

## Contributed Mini Review

## Tumor bioenergetics: An emerging avenue for cancer metabolism targeted therapy

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Cell proliferation is a delicately regulated process that couples growth signals and metabolic demands to produce daughter cells. Interestingly, the proliferation of tumor cells immensely depends on glycolysis, the Warburg effect, to ensure a sufficient amount of metabolic flux and bioenergetics for macromolecule synthesis and cell division. This unique metabolic derangement would provide an opportunity for developing cancer therapeutic strategy, particularly when other diverse anti-cancer treatments have been proved ineffective in achieving durable response, largely due to the emergence of resistance. Recent advances in deeper understanding of cancer metabolism usher in new horizons of the next generation strategy for cancer therapy. Here, we discuss the focused review of cancer energy metabolism, and the therapeutic exploitation of glycolysis and OXPHOS as a novel anti-cancer strategy, with particular emphasis on the promise of this approach, among other cancer metabolism targeted therapies that reveal unexpected complexity and context-dependent metabolic adaptability, complicating the development of effective strategies. [BMB Reports 2014; 47(3): 158-166]

## CANCER METABOLISM: THE WARBURG EFFECT REKINDLED, AND REVISITED

The pathognomonic feature of cancer is virtually unlimited growth and propagation (local and metastatic growth) of malignant cells. Uncontrolled cancer cell proliferation requires both oncogenic aberrant growth signals, and sufficient metabolic provision of bioenergetics and biosynthetic precursors. Intriguingly, the metabolic flux is principally derived from the glucose in cancer cells, known as the Warburg effect. Unlike

normal cells, which can also burn amino acids and fatty acids for energy, cancer cells seem 'addicted' to glucose, which serves as both a principal energy source, and the cellular building blocks required for cell proliferation. Recent advances in molecular imaging technology provide compelling clinical evidence that cancer tissues vividly uptake glucose, and display increased glycolysis, compared to neighboring normal tissues, by 18-FDG PET scan (1), reflecting dysregulated glucose metabolism in cancer cells. Considering the high growth and proliferation rate of cancer cells, an unusual amount of metabolic flux is indispensable to meet the need for rapid provision of ATP and intermediary metabolites, for macromolecule synthesis, such as nucleotides, lipids, and proteins. Thus a high glycolytic feature should allow cancer cells to have a selective growth advantage, in terms of bioenergetics and biosynthesis.

In 1920s, Warburg pioneered the investigation of the tumor cell energetics metabolism, and reported that tumor cells produce a large amount of lactate, even in the presence of oxygen (2). Glycolysis, conversion of glucose to lactate, only produces two ATP molecules, compared to 36 for the complete oxidation of glucose to CO<sub>2</sub> and H<sub>2</sub>O. Thus, incomplete oxidation of glucose to lactate yields only 5% of the energy available from glucose. Considering the immense metabolic necessity for cancer cells, this phenomenon poses a paradox: Why do cancer cells, which obviously are in great demand for ATP and intermediary metabolites, heavily depend on such a wasteful version of metabolism? This apparently senseless waste of glucose prompted Warburg to postulate a hypothesis, that a defect in mitochondrial respiration is the ultimate cause of cancer, the Warburg theory (3); although it has been discredited.

Accumulating evidence suggests that malignant transformation is associated with alterations in cellular metabolism, and cancer-associated metabolic derangement is linked to the activation of oncogenes, and to the inactivation of tumor suppressor genes (4, 5). Subsequently, it has now become clear that the Warburg effect is, at least in part, the outcome of a complex and intricate network of signaling pathways, which once used to be regarded as a separate entity of cell biology. Further, the Warburg effect represents only one aspect of multifaceted cancer-associated metabolism, which spans from aerobic glycolysis, increased pentose phosphate pathway (PPP), increased macromolecule biosynthesis, through redox

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homeostasis, to autophagy (6). Although the detailed mechanistic link between oncogenic growth signaling and the control of cancer-associated metabolism is not yet fully unveiled, it is now widely accepted that the signal transduction that controls tumor cell growth, and cancer metabolism, are closely interconnected (7). One of the frequently deranged growth factor signals in cancer, phosphatidylinositol 3-kinase (PI3K)-AKT/PKB signaling, is important in the physiological role of insulin in normal cells (8). The insulin-PI3K-AKT/mammalian target of rapamycin (mTOR) pathway plays a critical role in coupling the energy status, and cellular growth. In animals, this mechanism provides the central metabolic homeostasis in nutrient sensing, and the regulation of cell growth, in response to environmental nutrient fluctuations.

