Warburg and pasteur phenotypes modulate cancer behavior and therapy

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Energetic pathways combine in the heart of metabolism. These essential routes supply energy for biochemical processes through glycolysis and oxidative phosphorylation. Moreover, they support the synthesis of various biomolecules employed in growth and survival over branching pathways. Yet, cellular energetics are often misguided in cancers as a result of the mutations and altered signaling. As nontransformed and Pasteur-like cells metabolize glucose through oxidative respiration when only oxygen is sufficient, some cancer cells bypass this metabolic switch and run glycolysis at higher rates even in the presence of oxygen. The phenomenon is called aerobic glycolysis or the Warburg effect. An increasing number of studies indicate that both Warburg and Pasteur phenotypes are recognized in the cancer microenvironment and take vital roles in the regulation of drug resistance mechanisms such as redox homeostasis, apoptosis and autophagy. Therefore, the different phenotypes call for different therapeutic approaches. Combined therapies targeting energy metabolism grant new opportunities to overcome the challenges. Nevertheless, new biomarkers emerge to classify the energetic subtypes, thereby the cancer therapy, as our knowledge in coupling energy metabolism with cancer behavior grows. ***Anti-Cancer Drugs*** **33: e69–e75** **Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.**

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Introduction

Energetic pathways combine in the heart of metabolism. Not only do they supply energy for biochemical processes through glycolysis and oxidative phosphorylation (OXPHOS), but also they support the synthesis of countless biomolecules employed in growth and survival over branching pathways. Cellular messengers together with starvation on oxygen and nutrients tightly manage the shifting between these routes. The metabolic switch regulates various cellular behaviors ranging from mitosis to cell death.

Cellular energetics are often misguided in cancers as a result of the mutations and altered signaling [1]. Glycolysis, Krebs cycle (TCA cycle) and electron transport chain (ETC) usually co-metabolize glucose in terminally differentiated cells if oxygen is sufficient. Moreover, hypoxic conditions affect the fate of glucose from mitochondrial substances to a cytoplasmic fermentation product lactate. The inhibiting effect of oxygen over fermentation is known as the Pasteur effect [2]. Even though it yields inefficient energy production, it supports cell survival under oxygen-limited conditions. Yet, a century ago, Otto Warburg observed cancer cells bypass this metabolic switch and run glycolysis at higher rates even in the presence of oxygen. The phenomenon is also known as aerobic glycolysis [3].

Cancerous cells took advantage of high glucose consumption and glycolysis rates to meet the energetic gap between OXPHOS and Warburg effect. Several studies indicate that the expression of glucose transporters, some glycolytic enzymes and lactate transporters are enhanced in tumors and reprogram the glycolysis [4,5]. Nevertheless, the phenomenon also supplies many anabolic pathways supporting biomass, such as glycerol synthesis, pentose phosphate pathway and folate cycle. While aerobic glycolysis influences the fate of glucose, also glutamine is another major player in cancer metabolism [6]. This amino acid could afford the Krebs cycle to boost the OXPHOS or assist other anabolic routes such as lipid synthesis and the methionine cycle.

As cancerous cells accumulate mutations as distinct from another in their ruthless microenvironment, so both behaviors and metabolic requirements of cancer metabolism also evolve and vary [7,8]. Altered signaling, miRNA, epigenetics as well as cellular and mitochondrial mutations drive these variants [1,7,8]. While some of these subtypes could bypass the Pasteur effect and exhibit aerobic glycolysis, others could metabolize glucose through mostly OXPHOS [7]. Moreover, some of these tumors could take advantage of glycolysis in the cancer-associated stroma to meet their metabolic needs in a process

