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Review

Targeting cellular metabolism to improve cancer therapeutics

Y Zhao[1,4], EB Butler[2,4] and M Tan*[,2,3]

The metabolic properties of cancer cells diverge significantly from those of normal cells. Energy production in cancer cells is abnormally dependent on aerobic glycolysis. In addition to the dependency on glycolysis, cancer cells have other atypical metabolic characteristics such as increased fatty acid synthesis and increased rates of glutamine metabolism. Emerging evidence shows that many features characteristic to cancer cells, such as dysregulated Warburg-like glucose metabolism, fatty acid synthesis and glutaminolysis are linked to therapeutic resistance in cancer treatment. Therefore, targeting cellular metabolism may improve the response to cancer therapeutics and the combination of chemotherapeutic drugs with cellular metabolism inhibitors may represent a promising strategy to overcome drug resistance in cancer therapy. Recently, several review articles have summarized the anticancer targets in the metabolic pathways and metabolic inhibitor-induced cell death pathways, however, the dysregulated metabolism in therapeutic resistance, which is a highly clinical relevant area in cancer metabolism research, has not been specifically addressed. From this unique angle, this review article will discuss the relationship between dysregulated cellular metabolism and cancer drug resistance and how targeting of metabolic enzymes, such as glucose transporters, hexokinase, pyruvate kinase M2, lactate dehydrogenase A, pyruvate dehydrogenase kinase, fatty acid synthase and glutaminase can enhance the efficacy of common therapeutic agents or overcome resistance to chemotherapy or radiotherapy.

Facts

- The metabolic properties of cancer cells are remarkably different from those of normal cells.
- Dysregulated cellular metabolism is linked to drug resistance in cancer therapy.
- Targeting metabolic enzymes improves the efficacy of cancer therapy.
- Targeting metabolic enzymes may overcome therapeutic resistance.

Open Questions

- Whether the dysregulated cellular metabolism contributes to therapeutic resistance?
- Is inhibition of metabolic enzymes a promising strategy to improve the efficacy of cancer therapy or overcome therapeutic resistance?

- Is targeting dysregulated metabolism a selective approach to inhibit cancer cells?
- What are the mechanisms by which targeting metabolic enzymes improves the efficacy of cancer therapy or overcomes chemoresistance?

The metabolic properties of cancer cells are different from those of normal cells. Cancer cells are more dependent on aerobic glycolysis, fatty acid synthesis and glutaminolysis for proliferation.[1] This difference suggests that targeting metabolic dependence could be a selective approach to treat cancer patients. In 1956, Warburg observed that the rate of glycolysis was abnormally high in cancer cells, yet a smaller fraction of this glucose is broken down by oxidative phosphorylation. This 'Warburg effect' indicates that cancer cells prefer glycolytic breakdown of glucose for energy, rather than mitochondrial oxidative phosphorylation.[1–9] Although the molecular mechanisms that define the Warburg effect are not

[1]Department of Biochemistry and Molecular Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, China; [2]Center for Cell Death and Metabolism Research, Mitchell Cancer Institute, University of South Alabama, Mobile, AL, USA and [3]Department of Cell Biology and Neuroscience, University of South Alabama, Mobile, AL, USA
*Corresponding author: M Tan, Mitchell Cancer Institute, University of South Alabama, 1660 Spring Hill Avenue, Mobile, AL 36604, USA. Tel: + 1 251 460 6993; Fax: + 1 251 460 6994; E-mail: mtan@usouthal.edu
[4]These authors contributed equally to this work.

yet fully understood, the increased glycolysis observed in cancer cells is well accepted to be important for the support of malignant phenotypes (Box 1).[8]

In addition to the dependency on glycolysis, cancer cells exhibit other metabolic characteristics such as increased fatty acid synthesis and glutamine metabolism. Enhanced fatty acid synthesis provides rapidly proliferating tumor cells lipids for membrane biogenesis, conferring both a growth and survival advantage.[10] Similarly, cancer cells are extremely sensitive to glutamine deprivation and cannot proliferate in culture without it. 'Glutamine addiction' results in enhanced production of byproducts necessary for rapidly proliferating cells, such as amino-acid precursors.[11,12]

Recently many review articles on cancer and metabolism[13–21] have been published. However, dysregulated metabolism in therapeutic resistance, a highly clinical relevant area in cancer research, has not been specifically addressed. Here we will discuss the relationship between cellular metabolism and drug resistance in cancer cells and how to improve cancer therapeutics and to overcome drug resistance by targeting dysregulated metabolic enzymes and pathways.