Indeed, the PI3K-AKT/mTOR signaling axis is a well-known principal cellular growth signaling pathway, and its aberrant activation is reported across many cancer types (9), establishing a relationship between tumor growth signaling, and central metabolism.

Another example of this intricate interconnection between oncogenic drivers and cancer metabolism is Myc, a pleiotropic transcription factor, and multi-function oncoprotein. Myc is known to stimulate glucose uptake (10) and expression of the M2 isoform of muscle-type pyruvate kinase (PK-M2), but it also leads to glutaminolysis, by complex transcriptional and post-transcriptional regulations (11, 12). Oncogenic Ras and Raf mutations are also associated with increased expression of glucose transporter 1 (GLUT1) (13), allowing cancer cells to survive a glucose limited tumor microenvironment (14, 15).

As exemplified by the interconnection between typical oncogenes and tumor metabolic regulation, tumor suppressor genes also have intimate relationship with cancer-associated metabolic aberrations. For example, p53 mutation, which occurs in more than 50% of all human cancers, results in a diverse spectrum of metabolic ramifications that stimulate glycolysis. Indeed, wild type p53 is able to transcriptionally repress the expression of a couple of GLUTs, GLUT1 and GLUT4 (16). Further, p53 stimulates the expression of TP53-induced glycolysis and apoptosis regulator (TIGAR) (17), glutaminase 2 (GLS2) (18), and synthesis of cytochrome c oxidase 2 (SCO2), along with various pro-autophagic genes (19, 20). Among these, TIGAR functions as a Fructose-2,6 bisphosphatase, which reduces fructose-2,6 bisphosphate, the allosteric activator of 6-phosphofructo kinase (PFK), a second committed enzyme in glycolysis, diverting metabolic flux towards pentose phosphate pathway (PPP), thereby decreasing glycolysis (21). Further, SCO2 is crucial for the assembly of the cytochrome c oxidase (COX) complex, a critical molecular player in oxidative phosphorylation, explaining why p53-deficient cells exhibit reduced baseline levels of mitochondrial energy transduction (19). The p53 system also cross-talks with PI3K-PTEN-AKT signaling pathway, thereby modulating their metabolic functions (5). Notably, p53 has recently been revealed to aid the adaptive response of cancer cells to serine and glutamine shortage (22,

23), indicating that p53-deficient cancer cells may require an ample supply of these amino acids, to support their survival and proliferation. Further research is required to document that the therapeutic exploitation of serine or glutamine deprivation, specific to cancer cells with p53 deficient tumors, is clinically feasible.