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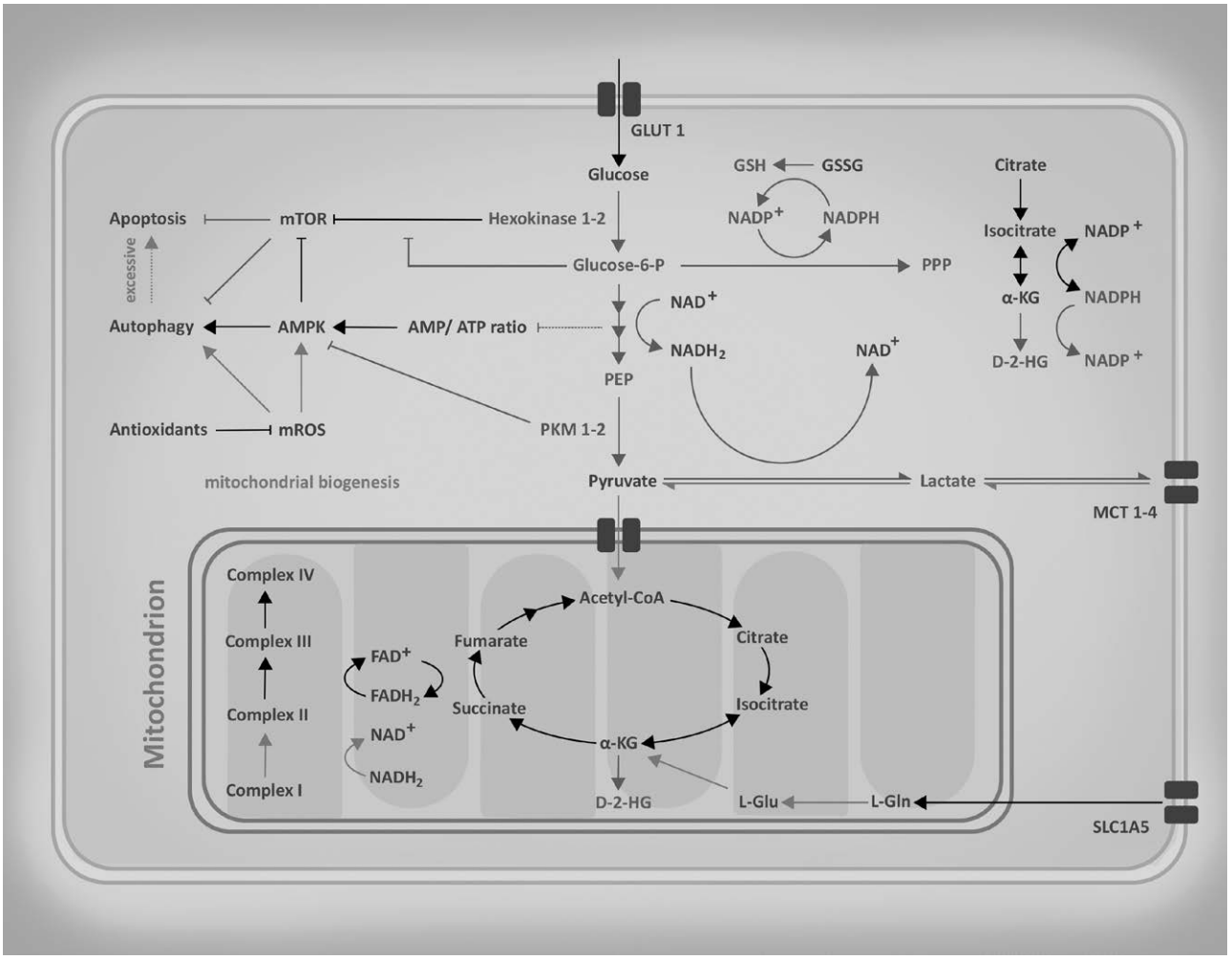
called reverse Warburg [9]. Eventually, heterogeneity in both the microenvironment and metabolism alters the cancer therapy.

Altered energy metabolism differs redox homeostasis, autophagy and apoptosis among Warburg and Pasteur phenotypes, thereby the drug resistance and the response of tumors to therapy. (Fig. 1) Within the framework of these criteria, this review illuminates the diverse metabolic behavior of cancers cells together with challenges in the cancer therapy.