Box 1 The Warburg effect and cancer.
The Warburg effect is defined by an increased utilization of glucose via glycolysis as a cellular resource, and is a common phenotype of cancerous cells. The characteristic enhanced glucose uptake observed in cancer cells has been used to detect and image cancers via PET detection of 2-^{18}F-2-deoxyglucose, which preferentially concentrates within tumors as a result of their rapid uptake of glucose. Although normal cells require growth factor signaling to utilize available resources for anything more than preservation, cancer cells often display dysregulated metabolism and freely take advantage of the abundant resources available within the body. Breaking these resources down via glycolysis and glutaminolysis is more to feed and protect the cell as it grows than provide energy to maintain cellular functions. Intermediates produced through glycolysis and glutaminolysis are diverted to biosynthetic pathways that are necessary to produce the basic building blocks of cellular growth. Carbon and nitrogen from glucose and glutamine fuel nucleoside and amino-acid synthesis, whereas pyruvate feeds the citric acid cycle supporting continued fatty acid synthesis by supplying acetyl- and malonyl-CoA. The metabolic changes, such as the Warburg effect, observed in cancer allow readily available resources to be converted into biomass in an efficient manner. This metabolic shift releases cells from the typical restraints on growth, and provides a potential way to distinguish them from healthy cells – allowing for treatments that may be selective for cancerous cells. In addition, there are many links between cancer metabolism and drug resistance and compounds that influence dysregulated cellular metabolism often have the ability to increase the effect or reduce resistance to current anticancer treatments.

Dysregulated Metabolism has been Linked to Drug Resistance

The ability to reduce chemoresistance would be a significant boon for cancer patients, demonstrating the importance of research into the mechanisms underlying how chemoresistance arises (Box 2). Mounting evidence supports the idea that dysregulated cellular metabolism is linked to drug resistance in cancer therapy.[22–25] In the glycolytic pathway, lactate dehydrogenase A (LDHA) contributes to paclitaxel/trastuzumab resistance in breast cancer and pyruvate dehydrogenase

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Box 2 Anticancer agents that can be potentiated by metabolic inhibitors.
In general, anticancer agents work by interrupting critical events within the cellular lifecycle resulting in either irreversible damage to the cell or induction of apoptotic pathways. DNA replication is directly or indirectly a feature targeted by a wide variety of compounds. Both the cisplatin and temozolomide families of compounds modify the DNA, either by forming bulky adducts or by alkylating the bases, preying on the limited or compromised DNA repair ability that is common within many cancers. Similarly, nucleoside mimics such as gemcitabine and 5-FU disrupt replication by inhibiting the synthesis of deoxynucleotides – through the inhibition of ribonucleotide reductase or thymidylate synthase, respectively. More indirect methods of disrupting DNA replication target the topoisomerases, using families of compounds such as adriamycin and doxorubicin. These intercalating drugs stop DNA replication by stabilizing topoisomerase II, which prevents progression of the replication fork and ultimately leads to cellular death. Similarly, taxol class drugs indirectly target replication by stabilizing tubulin. This blocks progression of the cellular cycle, as metaphase chromosomes can no longer achieve the correct configuration, ultimately resulting in cell checkpoint activation and/or stalling of the cell cycle. Although DNA replication is a common target for current clinical anticancer drugs, it is not the only clinically effective target. Other classes of drugs affect the signaling pathways that have gone awry within cancer. The unchecked activation of these signaling networks often results in increased angiogenesis and unregulated growth. Selective estrogen receptor modulators, such as tamoxifen and raloxifen, modulate signaling through the estrogen receptor-mediated pathways and have been particularly effective for patients with certain types of breast cancer. Similarly, the EGFR family has been effectively targeted using drugs such as lapatinib (tyrosine kinase inhibitor active against EGFR and HER2) and trastuzumab (a humanized antibody targeting the HER2 receptor). The EGFR family, and specifically HER2, are aberrantly active in many types of cancers and initiate signaling pathways that lead the cells to grow aggressively and often results in a less positive outcome than seen in non-HER2 expressing tumors. We anticipate that as the molecular mechanisms defining differing subsets of cancer are better understood new drug families exploiting those characteristics will be successfully developed for clinical use.

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kinase 3 (PDK3) contributes to hypoxia-induced drug resistance in cervical and colon cancer. Fatty acid synthase (FASN), a key complex catalyzing fatty acid synthesis, is linked to acquired docetaxel/trastuzumab/adriamycin resistance in breast cancer or intrinsic gemcitabine and radiation resistance in pancreatic cancer. Finally, glutaminolysis is linked to cisplatin resistance via the activation of mammalian target of rapamycin complex 1 (mTORC1) signaling in gastric cancer (Table 1). In this review, we will discuss the role of these enzymes or processes in drug resistance in detail below.

Targeting Cellular Metabolism to Improve Cancer Therapeutics

Targeting glycolytic enzymes. As a central energetic resource for the cell, glucose metabolism is quite complex. Many enzymes contribute to the series of reactions necessary for the glycolytic breakdown of glucose. Below we will discuss glycolytic inhibition as an anticancer strategy in the context of selected components of the glycolytic pathway, such as glucose transporters (GLUTs), hexokinase (HK), pyruvate kinase M2 (PKM2) and LDHA.