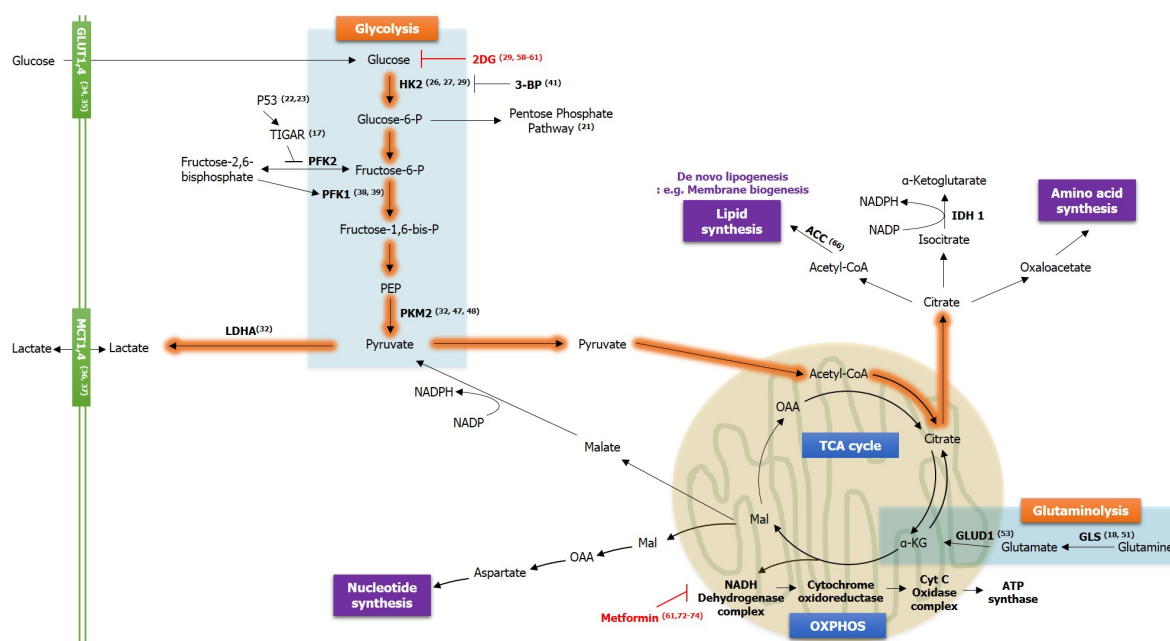
Taken together, these observations indicate that cancer growth is intimately connected to metabolic regulation by diverse oncogenes and oncosuppressors. Further, the cross-talk between tumor-relevant genes and glycolytic enzymes provides potential molecular explanations of why cancer cells exhibit the Warburg effect, a seemingly senseless wasteful version of metabolism, despite heightened metabolic demands.

## TARGETING CANCER METABOLISM

During the past decade, the understanding of cancer-associated metabolic rewiring has been deepened, and this metabolic derangement of cancer cells has recently been viewed as a novel therapeutic target (Fig. 1). Several different approaches to tackle this Achilles' heel of cancer have been explored, with some extent of preclinical successes, resulting in the identification of drug candidates that are now being evaluated through clinical trials (24). A detailed compendium of such metabolic targets and therapeutics is beyond the scope of this mini Review, and has been covered elsewhere in the literature.

One of the prototypical metabolic inhibitors used to treat cancer is 2-deoxy-D-glucose (2DG). 2DG serves a couple of distinct roles, in terms of core energy metabolism. First, it competes with naïve glucose for GLUTs, which enhance the facilitated diffusion of glucose inside the cell, thereby decreasing glucose uptake by cancer cells. Second, the unselective inhibition of hexokinase (HK), which is the first committed enzyme, and which entraps glucose inside the cell by phosphorylation, further decreases the overall glycolytic flux. Once phosphorylated by HK, the 2DG is a dead-end metabolite that is not allowed to be involved in downstream metabolism (25, 26). Theoretically, 2DG is an attractive candidate for inhibiting the Warburg effect; however, 2DG alone has proven unsuccessful in clinical evaluation, and subsequent trials have focused on combination strategy, with conventional chemotherapeutics (27), or radiotherapy (28).

A large body of preclinical researches suggests that several enzymes and transporters in cancer-associated metabolic circuitries, such as glycolysis, the TCA cycle, lipid biogenesis, and glutaminolysis, may be potential drug targets. These include HK2 (29), PFKFB3 (30), GAPDH (31), PK-M2 (32), LDHA (33), GLUT1 (34), GLUT4 (35), and monocarboxylate transporter 4 (MCT4, SLC16A4) (36, 37). Among these, small molecule inhibitors of PFKFB3 have been shown to suppress the growth of breast cancer cells and promyelocytic leukemia cells xenografted in immune compromised mice (38). Further, it inhibits the development of murine lung cancer transplanted to immune competent hosts (39). Similarly, the pharmacological or genetic