Energy metabolism involves in drug resistance mechanisms

Redox homeostasis is highly regulated in most cancer cells. As major players of Redox metabolism, reactive oxygen species (ROS) are inevitable products of OXPHOS as the ETC complexes I-IV play essential roles in superoxide generation. Not only does ROS emerge as secondary messengers regulating energy metabolism and survival, but also they cause random radical damage on cellular structures which induces programmed cell death apoptosis and autophagy [10]. While ROS-mediated autophagy is a survival mechanism to recycle aberrant organelles and proteins, sustained and even higher ROS signal eventually leads to over-activation of this self-digestive metabolism causing autophagic cell death. This accumulated stress signal also triggers apoptosis and Fenton-mediated ferroptosis. On the other hand, cells also synthesize antioxidants such as glutathione and catalase to protect cellular structures through scavenging these radicals. Hence, the balance between ROS levels and antioxidants induces the cell to rather survive or die. Therefore, most of the anticancer therapeutics hinder redox homeostasis through increasing radicals or

Fig. 1



Glycolysis and mitochondrial oxidative phosphorylation have been related to redox homeostasis, autophagy and apoptosis as well as branching pathways. All red arrows indicate aerobic glycolysis whereas green arrows are associated with oxidative metabolism and Pasteur phenotype. Finally, black arrows and lines claim no correlation with neither Warburg nor Pasteur characters.

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reducing antioxidant metabolites [11,12]. Because the ROS and glutathione are co-metabolites of OXPHOS and glycolysis, the fate of the cancer cells not only regulated by redox homeostasis but also cancer phenotypes with different energy metabolisms.

Many therapeutic approaches induce apoptosis through DNA damage or targeting the disturbances in the apoptosis [13]. Fasting and altered energy metabolism manage both intrinsic and extrinsic apoptotic signals. Aerobic glycolysis and increased expression of glycolytic enzymes support the activity of antiapoptotic B-cell lymphoma-2 proteins while they inhibit pro-apoptotic regulators [14,15]. Moreover, both metabolic alterations and apoptosis are often related to cellular signaling. Activation of these pathways is correlated with the enhanced glycolytic phenotype. For example, glucose transporter protein 1 (GLUT1) localizes on the plasma membrane upon Akt-dependent activation [16]. Active protein kinase B also suppresses another pro-apoptotic factor, p53 upregulated modulator of apoptosis, under the influence of high glucose levels [14]. Along with Warburg metabolism, oxidative phosphorylation and mitochondria have also major roles in cell death via cytochrome C leakage. High levels of ROS induce this key molecule into the oxidized form which can trigger apoptosome assembly [17]. As we mentioned earlier, aberrant glycolysis could support the pentose phosphate pathway resulting in enhanced nicotinamide adenine dinucleotide phosphate hydrogen levels which manage the redox state of cytochrome C leading decrease level of apoptosis through the production of glutathione [18].

Another drug resistance mechanism, autophagy has a versatile nature that is highly relevant to alteration in energy metabolism because its role is based on the energetic needs of cells. Therefore, Warburg and nonglycolytic phenotypes differ cell behavior in autophagy-related drug resistance. Combined therapies and fasting often target overexpressed and mutated glycolytic or mitochondrial enzymes to induce cell death. Yet, recent studies point out that inhibiting glycolysis also enhances autophagic survival mechanisms along with the apoptotic signal. Hexokinase-2 inhibitors such as 3-bromopyruvate (3-BP) and 2-Deoxy-d-Glucose (2-DG) treatments coupled with several inhibitors including UO126 (MEK inhibitor), tamoxifen and ULK-1 inhibiting chloroquine to rewire Warburg phenotype and induce apoptosis while overcoming anti-Warburg mediated autophagy [19–21]. Both glycolytic and OXPHOS metabolism regulates autophagy through energy-sensing motifs such as Sirtuins and AMP-activated protein kinase (AMPK) together with signaling pathways, especially mechanistic target of rapamycin (mTOR) [22,23]. One should also consider activity levels of these motifs and signaling intensities when planing combined therapies. We recommend some excellent reviews for more details in the mechanism of these energy-sensing motifs and autophagy [24,25].