Glucose transporters. The first rate-limiting step of glucose metabolism is the transport of glucose across the plasma membrane. The GLUT family of proteins is responsible for this, and are often found dysregulated or overexpressed in malignant cells.[26] The human GLUT family consists of 14 members (GLUT1-14 or SLC2A1-14).[26–28] Here we will focus on targeting GLUT1, GLUT3 and GLUT4 for improving cancer therapy.

WZB117 is an inhibitor of GLUT1 that decreases glucose uptake, intracellular ATP levels and glycolytic enzymes leading to a lowered rate of glycolysis and cellular growth. Exogenous ATP rescues growth of WZB117-treated cancer cells, suggesting that reduction of ATP is an important mechanism of WZB117's anticancer effect. WZB117 also induces endoplasmic reticulum (ER) stress leading to cell-cycle arrest. The combination of WZB117 and cisplatin or paclitaxel displayed synergistic anticancer effects (Table 1).[29,30] Under hypoxia, the GLUT1 inhibitor phloretin significantly enhances daunorubicin's anticancer effects (Table 1) and overcomes hypoxia-conferred drug resistance. Inhibition of glucose uptake by phloretin sensitizes P-glycoprotein overexpressed doxorubicin-resistant cells to daunorubicin via enhancing daunorubicin-induced apoptosis only under hypoxia.[27]

Multiple myeloma (MM) cells are dependent on GLUT4 activity for basal glucose consumption, maintenance of Mcl-1 protein levels, growth and viability. Ritonavir displays

Table 1 Targeting cellular metabolism improves cancer therapeutics

Targeted metabolism	Targeted metabolic enzymes	Metabolic inhibitors	Cancer therapeutics/ other inhibitors	Cancer types (in vitro and/or in vivo)	Refs
Glycolysis	GLUT1	Phloretin	Daunorubicin	Colon cancer (in vitro), leukemia (in vitro)	27
		WZB117	Cisplatin/paclitaxel	Lung cancer (in vitro), breast cancer (in vitro)	29
	GLUT4	Ritonavir	Doxorubicin	Multiple myeloma (in vitro)	28
	HK	2-DG	ABT-737/ABT-263	Leukemia (in vitro), cervical cancer (in vitro), hepatocarcinoma (in vitro), breast cancer (in vitro), small lung cancer (in vitro), lymphoma (in vitro), prostate cancer (in vitro and in vivo)	38–40
			Trastuzumab	Breast cancer (in vitro and in vivo)	23
			Prednisolone	Leukemia (in vitro)	54
		3-BrPA	Daunorubicin	Leukemia (in vitro)	52
			Doxorubicin	Multiple myeloma (in vitro and in vivo)	52
			Oxaliplatin/5-FU	Colon cancer (in vitro)	53
			Prednisolone	Leukemia (in vitro)	54
		LND	ABT-737	Leukemia (in vitro), lymphoma (in vitro)	39
			Prednisolone	Leukemia (in vitro)	54
	PKM2	shRNA	Cisplatin	Lung cancer (in vivo)	62
			Docetaxel	Lung cancer (in vitro and in vivo)	63
	LDHA	FX11	FK866	Lymphoma (in vivo)	66
		Oxamate	Paclitaxel	Breast cancer (in vitro)	24
			Trastuzumab	Breast cancer (in vitro and in vivo)	23
Citric acid cycle	PDK3	siRNA	Paclitaxel	Cervical cancer (in vitro)	71
			Cisplatin/paclitaxel/ oxaliplatin	Colon cancer (in vitro)	72
	PDK	DCA	Omeprazole	Fibrosarcoma (in vitro and in vivo) colon cancer (in vitro)	79
			Omeprazole + tamoxifen	Fibrosarcoma (in vitro)	80
			5-FU	Colon cancer (in vitro)	81
			Sulindac	Lung cancer (in vitro), squamous cell carcinoma (in vitro)	82
			Irradiation	Prostate cancer (in vitro)	75
Fatty acid synthesis	FASN	Cerulenin	Docetaxel	Breast cancer (in vitro)	84
			Trastuzumab	Breast cancer (in vitro)	92
			5-FU	Breast cancer (in vitro)	93
		C75	Trastuzumab	Breast cancer (in vitro)	85
		Orlistat	Adriamycin/ mitoxantrone	Breast cancer (in vitro)	22
			Gemcitabine	Pancreatic cancer (in vitro)	86

off-target inhibitory effects on GLUT4 and inhibits glucose consumption and proliferation by reducing Mcl-1 expression to induce apoptosis. Ritonavir also inhibits viability of primary myeloma cells and increases the sensitivity to doxorubicin (Table 1).[28] Temozolomide is used with radiation and chemotherapy to treat glioblastoma, yet nearly all glioblastoma patients develop resistance. Long-term treatment of glioblastoma cells with temozolomide *in vitro* induces partial resistance *in vivo* through upregulation of GLUT3, suggesting involvement in temozolomide resistance and that selective targeting of GLUT3 could delay the acquisition of such resistance in glioblastoma cells.[31] Inhibiting glucose uptake may potentiate cancer therapeutics or overcome hypoxia/drug-induced resistance.