**Fig. 1.** Schematic representation of cancer energy metabolism and potential bioenergetics targeted strategy. Simplified overall metabolic network features are illustrated. In tumor cells, enhanced metabolic fluxes are highlighted with orange lines. When glycolysis is inhibited by 2DG, the metabolic flux to TCA cycle and OXPHOS (Oxidative phosphorylation) is increased. Metformin, which inhibits ETC complex I (NADH dehydrogenase complex), a major entry point of electron to electron transfer chain, can suppress tumor cell bioenergetics by decreasing ATP generation, through OXPHOS complementary to glycolysis inhibitors.

intervention of HK2 (29), GAPDH (40), PK-M2 (32), LDHA (33), GLUT1 (34), GLUT4 (35), and MCT4 (36) have demonstrated *in vivo* anti-cancer effects in several tumor models. Of note, promising preclinical results have been reported with HK2 inhibition using 3-bromopyruvate (3-BP) (41) and methyl jasmonate, in a range of rodent human tumor xenograft models (42). However, subsequent studies revealed that the specificity of 3-BP for HK2 is limited: 3-BP has been shown to inhibit GAPDH and LDH, at least, among other glycolytic enzymes (41, 43), complicating the further clinical development. In line with this, the development of another HK2 inhibitor Ionidamine once was promising, but has failed to prove clinical efficacy in clinical trials (44-46).

Despite early enthusiasm, only a small number of metabolic inhibitors have advanced to clinical development. One of these winners, a PK-M2 inhibiting approach, has been assessed as a single therapeutic agent, in patients with metastatic renal cell carcinoma and recurrent skin cancers (ClinicalTrials.gov identifiers: NCT00735332; NCT00422786). PK-M2 is one of the three committed enzymes in glycolysis, making this enzyme a promising therapeutic target, as is the case with HK2 and PFKBP3. Notably and paradoxically, however, PK-M2 activation is also being investigated for another anti-cancer therapeutic strategy in preclinical setting (47). Further, recent evidence suggests that PK-M2 is not required for some form of tumors to proliferate, indicating the dispensability (48), and cellular context-dependency, of this enzyme. Collectively, these

results make cancer metabolic enzyme targeted therapy difficult to advance into clinical developments.

Another cancer specific metabolic alteration, Glutaminolysis, that cancer cells also depend heavily on glutamine for proliferation and survival (49, 50), prompted the evaluation of glutamine addiction in cancer. Glutaminase 1 (GLS1) targeting agents have been demonstrated to selectively suppress the malignant transformation of murine fibroblast induced by GTPase of the RHO family protein, and to inhibit the growth of human breast cancer, B cell lymphoma and glioma cells (51, 52). It is thought that glutamine might provide both carbon and nitrogen sources to tumor cells' either baseline or proliferative phase requirement of those essential metabolic atoms. Indeed, glutamine can feed the TCA cycle (i.e. anaplerosis), by being converted to  $\alpha$ -KG catalyzed by glutamine dehydrogenase 1 (GLUD1). Interestingly, RNAi-mediated knock down or pharmacological inhibition of GLUD1 shifts the metabolic scheme of cultured glioblastoma cells from glutamine to glucose (53), indicating that the metabolic adaptation of tumor cells, once thought "addicted", quite frequently takes place.

Taken together, these metabolic adaptabilities, redundancy in similar metabolic modules, and circumventing pathways interconnected with the unique molecular make-up of individual tumors, complicate the development of clinically usable metabolic targeted therapeutics.

## TUMOR CELLULAR BIOENERGETICS AND ENERGY SENSING SIGNALING PATHWAY

As discussed above, the clinical development of cancer-associated metabolism targeted drugs has been faltering, despite the initial enthusiasm since the Warburg effect has triumphantly been revisited.

