization in anabolic pathways, such as the pentose phosphate pathway and nucleotide synthesis [65].

Tumor cells engage in de novo fatty acid synthesis, in contrast to importation of circulating lipids by most normal cells in the body [66, 67]. Several recent reports have indicated that cancer cells may also exhibit high rates of peroxisomal and mitochondrial β -oxidation [68, 69]. Prostate cancers with low 2-deoxy-2-fluoro-D-glucose (FDG) avidity on PET scans may engage in fatty acid β -oxidation as a principal bioenergetic pathway [69]. Respiration in glioblastoma cells is inhibited 30–40 % by treatment with the carnitine palmitoyltransferase-1 (CPT1) inhibitor, etomoxir, with a 50 % decrease in ATP levels [70]. Fatty acid β -oxidation has been associated with uncoupling protein-2 expression and chemoresistance [71, 72]. The brain CPT1C isoform is upregulated in non-small-cell lung cancers and confers resistance to hypoxia, glucose deprivation, and the mTOR inhibitor, rapamycin [73]. Adipocytes promote the growth of ovarian cancer cells by mobilizing free fatty acids for β -oxidation by tumor cells [74]. Inhibition of fatty acid β -oxidation (etomoxir, ranolazine) or fatty acid biosynthesis (cerulenin, C75, orlistat) is selectively cytotoxic to some cancer cells [70, 75].

2.5 Screening for Additional Metabolic Dependencies

The Recon 1 genome-scale metabolic reconstruction includes 1,496 open reading frames and 3,311 metabolic and transport reactions [76]. Enzymes are optimal targets for drug development, with active sites and allosteric binding pockets well suited for drug interactions. The extent of metabolic differences between cancer and normal cells is currently not known, although it is evident that the Warburg effect is only one aspect of “cancer metabolism”. In addition to glutaminolysis and lipid metabolism, cancer cells exhibit auxotrophy for specific amino acids and increased NAD⁺ turnover [77–80].

Several approaches have been employed recently to identify metabolic pathways that are required for growth or survival in cancer cells, but not normal cell types. Analogous to other strategies for identifying “driver” genes in cancer, analysis of gene expression may reveal candidate targets for functional genomic studies. Possemato et al. generated a high-priority set of 133 metabolic and transporter genes based on high expression in cancers versus normal tissues, high expression in aggressive breast cancers, and expression in stem cells [81]. Tumorigenic breast cancer cells were transduced with pools of shRNAs targeting the 133 genes and grown as orthotopic tumors in mice. 16 genes were identified with significant depletion of targeting shRNAs during 28 days of *in vivo* growth. One of the genes, phosphoglycerate dehydrogenase (PHGDH), is amplified in breast cancers and melanomas, and PHGDH expression is associated with poor prognosis in ER-negative breast cancers. PHGDH catalyzes the oxidation of the glycolytic intermediate, 3-phosphoglycerate, to phospho-hydroxypyruvate, which can subsequently be converted to serine. Serine is used for the synthesis of phosphatidylserine, sphingosine, cysteine, and glycine, as well as protein synthesis. Additional stable isotope analysis demonstrated increased serine biosynthetic flux in cancer cells overexpressing PHGDH, accounting for 8–9 % of total glycolytic flux. RNAi-mediated suppression of PHGDH in these cells induced non-apoptotic cell death and reduced tumor growth *in vivo*. Finally, metabolomic studies demonstrated large decreases in α -ketoglutarate concentrations in these cells, indicating that phosphoserine aminotransferase provides a major route for conversion of glutamate to α -ketoglutarate.

Unlike transcription factors or signal transduction pathways, metabolic enzyme activity is not manifested by accumulation of downstream gene products or post-translationally modified substrates, but rather by increased substrate to product flux. Furthermore, enzyme regulation often includes allosteric effects. Locasale et al. used [U-¹³C] glucose stable isotope metabolic flux measurements to identify high levels of glycine enrichment in cancer, but not immortalized, cell lines [82]. As in the previous study, PHGDH amplification was observed to correlate with high flux in the serine biosynthetic pathway.