Hexokinase. HK has important roles in both glycolysis and apoptosis and inhibitors of HK, such as 2-deoxyglucose (2-DG), 3-bromopyruvate (3-BrPA) and lonidamine (LND) are in pre-clinical and early phase clinical trials. The effects of 2-DG, 3-BrPA and LND on cell death in combination with chemotherapy or radiotherapy have been reviewed in detail.[17] We will discuss the impact of these inhibitors on cell death and their use to combat drug resistance.

2-DG is a glucose analog that is phosphorylated by HK to 2-DG-phosphate, which cannot be further metabolized. Accumulation of 2-DG inhibits glycolysis causing ATP depletion, cell cycle inhibition and cell death.[32,33] Under normoxic conditions, 2-DG can interfere with N-linked glycosylation and induce an unfolded protein response, leading to subsequent induction of some proapoptotic BH3-only proteins.[17,34] There are no ongoing clinical trials using 2-DG as a single agent as in some systems it does not have a significant effect on tumor growth *in vivo*.[35] However, combining 2-DG with radiation or chemotherapeutic treatments potentiates the tumor-destroying effects and enhances the clinical efficacy.[36]

Bcl-2 family proteins have an important role in the regulation of apoptosis, tumorigenesis and cellular response to cancer therapeutics. Bcl-2 family proteins are divided into three groups: anti-apoptotic members (Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and A1); pro-apoptotic members (Bax and Bak); and those with only a BH3 domain that promote apoptosis by binding anti-apoptotic proteins (Bad, Bid, Bim, Noxa and Puma).[37] BH3-mimetics, such as ABT-737 and ABT-263, are small-molecule inhibitors of Bcl-2, Bcl-XL, Bcl-w, but not Mcl-1. Several recent studies have demonstrated that 2-DG or LND enhances ABT-263/737-induced apoptosis both *in vitro* and *in vivo* (Table 1).[38–40] There are two proposed mechanisms explaining the effect of 2-DG on ABT-263/737-induced apoptosis. In the first 2-DG decreases Mcl-1 levels indirectly by inhibiting glycolysis and depleting ATP levels, leading to activation of AMP-activated protein kinase and inhibition of Mcl-1 translation.[38,39,41] In the second mechanism, 2-DG weakens the interaction between Bak and Mcl-1, which increases the ability of ABT-263/737 to release Bak from the Mcl-1/Bcl-XL/Bak heterotrimer, thus inducing apoptosis.[40] Both 2-DG and ABT-737 are well tolerated by patients and in clinical trials, suggesting 2-DG-ABT-737 co-treatment has the potential to be developed in treating ABT-737 resistance.

Trastuzumab is a humanized monoclonal antibody against ErbB2 and has shown efficacy treating ErbB2-positive breast cancer patients, yet acquired trastuzumab resistance occurs in most patients.[42–48] Our previous studies showed that overexpression of ErbB2 promotes glycolysis and increases their sensitivity to glycolytic inhibition.[49] Trastuzumab-resistant human cells also have increased glucose uptake and lactate production, indicative of increased glycolysis. Trastuzumab also inhibits glycolysis via downregulation of HSF1 and LDHA in breast cancer (Figure 1).[23] We found 2-DG/trastuzumab combination therapy synergistically inhibits growth of both trastuzumab-sensitive and trastuzumab-resistant human breast cancers *in vitro* and *in vivo* (Table 1), because of more efficient glycolysis inhibition.[23] These results suggest that 2-DG can effectively enhance efficacy of trastuzumab in treating ErbB2-positive human breast cancer cells and overcome trastuzumab resistance.

3-BrPA is a glycolysis inhibitor that targets HKII and depletes cellular ATP reserves, a key determinant of chemoresistance in certain cancer types.[50,51] In leukemia and MM cells increased glycolysis raises ATP levels, which activates ATP-binding cassette (ABC) transporters and confers drug resistance via enhanced drug efflux activity (Figure 1). 3-BrPA causes ATP depletion, decreasing ABC transporter activity and drug efflux, therefore enhancing drug retention in cells producing preferential cell death in malignant cells. Glycolysis inhibition by 3-BrPA not only enhances the cytotoxic effects of daunorubicin and doxorubicin, but also markedly suppresses tumor growth when used with doxorubicin to treat MM-bearing mice (Table 1).[52] In addition to activating ABC transporters, increased ATP levels from elevated glycolysis upregulate HIF-1α and enhance HIF-1α-mediated signaling, which can confer chemoresistance (Figure 1). ATP depletion by 3-BrPA partially reversed the resistant phenotype and resensitized cells to chemotherapeutic agents such as oxaliplatin and 5-fluorouracil (5-FU; Table 1).[53] These findings demonstrate that glycolysis inhibition by 3-BrPA causes ATP depletion, which can improve cancer therapeutics or overcome chemoresistance.