Among others, the unexpected metabolic flexibility, redundancy in metabolic network, and emergence of circumventing strategy by cancer cells are the major culprits. Further, several agents that were apparently promising at the pre-clinical stage have failed to enter the clinical development, due to unacceptable high toxicity, such as hepatotoxicity caused by carnitine palmitoyltransferase 1A (CPT1) inhibitor etomoxir (54).

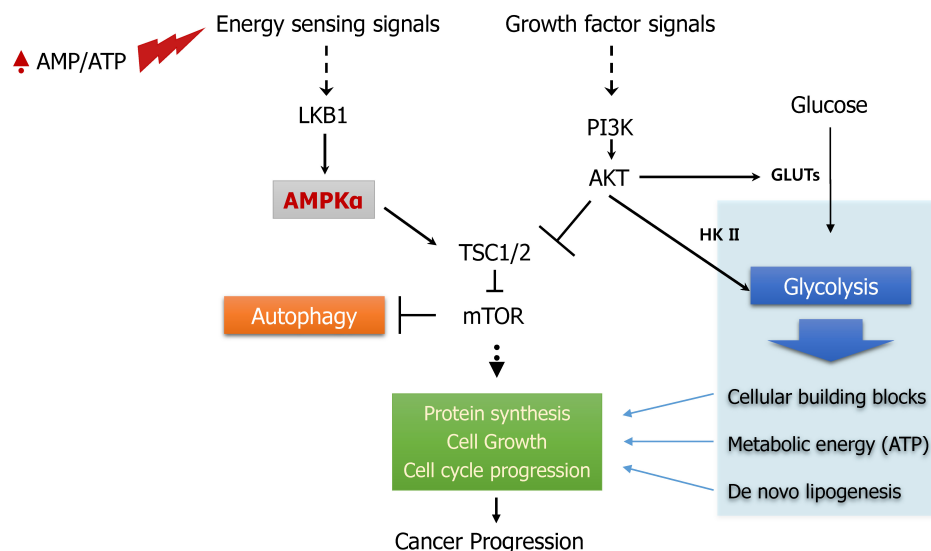
In this context, the Warburg effect itself is still an attractive therapeutic target over many metabolic enzymes, since many cancers exhibit glycolytic phenotype. An accumulating body of evidence indicates that the tumor bioenergetics are primarily derived from glucose in most tumor types, consolidating the idea that blocking glucose utility holds promise in cancer therapy. Clinically, tumors with high glucose uptake detected by the FDG-PET scan demonstrate a worsened outcome (55), further underscoring the therapeutic potential of the inhibition of glycolysis.

Cellular energetics are primarily supplied by either glycolysis, or mitochondrial oxidative phosphorylation (OXPHOS). Consi-

dering the clinical demonstration of high glycolytic phenotype of most tumors, it looks reasonable that drugging the Warburg effect could result in an effective anti-cancer effect. Indeed, many malignant cells have a demonstrably greater sensitivity to glucose deprivation-induced cytotoxicity than normal cells (56). An inhibitor of glycolysis, 2DG is a glucose analog that cannot be metabolized, and that is a useful drug of many diseases, including autosomal dominant polycystic kidney disease (27), and cancer. In spite of promising preclinical results, however, in clinical studies on its own, 2DG failed to realize its expectation.

Subsequently, in experimental cancer therapeutics, 2DG is now used in combination with radiotherapy or classical cytotoxic anticancer drugs (28, 57). It is highly speculated that the concentrations of 2DG used in preclinical evaluation do not represent clinical dose, as most *in vitro* studies utilized non-physiological levels of 2DG, ranging from 8-100 mM. In reality, the acceptable level of 2DG in clinical setting might be 4-5 mM, which can maintain the molar ratio between naïve glucose to 2DG at approximately 1 : 1 (58). As a result, clinically applicable doses of 2DG make cell growth slower, but rarely cause cell death *in vitro* and *in vivo*, when used alone (59, 60).