Finally, *in silico* network models of cancer metabolism based on flux balance analysis have been used to predict metabolic pathways required for growth in specific genetic contexts [83]. Germline mutations of the TCA cycle enzyme,

fumarate hydratase (Fh1), cause hereditary leiomyomatosis and renal cell cancer (HLRCC). The accumulation of fumarate inhibits prolyl hydroxylases, leading to an increase in HIF expression under normoxic conditions. Incorporation of cellular growth as consumption of biosynthetic precursors in the *in silico* model allowed testing of specific gene knockouts for effects on growth. Frezza et al. predicted 24 reactions to be synthetically lethal with Fh1 deletion, 18 of which involved heme metabolism. Heme biosynthesis utilizes TCA-derived succinate, providing an alternative route to fumarate synthesis. Subsequent analysis of *Fh1*^{-/-} cells demonstrated increased excretion of bilirubin, the degradation product of heme. Only three enzymes involved in heme degradation were overexpressed in *Fh1*^{-/-} cells, highlighting the power of the computational approach to identify significant pathways in the absence of expression criteria. Several strategies to inhibit heme pathway flux reduced growth of *Fh*^{-/-} cells.

Toward an unbiased screening strategy, we have generated a chemical library with inhibitors for enzymes of intermediary metabolism, as validated in the literature by direct enzymatic assay. Inhibitors were chosen from the BRENDA enzyme database (www.brenda-enzymes.org) and review of published literature, purchased from Sigma-Aldrich or other chemical supply houses, or donated by academic labs. Inhibitors of 585 enzymes have been identified. Each inhibitor is plated at 100X EC50 concentration in DMSO. Transcriptome analysis in mouse and human databases indicates ~750 enzymes of intermediary metabolism encoded in the genome with unique EC numbers [84]. Such a library has several applications relevant to cancer research. Screening cancer cell lines versus normal cellular counterparts for loss of viability or cell growth can reveal critical metabolic dependencies associated with the transformed phenotype. Altered expressions of metabolic enzymes, in particular, as revealed by Gene Set Enrichment analyses, are frequently observed in RNA microarray profiles. As yet, there is no rapid method to determine the essentiality of a metabolic pathway for cancer cell viability. Metabolomic studies of cancer cells are increasingly being reported. A chemical inhibitor library can rapidly ascertain which pathways connecting to a given metabolite are casually linked to cell growth or viability.

We have tested 217 causally compounds out of a total of 585 in our enzyme inhibitor compound library in the MCF10A cell line expressing Myc-ER^{TAM}. As shown in Fig. 2.2, inhibitors are available for a high proportion of enzymes in major metabolic pathways. MCF10A-MycER^{TAM} cells were treated with tamoxifen to activate Myc for 24 h and compared with comparably treated vehicle controls. Cell metabolism was evaluated using a Seahorse analyzer, which demonstrated a shift toward glycolytic metabolism in tamoxifen-treated, but not control, cells.

Cells were tested with each compound in triplicate at a single dose, based on published IC₈₀ values for enzymatic inhibition. Cell viability was assessed after 24 h by Alamar Blue assay. Log₂-transformed results were analyzed according to SSMD scores (strictly standardized mean difference; [85]) with respect to negative vehicle controls. The results are graphed in Fig. 2.3 (high SSMD values correspond to selective killing of tamoxifen-treated cells). A secondary assay for cell number (Hoechst 33342 fluorescence of adherent cells) confirmed these results.

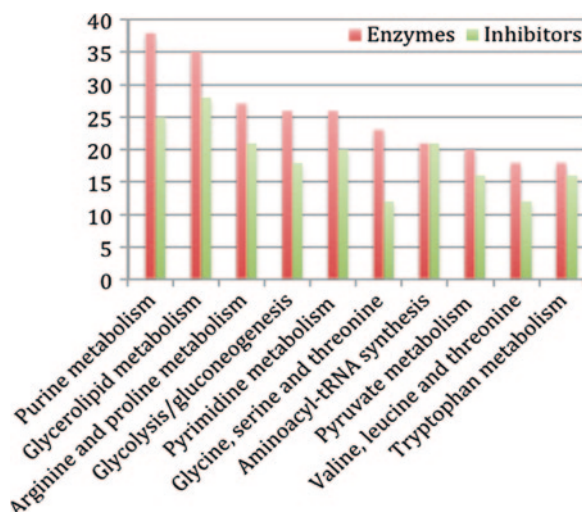


Fig. 2.2 Comparison of enzymes in major primary metabolic pathways and availability of specific inhibitors