Most treatment failure in childhood acute lymphoblastic leukemia (ALL) is ascribed to glucocorticoid (e.g., prednisolone) resistance. Increased glycolysis is directly associated to glucocorticoid resistance and inhibition of glycolysis by 2-DG, 3-BrPA or LND increases prednisolone-induced toxicity in leukemia cells (Table 1).[54] Importantly, 2-DG can reverse glucocorticoid resistance in primary leukemia cells isolated from pediatric ALL patients.[54]

Pyruvate kinase M2. Pyruvate kinase (PK) is the last rate-limiting enzyme in the glycolytic pathway and catalyzes the conversion of phosphoenolpyruvate and ADP into pyruvate and ATP. There are four isoforms of PK in mammals (M1, M2, L and R), which are expressed in different cell types.[14,55] PKM2 is expressed predominantly in tumor cells[56] and is important for cancer metabolism and tumor growth.[57] Several studies showed a negative correlation between PKM2 expression and drug resistance.[58–60] Decreased PKM2 protein and activity is linked to cisplatin resistance while suppression of PKM2 expression by siRNA increased cisplatin resistance.[60] Both PKM2 mRNA and protein levels are downregulated in oxaliplatin-resistant cells and PKM2 mRNA levels are inversely correlated with oxaliplatin
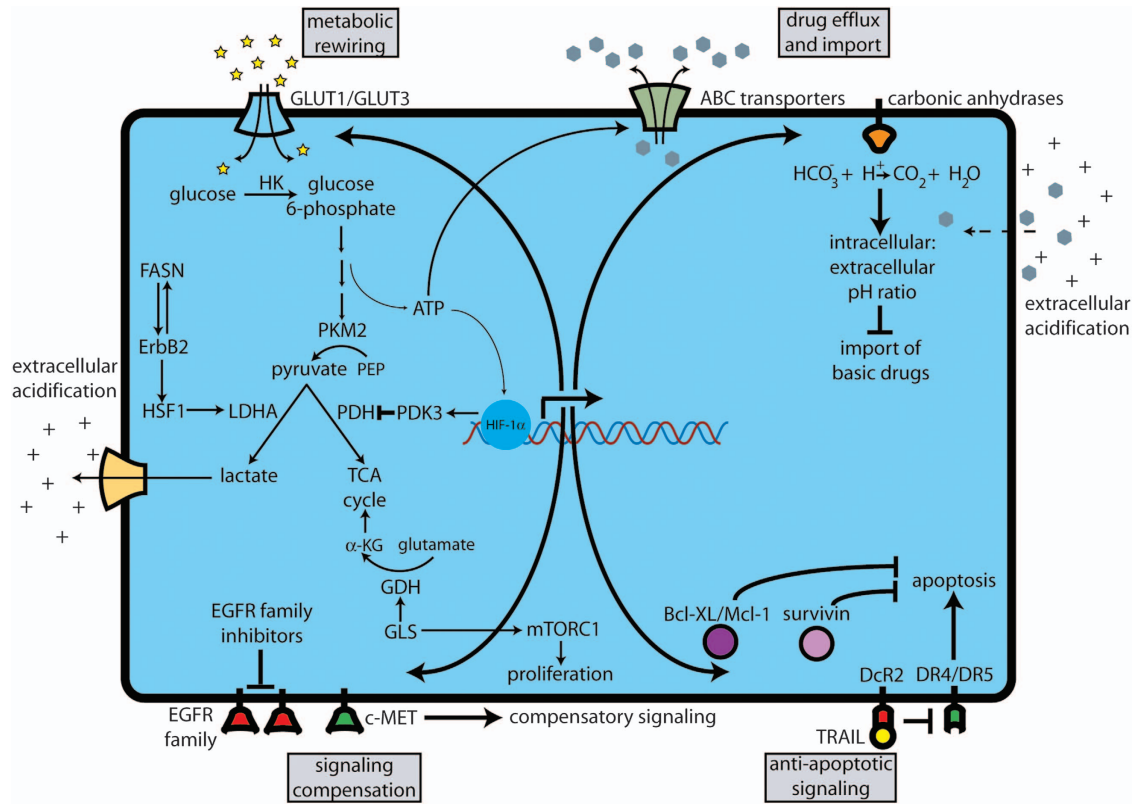
Figure 1 Dysregulated metabolism affects chemoresistance via multiple cellular pathways. Glycolytic intermediates generated by dysregulated cancer metabolism fuel expanded cellular growth and contribute to clinical resistance. ATP generated by the glycolytic breakdown of glucose fuels the active export of chemotherapeutic agents by the ABC transporters and induces HIF-1α expression. Export of the glycolytic end product, lactate and expression of carbonic anhydrases shift the pH ratio of the interior and exterior of the cell resulting in decreased passive transport of basic drugs. Signaling pathways activated by dysregulated metabolism also contribute to resistance, either via repressing pro-apoptotic signaling or activating compensatory pathways to circumvent drug-induced signal inhibition

resistance in a panel of eight colorectal cancer cell lines. Low PKM2 mRNA levels in patients are associated with high p53 protein levels and predict poor response to oxaliplatin.[59] In contrast, PKM2 levels are significantly upregulated in secreted proteins of the 5-FU-resistant colon cancer cell line. Moreover, increased PKM2 is also observed in sera and tissues from colorectal cancer patients with poor response to 5-FU. These findings suggested that upregulation of PKM2 is linked to 5-FU resistance in colon cancer.[61]