Indeed, when these clinically achievable concentrations of 2DG were used *in vitro*, the anti-cancer effect was not substantial, across a broad range of human cancer cell lines (61). Further, 2DG at the clinical doses did not substantially activate AMP-activated kinase (AMPK), reflecting insufficient sup-



**Fig. 2.** Integrated signaling networks and tumor cellular bioenergetics. Tumor cell growth and survival require both oncogenic growth signals and sufficient metabolic flux that supports both biosynthesis of macromolecules like nucleotides, proteins and lipids, as well as ATP production. Oncogenic growth factor receptor signaling conveyed through PI3K-Akt can upregulate glycolysis, thereby providing sufficient cellular bioenergetics to support mTOR mediated cancer progression. AMPK signaling senses cellular energy stress, and downmodulates energy-consuming processes (e.g. mTOR driven biosynthetic pathways). The LKB1-AMPK pathway and PI3K-AKT-mTOR pathway have a cross-talk through a molecular convergence point TSC2, consolidating the emerging intimate relationship between physiological control of metabolism and cancer growth.

pression of tumor bioenergetics. AMPK, which is a conserved serine/threonine protein kinase, maintains and monitors energy homeostasis, as a cellular energy sensor and signal transducer (62, 63). AMPK phosphorylates many substrates that modulate the balance between intracellular energy levels and cellular metabolic status, switching from anabolic to catabolic metabolism during energy stress period (64). Among the downstream substrates of AMPK, tuberous sclerosis complex 1 and 2 (TSC1/2), as well as Acetyl CoA carboxylase (ACC1 and 2), have particular importance in cancer. mTOR pathway constitutes a key cellular growth machinery, transducing signals to promote protein synthesis and cell cycle progression (65). mTOR activity is positively regulated by upstream AKT, which phosphorylates and suppresses TSC1/2, thereby reversing its inhibitory effect on mTOR. On the other hand, AMPK activates TSC1/2, resulting in mTOR signaling downregulation. In this way, AMPK activation can switch off the cell's major growth signaling and biosynthetic pathway, to stringently keep energy expenditure in control (Fig. 2). Further, AMPK-mediated mTOR suppression activates autophagy, which serves the catabolic mobilization of endogenous energy substrates, such as ribosome, obsolete subcellular organelles - aged mitochondria for example - portion of cytosol, and lipid membrane, and degrades them in the lysosome.

ACC1 and ACC2 are also phosphorylated and inhibited by AMPK. Since ACC1/2 catalyze the first step of fatty acid biosynthesis by carboxylating acetyl-CoA, inhibition of their catalytic activity by AMPK serves a gatekeeper role in fatty acid synthesis (66).

Taken together, AMPK activation suppresses energy-consuming anabolic metabolism, and shifts toward energy-generating catabolic metabolism, indicative of a bioenergetics stress induced salvage mechanism.

Recently, it has been demonstrated that glucose deprivation of cultured cancer cells results in the activation of AMPK signaling system (67-69). Further, the AMPK activation by AICAR, an AMP-mimetic thus mechanism specific, leads to transcriptional reprogramming, favoring augmentation of mitochondrial OXPHOS, by the coordinated upregulation of genes encoding ETC complexes and TCA cycle (58). Subsequent functional analysis of mitochondrial energy generation and cellular phenotype assay confirmed that AMPK activation provides protective effect on cancer cells in bioenergetics stress (70). To assess the role of AMPK in glucose deprivation context, AMPK<sup>-/-</sup>MEFs were cultured in either glucose repleted or deprived media, and confirmed that AMPK indeed protects cells from apoptosis in glucose depleted culture. This observation poses a clinically important notion that AMPK is a double-edged sword, which might act as a tumor suppressor, or a pro-oncogenic regulator, depending on the cellular energetic context. Indeed, when tumor cells are in proliferative phase, when nutrients are ample, and matched with growth promoting signals, AMPK can exert tumor suppressive effect, through inhibition of mTOR. In contrast, when tumor cells are in energy stress

condition, AMPK can promote the survival of tumor cells, by shifting energy pathway to OXPHOS. Therefore, AMPK-activating drugs should be carefully used, considering the tumor bioenergetic status; and the improved understanding of AMPK effects on cancer progression should be further investigated.