Drug resistance and metabolic targeting

Energy metabolism dawns as a major influencer of cancer progression and therapy. On one hand, a bunch of therapeutic approaches emerges to reverse Warburg's character to overcome drug resistance and induce apoptosis. On the other hand, several studies target OXPHOS related pathways to prone cancer cells with Pasteur phenotype to energy-depletion and cell death (Table 1).

Rate-limiting glycolytic enzymes and glucose transportation appears as favored therapeutic targets to reverse the Warburg phenotype. One such enzyme, hexokinase II (HK-2) drives chemoresistance in colon, breast, ovarian cancers along with several others through autophagy [26–29]. Several studies have been published on HK-2 knockdown to resensitize cells to chemotherapy [26,27]. Recently, Zhang *et al*., (2018) point out that the enzyme was downregulated in cisplatin-sensitive but not in resistant ovarian cancer cells. They also refer that the enzyme enhances the cisplatin effect on autophagy as well as extracellular-signal regulated kinase 1/2 (ERK1/2) phosphorylation which induces survival signal [30]. When HK-2 is knockdown in ovarian tumor xenografts, they report that the procedure discourages cisplatin resistance by drug-induced ERK-mediated autophagy. Similarly, another research group co-target HK-2-mediated Warburg effect along with ULK-1-dependent autophagy with 2-DG and chloroquine to enhance intrinsic apoptosis whereas they inhibit 2-DG induced autophagy in Pten and p53 deficient prostate cancer xenograph [31]. Therefore they suggest the treatment for phase II clinical trials.

As a late metabolite of glycolysis, pyruvate encourages either lactate fermentation or OXPHOS. Its role in the crossroads endorses pyruvate-catalyzing enzymes in energy metabolism as potential targets. When oxygen is sufficient, pyruvate dehydrogenase irreversibly catalyzes pyruvate into acetyl CoA which is an essential substrate feeding the TCA cycle in mitochondria. Pyruvate dehydrogenase (PDH) kinase (PDK) inhibits OXPHOS by phosphorylating the enzyme into its inactive form. Lately, an increasing number of studies associate overexpression of PDK with drug resistance and recurrence [32–34]. Shen *et al*., (2012) overcome the chemoresistance to frequently used kinase inhibitor sorafenib in hepatocellular carcinoma through inhibition of PDK with dichloroacetate, thereby inducing a more Pasteur-like phenotype [35]. Moreover, dichloroacetate and cisplatin co-treatment also resulted in enhanced overall cell death in bladder cancer [32]. Nevertheless, inhibition of PDK also radiosensitizes triple-negative breast cancer cell lines in the account of ROS production result of rewired OXPHOS metabolism [36]. However, the combination therapy failed to delay cell growth *in vivo*.

Lactate is the inevitable final product of aerobic glycolysis. As pyruvate accumulates in cells and the PDH

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Table 1 Different therapeutic approaches targeting Pasteur and Warburg phenotypes