Changes in PKM2 expression are associated with drug resistance in different tumor. This indicates that PKM2 is a potential target for adjuvant cancer therapy. For example, shRNA targeting PKM2 improves the therapeutic efficacy of cisplatin by increasing apoptosis and inhibiting proliferation (Table 1).[62] Silencing of PKM2 enhances the efficacy of docetaxel because of increased inhibition of proliferation and apoptosis-inducing activity both in vitro and in vivo (Table 1).[63] A possible mechanism for the sensitization of lung cancer cells to docetaxel is that shPKM2 decreases ATP levels leading to intracellular accumulation of docetaxel.[63] These results indicated that targeting PKM2 can effectively improve the efficacy of chemotherapeutic drugs.

Lactate dehydrogenase A. LDHA catalyzes the final step in the glycolytic pathway, the conversion of pyruvate and NADH to lactate and NAD$^+$, and has a critical role in tumor

maintenance. Knockdown of LDHA in tumor cells produces increased mitochondrial respiration, decreased cellular ability to proliferate under hypoxic conditions, and suppressed tumorigenicity.[64] LDHA knockdown in the fumarate hydratase knockdown background results in increased apoptosis via ROS production, resulting in a reduction in tumor growth and indicating that LDHA might be a promising therapeutic target.[65] Inhibition of LDHA by siRNA or FX11 treatment reduces ATP levels and induces significant oxidative stress resulting in cell death.[66] Importantly, combining FX11 with FK866, an NAD$^+$ synthesis inhibitor, induces lymphoma regression in a xenograft model (Table 1).[66]

Paclitaxel (taxol) is a widely used chemotherapeutic agent in the treatment of a variety of human cancers (Table 1). LDHA expression and activity is higher in taxol-resistant breast cancer cells than in taxol-sensitive cells, and down-regulation of LDHA resensitizes taxol-resistant cells to taxol. Taxol-resistant cells are more sensitive to oxamate, a pyruvate analog that inhibits glycolysis by inhibiting the conversion of pyruvate to lactate. These results indicate that LDHA and lactate metabolism have an important role in the resistance to paclitaxel. Moreover, combination of paclitaxel with oxamate shows synergistic inhibitory effect on taxol-resistant cells (Table 1) by promoting cellular apoptosis.[24]

Heat shock factor 1 (HSF1) is the master regulator of the heat shock response in eukaryotes. HSF1 functions primarily

to coordinate the response to heat shock, but recent studies demonstrate HSF1 exhibiting non-heat shock functions important for cancer development.[67–69] Dai et al[70] reported that HSF1 increases glucose uptake, lactate production and LDH activity. Our previous study showed that ErbB2 promotes glycolysis partially through upregulation of HSF1 and LDHA (Figure 1), whereas downregulation of HSF1 leads to decreased glycolysis.[49] Our recent studies showed that trastuzumab-resistant cells have significantly higher HSF1 protein levels than trastuzumab-sensitive cells. Moreover, we found that inhibition of HSF1 sensitizes cells to trastuzumab and overexpression of HSF1 increased trastuzumab resistance, demonstrating that HSF1 can have an important role in resistance to trastuzumab.[23]

We reported that increased glycolysis via HSF1 and LDHA contributes to trastuzumab resistance. Importantly, we found that combination of trastuzumab and oxamate synergistically inhibits growth of both trastuzumab-sensitive and trastuzumab-resistant cancer both in vitro and in vivo (Table 1), because of more efficient glycolysis inhibition.[23] Overall, high-rate glycolysis confers chemoresistance and HSF1 and LDHA may potentially act as excellent targets for overcoming this resistance in cancer patients.

Targeting PDK. Pyruvate dehydrogenase (PDH) is responsible for the rate-limiting conversion of pyruvate to acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle to generate ATP. PDK phosphorylates PDH and inhibits its enzymatic activity. Four isotypes of PDK (PDK1–4) have been identified with PDK3 demonstrating the highest activity coupled with a lack of inhibition in response to high concentrations of pyruvate.[71] Hypoxia induces PDK3 expression via upregulation of HIF-1α, which binds to the promoter of PDK3, resulting in a switch from mitochondrial respiration to glycolysis for energy production. Hypoxia-mediated PDK3 induction or forced PDK3 overexpression significantly inhibits cell apoptosis and increases resistance to cisplatin or paclitaxel (Figure 1). Knockdown of PDK3 inhibited hypoxia-induced glycolysis and increases susceptibility of cancer cells to anticancer drugs such as cisplatin, paclitaxel or oxaliplatin (Table 1).[71,72] Moreover, PDK3 levels are elevated and correlated with the HIF-1α level in patient colon cancer tissues and strongly correlates with the severity of the cancer while predicting poor disease-free survival outcomes.[72] These findings indicate that PDK3 contributes to hypoxia-induced drug resistance and is potentially a novel target for improving chemotherapy or overcoming drug resistance.