## TARGETING BIOENERGETICS: DRUGGING THE WARBURG EFFECT AND COMPENSATORY MECHANISM

Recently, Sahra *et al.* (71) and Cheong *et al.* (61) reported the anti-cancer effect of a combination of 2DG and metformin to target cancer energy metabolism. The fundamental concept of their approaches is to target two sources of cellular bioenergetics, thereby suppressing tumor growth, and inducing cell death.

Metformin is a biguanide drug, widely used for the treatment of type-2 diabetes, which is known to increase insulin sensitivity, and to lower hyperglycemia (72). Metformin has been known to inhibit mitochondrial ETC complex I activity, thereby indirectly activating AMPK, as a result of ATP depletion (73). So far, mechanisms of the action of metformin have been reported in diverse ways. Among those, the inhibition of cancer cell growth through regulation of LKB1-AMPK signaling or mTOR signaling have mostly been reported. AMPK phosphorylates and activates tumor suppressor gene, p53, which causes the inhibition of cell division, and induced apoptosis. Unfolded protein response (UPR) is a complex signaling network, and metformin selectively disrupted UPR transcriptional activity, by mimicking glucose deprivation (74). In their report, Cheong *et al.* provided compelling evidence that metformin actually coordinately downregulates genes encoding ETC complex I (61). This observation is in line with previous reports, and further suggests the potential explanations of how metformin exerts inhibitory effect on mitochondrial respiration. In the absence of glycolysis, mitochondrial OXPHOS is the only potential source for ATP generation. Therefore, when inhibition of glycolysis with 2DG is combined with a compromise of mitochondrial energy transduction pathway with metformin, it would result in bioenergetics crisis, leading to cell death. Interestingly, a combination of metformin and 2DG induced significant amount of autophagy, leading to cell demise at later time point, in cultured cancer cells (61). This is somewhat discordant from the observations by Sahra *et al.*, where they showed that the combination shifts surviving autophagy to apoptosis (71). However, these observations are evident in p53-dependent manner; thus, further investigation of the mode of action should be warranted, in the range of tumor cells harboring p53 mutations.

Regardless, a combination strategy using 2DG and metformin showed *in vivo* efficacy, and the combination should be readily applicable to clinical test, since these two drugs have been used in clinic for a long time, without demonstrable serious toxicities.

In the foreseeable future, drugs targeting metabolic enzymes in biogenesis or anabolic metabolism are far fetching, since redundancy and circumventing metabolic networks might exist, hampering the clinical benefit, while imposing unexpected toxicity to normal cells, which do not have such metabolic redundancy, or have only limited circumventing mechanisms.

In contrast, targeting bioenergetics with agents that smartly exploit tumor-cell specific traits, such as tumor mitochondrial transmembrane potential and CPT1 expression, thereby sparing normal cells from unwanted collateral damage, can be a promising low-hanging fruit, which can deliver immediate clinical benefit.

## CONCLUDING REMARKS

The Warburg effect confers metabolic advantages on tumor cells. Increased glycolysis is driven by oncogenic pathways that allow tumor cells to couple growth machinery and biomass production. Consequently, drugging the Warburg effect seems a promising therapeutic target. However, realizing the clinical benefit of drugging the Warburg effect requires simultaneous blockade of the mitochondrial oxidative energy pathway.

Further, comprehensive understanding of the cross-talk between signaling pathways and metabolic control in cancer cells, could extend the current molecular targeted therapy to a combinatorial strategy with cancer metabolic manipulation.

Importantly, the genetic alterations unique to individual cancer types should be assessed; thereby, how genomic changes influence, and modify the response to bioenergetics targeted therapy. Regardless, it is not easily foreseeable that cancer cells circumvent energy-targeted therapeutics, since cellular bioenergetics is a nonnegotiable necessity for tumor cell survival and proliferation. Therefore, we envision that targeting cancer metabolism and bioenergetics would provide an exciting opportunity on the horizon, to control human cancer.

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