Metabolic target	Therapy	Targeted metabolic phenotype	Mechanism of action	Reference
GLUT1	Resveratrol	Warburg-like	Resveratrol decrease Akt-regulated GLUT1 localization	[58]
HK-II	2-deoxy-d-glucose (2-DG)	Warburg-like	2-DG hinders glycolysis through inhibition of HK-2	
	2-DG + Cisplatin	Warburg-like	2-DG reverse Warburg and overcome Ciplatin resistance	[21]
	2-DG + CQ	Warburg-like	2-DG reverse Warburg effect and enhanced apoptosis while CQ overcome anti-Warburg mediated autophagy	[20]
	2-DG and radiotherapy	Warburg-like	2-DG sensitize glioma to radiotherapy	[59]
	Ginsenoside	Warburg-like	Ginsenoside inhibits Warburg effect through downregulating HK-II expression via increase in miR-603	[60]
PKM2	Curcumin	Warburg-like	Curcumin downregulates PKM2 through mTOR-HIF1a inhibition and attenuates Warburg effect	[61]
PDK	DCA + Sorafenib	Warburg-like	Inhibition of PDK rewires glycolysis to OXPHOS, thereby inducing pasteur phenotype and resensitize HCC to Sorafenib	[35]
	DCA + Cisplatin	Warburg-like	Inhibition of PDK enhance PDH activity and G0-G1 arrest as well as cisplatin vulnerability in bladder cancer	[32]
	DCA + Radiotherapy	Warburg-like	Inhibition of PDK resensitize breast cancer to radiotherapy through enchanced OXPHOS capacity	[36]
LDH-A	Oxamate + Transtuzumab	Warburg-like	Inhibition of LDH and HSF mediated Warburg attenuates Transtuzumab resistance	[62]
	Cetuximab	Warburg-like	Blocking EGFR downstream signal reverse warburg effect through inhibition of HIF1 alpha related LDH	[44]
	Oxamate + N-acetyl cysteine	Warburg-like	Oxamate downregulates mTOR signaling through LDH-A inhibition as NAC overcomes oxamate-mediated autophagy	[39]
	Oxamate + Doxorubicin + Metformin	Warburg-like	The combination inhibits mTOR signal and LDH-A mRNA expression while it enhances apoptosis and autophagy	[63]
	Oxamate + Taxol	Warburg-like	Combined Therapy reduces ATP, MDA, TNF-α, Il-17 and increases antiangiogenic effect and apoptosis through inhibiiton of LDH-A related Warburg effect	[64]
	GSK 2837808A + Rapamycin	Warburg-like	Rapamycin inhibits mTORC1 which is enhanced by LDH-A inhibition-related Gln uptake	[65]
IDH-1	Ivosidenib	Warburg-like	Ivosidenib inhibits mutant IDH-1 which hinders OXPHOS and promotes Warburg via 2-HG accumulation in glioma	[66]
IDH-2	Enasidenib	Warburg-like	Enasidenib inhibits mutant IDH-2 which hinders OXPHOS and promotes warburg via 2-HG accumulation in AML	[67]
GLS	CB-839 + ABT-119	Pasteur-like	CB-839 attenuates OXPHOS through inhition of glutaminolysis and increased cell death with BCL-2 inhibiting ABT-119	[68]
	CB-839 + Erlotinib	Pasteur-like	Combined therapy induce autophagic cell death through AMPK activation and energy crisis	[69]
GLUD	EGCG	Pasteur-like	EGCG trigger cell cycle arrest and induce mitogenic apoptosis through decreased OXPHOS	[70]
Complex I	Metformin	Pasteur-like	Metformin hinders cell proliferation by inhibing complex I whereas cells bypassing mitochondrial-dependent biosynthesis resist metformin effect	[71]
	Metformin + GNE-140	Pasteur-like	Complex I inhibition prone cells to NAD+ depletion and increase LDH dependency in NAD+ regeneration, thereby re-sentisize cells to LDH-A inhibitor GNE-140	[72]
	Phenformin + Gossypol + Irinotecan	Pasteur-like	Irinotecan-induced autophagy induce cell death by ATP depletion by means of Complex I and ALDH inhibition	[73]
	Phenformin + Gemcitabine	Pasteur-like	Phenformin prone PDAC with high OXPHOS rate to gemcitabine via overexpressed Complex I inhibition	[74]

AML, acute myeloid leukemia; AMPK, AMP-activated protein kinase; BCL-2, B-cell lymphoma 2; DCA, dichloroacetate; EGCG, epigallacatechin-3 gallate; GLUD, glutamate dehyrogenase I; GLUT1, glucose transporter protein 1; HIF1a, hypoxia-inducible factor 1 alpha; mTOR, mechanistic target of rapamycin; PDH, pyruvate dehydrogenase.

enzyme loses its function, lactate dehydrogenase (LDH) destined pyruvate into lactic acid in Warburg phenotype. Thereafter monocarboxylic acid transporters (MCT) secrete this byproduct to the extracellular environment, so that boosts acidity. In recent years, there have been a considerable amount of therapeutic approaches to inhibit Warburg's character through targeting lactate production and secretion [30,37,38]. However, oxamate-mediated inhibition of LDH promotes autophagy as a survival mechanism in gastric cancer cells concerning oxamate inhibits mTOR [39]. However co-therapeutic strategies could take advantage of mTOR inhibition while they counteract anti-Warburg mediated autophagy through ULK-1 inhibitors such as chloroquine [39]. Nevertheless, Warburg-induced acidity is also another promising mediator in targeted therapy through pH-responsive nanoparticles that dissolve in an acidic microenvironment [40]. Combined with other targeted agents such as antibodies, these drug delivery systems are associated with lesser side effects as well as higher survival rates.