Dichloroacetate (DCA) inactivates PDK leading to reactivation of PDH and a metabolic switch from glycolysis to mitochondrial respiration.[55,73] The preclinical trials on DCA have shown its effectiveness in a variety of tumors via induction of apoptosis.[74–78] However, its effect as a solitary agent is limited in the ongoing clinical trials.[79,80] Combinational therapy has displayed more effectiveness; cotreatment with DCA and omeprazole exhibits synergistic antitumor activity (Table 1).[79] Cotreatment of DCA, omeprazole and tamoxifen completely blocks the proliferation of fibrosarcoma cells (Table 1), whereas the same combination does not affect the proliferation of human normal fibroblast cells. Moreover, these three drugs were prescribed to a cholangiocarcinoma

patient and successfully blocked the disease progression for 3 months.[80] Owing to its low price, low toxicity, oral administration, long history of clinical use and ability to overcome cancer cells apoptosis resistance DCA serves as a potential metabolic-targeting molecule for sensitizing cancer cells to chemotherapy or radiotherapy.[76] DCA potentiates the anticancer effects of 5-FU (Table 1) via inducing more mitochondrial-mediated apoptosis.[81] Sulindac, a FDA-approved non-steroidal anti-inflammatory drug, has anticancer activity. The combination of DCA and sulindac enhances killing of lung and squamous cell carcinoma cells (Table 1), but not normal cells. The selective killing mechanism involves ROS production, loss of mitochondrial membrane potential, JNK-mediated signaling and apoptotic death.[82] DCA can also increase the sensitivity to radiotherapy.[75] Cao et al[75] reported that DCA sensitizes both wild-type and Bcl-2-overexpressing cancer cells to radiation (Table 1) by potentiating the apoptotic machinery via interaction with Bcl-2. In summary, targeting PDK can sensitize cancer cells to chemotherapy, radiotherapy or overcome drug resistance.

Targeting FASN. The fatty acid biosynthesis pathway catalyzes lipid synthesis from basic metabolites like acetyl- and malonyl-CoA. The FASN complex facilitates lipogenesis by synthesizing palmitate from its base components. FASN expression in normal adult tissues is generally very low or undetectable, and it is significantly upregulated and correlates with poor prognosis in many types of cancer. The metabolic products of the FASN complex are rapidly consumed by actively dividing cells and recent data demonstrates that FASN expression is important for tumor growth and survival, suggesting that FASN is a metabolic oncogene.[83]

FASN has an active role in ErbB2-induced breast cancer chemoresistance to docetaxel,[84] while trastuzumab-resistant breast cancer cells gain high sensitivity to FASN inhibition indicating that FASN is also important in ErbB2-induced resistance in breast cancers.[85] FASN is overexpressed and its activity is increased in the multidrug-resistant breast cancer cell line MCF7/AdVp3000.[22] Increased palmitic acid production from ectopic FASN overexpression is also shown to decrease adriamycin and mitoxantrone-induced apoptosis.[22] In pancreatic cancer, there is also a positive correlation between FASN expression and resistance to chemo- or radiotherapy. FASN expression is significantly upregulated in pancreatic cancer and inhibition of FASN by siRNA or the FAS inhibitor orlistat reduces gemcitabine resistance, whereas ectopic overexpression of FASN contributes to intrinsic resistance to gemcitabine and radiation. FASN-induced radiation resistance may result from decrease in radiation-mediated ceramide production, leading to reduced caspase 8-induced apoptosis. However, the mechanism of FASN-induced gemcitabine resistance remains to be elucidated.[86]

To date, several FASN inhibitors have shown antitumor activity including cerulenin, C75, orlistat, C93, GSK 837149A and natural plant-derived polyphenols. Both cerulenin and C75 are early small-molecule FASN inhibitors. Cerulenin is a natural compound isolating from Cephalosporium caerulens and contains an epoxy group that reacts with FASN to inhibit its activity. C75 is derived from cerulenin and interacts with FASN to inhibit its activity.[83] Both cerulenin and C75 induce