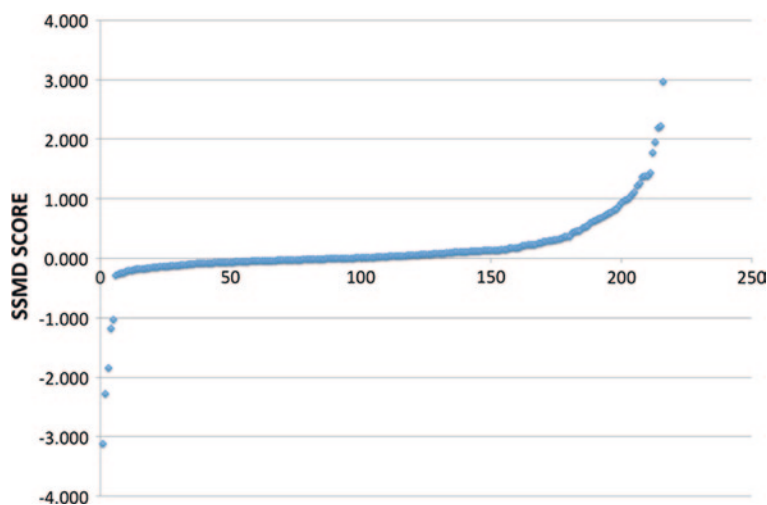


Fig. 2.3 Plot of SSMD scores for 217 compounds tested against MCF10A-MycER cells +/- tamoxifen, in relation to negative (vehicle) controls

The top hits include aldehyde dehydrogenase, malic enzyme, aromatic L-amino acid decarboxylase, and threonine tRNA ligase. Aldehyde dehydrogenases are markers for cancer stem cells. Disulfiram, the aldehyde dehydrogenase inhibitor in the screen, is reported to have anticancer activity [86], although the relevant target has not been validated. Aromatic L-amino acid decarboxylase (also known as L-dopa decarboxylase) is expressed in several epithelial cancers in addition to neuroendocrine tumors and has co-activator activity for the androgen receptor [87, 88].

Borrelidin, the threonine tRNA ligase inhibitor, induces apoptosis in acute lymphoblastic leukemia cells associated with the activation of the GCN2 stress kinase and proapoptotic CHOP transcription factor [89].

2.6 Summary

It is not surprising, based on the intimate role of catabolic and anabolic metabolism in furnishing biochemical energy and building blocks for macromolecular synthesis and repair, that metabolic pathways are vital to cancer cell growth and survival in different tumor microenvironments. What we have learned more recently is that metabolic pathways can also direct chromatin structure and gene expression, differentiation, and stemness [90–95], typified by the discovery of the oncometabolite, D-2-hydroxyglutarate (2HG). Specific gain-of-function mutations in the isocitrate dehydrogenase-1 and dehydrogenase-2 enzymes occur in glioblastomas and acute myeloid leukemia and alter enzymatic function to produce 2HG from α -ketoglutarate (α -KG) [96]. 2HG is an inhibitor of α -KG-dependent Jumonji-C domain histone demethylases [97, 98] and is associated with increased histone methylation and altered gene expression [99, 100]. Comprehensive mapping of cancer cell metabolism is at an early stage, but current knowledge suggests that the Warburg effect may be the “tip of the iceberg”. The ultimate value of cancer therapies directed against metabolic dependencies is still to be determined, as the links between metabolism and cell death, and extent of metabolic flexibility are largely unknown. Further exploration of metabolic networks in cancer will require novel strategies, but seems likely to yield novel targets for cancer therapy.

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