As glycolytic phenotype affect the fate of cancer therapy, mutations that alter the cell signaling also influence the strategies targeting energy metabolism. Kirsten Rat Sarcoma viral oncogene homolog gene (KRAS) mutations are frequently observed especially among colon and non-small cell lung cancers [41,42]. Amendola *et al.*, (2019) studied the association of KRAS4A mutations with Warburg-inducing glucose uptake and hexokinases in several cancer cell lines [43]. Their research

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illuminates that KRAS4A not only supports glucose flux but also restores HK-1 activity diminished by 2-DG through blockage of allosteric inhibition. Likewise, the inhibiting epidermal growth facto receptor downstream decreases a critical player called hypoxia-inducible factor 1 alpha (HIF-1 alpha) in reprogramming aerobic glycolysis. In their research, Haiquan *et al*., (2013) reversed Warburg phenotype by inhibition of HIF1 alpha regulating LDH-A through an EGFR blocking mAb Cetuximab [44]. The glycolysis is only inhibited in cetuximab-sensitive head and neck squamous cell carcinoma but does not affect the nontransformed cells with Pasteur phenotype.

Other favored targets in a clinical setting, MEK1 and MEK2, encourage cancer metabolism to a more Warburg-like state [45–47]. These oncogenes take the role of MAP2K in the ERK-MAPK signaling pathway. Because oncogenic mutations frequently activate the cascade, they eventually urge activation of the downstream element termed ERK1. This driver induces proliferation as well as glycolysis through transcriptional factors such as HIF1 alpha and c-MYC. An increasing number of studies point out that these factors promote most of the glycolytic enzymes such as GLUT1, LDH-A and HK-II. [48] Moreover, ERK 1-2 also phosphorylates phosphoglycerate kinase 1 (PGK1), an enzyme that activates PDK which in return inactivates gate-keeper PDH. By targeting the upstream of ERK, MEK inhibitors abate the glycolytic signal and manage a shift in Warburg cells to a more oxidative phenotype. Combined therapies coupling PDK and MEK inhibitors emerge synergic antitumor effect considering both inhibitors support a shift to Pasteur-like phenotype and ROS stress [46,47,49]. Interestingly, Guilia Cesi *et al*., (2017) suggest that MEK inhibitor (Trametinib) and BRAF inhibitor (PLX4032) inactivate PDH due to ROS-mediated activation of PDK in BRAF mutant melanoma even though the combined therapy rewires the cancer metabolism to OXPHOS. Therefore, they suggest PDK inhibitors such as AZD7545 for combination therapies in BRAF mutant and BRAF-inhibitor-resistant melanoma [50]. Targeting ETC complexes and the TCA cycle emerges as another approach to combined therapies. Phenformin, a biguanide drug inhibiting complex I, synergize with the antitumor effect of MEK inhibitor selumetinib in KRAS mutant non-small cell lung carcinoma [51]. What's more, Meng Xia *et al*., (2021) indicate that glutaminase inhibitor (CB836) combined with selumetinib suppresses glycolysis and glutaminolysis resulting in decreased OXPHOS, thereby induce autophagic cell death due to increased levels of ROS and energetic stress [52].

A great variety of biomarkers, including enzymes, receptors, channels as well as nucleic acids and peptides emerge to classify energetic subtypes. Tumor cells also alternate between Pasteur or Warburg phenotypes via miRNAs as well as metabolic intermediates and enzymes. Kha *et al*., (2020) indicate that a wide range of miRNAs modulates mitochondrial OXPHOS, de novo lipid synthesis, and glycolysis through not only targeting key enzymes and channels but also differentiating hypoxia and energy-sensing machineries such as HIF1 alpha, AMPK and SIRTs [53]. Likewise, Capula *et al*., (2019) reviewed tissue and circulating miRNAs to optimize FOLFIRINOX therapy and gemcitabine-based combinations in pancreatic cancer patients. They point out that miRNAs might be promising predictive markers when dealing with aberrant metabolism because they manage key features in glycolytic and oxidative characters [75]. For instance, LDH-A mRNA targeting miR-34a predicts the response to 5-FU and LDH-A inhibitor co-therapy [76]. Their availability in liquid biopsy also encourages the idea.

Recently, Feichtinger *et al*., (2018) point out a distinct metabolic signature of melanoma indicating that one-third of the tumors exhibit Warburg phenotype whereas the remaining are Pasteur character [7]. Moreover, they suggest OXPHOS-deficient melanomas might be vulnerable to therapeutic targeting of the energy metabolism whereas OXPHOS-competent cancers might not. Schöpf *et al*., (2020) investigate mitochondrial transcriptome profile and point out that mitochondrial DNA (mtDNA) has a distinct metabolic signature defining respiratory subgroups [8]. Moreover loss of function in Complex I support Succicinate associated pathway whereas it hinders nicotinamide adenine dinucleotide hydride-linked substrate capacity in ETC. They claimed that this phenotype is associated with worsen prognosis in pancreas cancer. Similarly, these deleterious mtDNA mutation loads also encourage impaired glucose transportation. Birsoy *et al*., (2014) draw our attention to complex I mutant cells that are more sensitive to a low glucose diet and antidiabetic biguanide treatments [77].

Conclusion

Energetic pathways combine in the heart of cancer metabolism. It is often altered through accumulating mutations, thereby rewiring cancer metabolism to a more Warburg-like state. However, recent studies indicate that both Warburg and Pasteur phenotyped cells emerge in the cancer microenvironment with several energetic subtypes. Alterations in both enzymes and signaling pathways not only differ these phenotypes but also induce diverse cellular behavior in survival and cell death. Therefore a challenge in cancer therapy dawns where the diversity in phenotypes calls for individual treatments.

Cancer profiling and unveiling heterogeneity are getting more essential because dual targeting of oncogenes and cancer metabolism drives synergic antitumor effects in most malignancies. Opportunely, new biomarkers emerge to classify the subtypes, thereby the cancer therapy, as our knowledge in coupling energy metabolism with cancer behavior grows. Indeed, glycolytic enzyme LDH is favored to figure out metabolic behavior and prognosis in the last decade [54]. Also, expression levels and mutations in rate-limiting glycolytic enzymes such

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as HK-2, PKM2 and PDK dawn as promising biomarkers along with ETC complexes and OXPHOS promoting enzymes. Moreover, recent studies suggest more comprehensive approaches like genomic and mitochondrial transcriptome as well as miRNA profiling to couple anticancer treatment and tumor metabolism. Availability of these metabolites and miRNAs harbored in extracellular vesicles such as exosomes also encourages liquid biopsies to detect subtypes concerning clonal evolution [55].

Targeting diverse metabolic phenotypes promises combined therapies coupled with conventional cancer treatment because important survival and death mechanisms serve in relation to energy metabolism. Inhibiting gate-keeping enzymes rewire these phenotypes to a more ROS producing as well as less antioxidant state. Therefore they synergize most ROS producing anticancer drugs and abate drug resistance. Furthermore, this approach could induce starvation to self-digestion when combined therapies such as the combination of biguanides and ERK-MAPK inhibitors hinder both aerobic glycolysis and OXPHOS. Similarly, managing the intake of nutrients via fasting as well as inhibitors could also be combined with certain phenotypes. Recently, comprehensive profiling studies dawn as promising approaches coupling metabolic diversity to anticancer therapies. As more resourceful profiling technologies such as single-cell and next-generation sequencing emerge, computational studies also come into prominence to forge personalized treatments targeting diverse metabolic behavior and anticancer drugs [56,57]. Overall, this area of expertise calls for further studies on combined therapies targeting subtypes in energy metabolism.

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Conflicts of interest

There are no conflicts of interest.

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