cancer cell apoptosis by similar mechanism including malonyl-CoA accumulation,[87] p53 accumulation,[88] induction of ER stress[89] and suppression of DNA replication.[90] FASN blockade by cerulenin synergistically enhances the efficacy of docetaxel against ErbB2-overexpressing and docetaxel-resistant SKBR3 cells (Table 1) in part via decreasing ErbB2 expression.[84] Inhibition of FASN activity with cerulenin/C75 or by siRNA upregulates the expression of PEA3, a transcriptional repressor of ErbB2, leading to downregulation of ErbB2 in ErbB2-overexpressing breast and ovarian cancer cells.[84] A combination of the FASN inhibitor cerulenin and trastuzumab synergistically downregulates ErbB2 expression, leading to more effective tumor growth inhibition (Table 1). Furthermore, inhibition of FASN activity synergistically enhances trastuzumab-induced apoptosis in ErbB2-overexpressing breast cancer cells.[91] The model proposed by Menendez et al[91] describes crosstalk between FASN and ErbB2 and suggests that FASN has a role in regulation of proliferation and cell survival by assisting in the maintenance of the cancerous phenotype. FASN inhibition affects the phospholipid partitioning and the formation of lipid rafts, which may result in the internalization and degradation of ErbB2 rather than successfully migration to the cell surface. This depletion of cell surface-associated ErbB2 could enhance the antitumor effects of trastuzumab (Table 1).[92] In addition to enhancing the efficacy of docetaxel and trastuzumab, cerulenin increases 5-FU-induced growth inhibition (Table 1).[93] Similarly, C75 and trastuzumab synergistically decrease ErbB2 expression and enhance apoptotic cell death (Table 1).[85]

Orlistat is a β-lactone compound and an irreversible inhibitor of FASN. Orlistat induces cell cycle G1/S arrest by downregulating Skp2, a component of the E3 ubiquitin ligase that controls the turnover of p27Kip1, leading to activation of the retinoblastoma protein pathway.[94] Orlistat inhibits endothelial cell proliferation and angiogenesis.[95] In addition to cytostatic effects, orlistat also has cytotoxic effects through activation of caspase-8-mediated apoptosis because of negative regulation of the mTOR pathway by upregulation of DNA damage-inducible transcript 4.[96] FASN inhibition with orlistat increases sensitivity to adriamycin and mitoxantrone in FASN-overexpressing breast cancer cells (Table 1) but not in the normal mammary epithelial cell line.[22] Orlistat treatment of pancreatic cancer cells increases the response to gemcitabine (Table 1).[86] In summary, FASN is a promising anticancer target that may result in chemosensitization or enhanced efficacy when FASN function is disrupted as part of a combinatorial treatment regimen.

Targeting glutaminolysis. Glutamine has an important role in cell growth and energy metabolism. Glutaminolysis, consists of two steps: the first is catalyzed by glutaminase (GLS) and converts glutamine to glutamate, whereas the second is catalyzed by glutamate dehydrogenase (GDH) and converts glutamate to α-ketoglutarate (α-KG).[97] There are two types of GLS in mammalian cells, kidney-type GLS (GLS1) and liver-type GLS (GLS2).[98] Metabolic flux experiments tracking ^{13}C show that cancer cells exhibiting Warburg-like metabolism do not stop utilizing the TCA cycle – instead these cells come to rely on glutamine as the carbon source for the TCA cycle.[99] This allows the intermediates

generated by the TCA cycle to feed other biosynthetic pathways as precursors.[98] Therefore, cancer cells are dependent on glutamine to maintain the TCA cycle. Glutaminolysis co-induced by glutamine and leucine activates mTORC1 signaling, which triggers cell growth and inhibits autophagy.[97] The mTOR pathway is involved in cisplatin resistance in highly malignant AFP-producing gastric cancer (AFPGC).[100] This indicates that elevated glutaminolysis is linked to drug resistance.

Bis-2-[5-phenylacetamido-1,2,4-thiadiazol-2-yl] ethyl sulfide (BPTES), an inhibitor of GLS, caused decreased aerobic cell proliferation and hypoxic cell death.[101] Inhibition of GLS by siRNA or BPTES slows the growth of glioblastoma cells with an isocitrate dehydrogenase 1 (IDH1) mutation. BPTES treatment inhibits GLS activity, lowers glutamate and α-KG levels and increases glycolytic intermediates, suggesting that simultaneous inhibition of GLS and glycolysis may be a more efficient strategy to treat mutant IDH1 patients.[102] An inhibitor of Rho GTPase-dependent cellular transformation, named 968, was found to block the growth of human breast cancer and B lymphoma cells without affecting normal cells. 968 Targets GLS C, a specific carboxy-terminal splice variant form of GLS1. Elevated levels of basal GLS activity has been shown to be dependent on Rho GTPases and NF-κB activity in transformed fibroblasts and breast cancer cells, which is blocked by 968.[25] This demonstrates that oncogenic transformation can be inhibited by targeting GLS activity, a potential therapeutic strategy against human malignancies.[11,16,25]

Rapamycin, a mTORC1 inhibitor, enhances the antitumor effect of cisplatin in AFPGC both in intro and in vivo.[100] Inhibition of mTORC1 by NVP-BEZ235, a dual PI3K/mTOR inhibitor, synergizes with chemotherapeutic agents such as cyclophosphamide, cytarabine and dexamethasone in T-cell ALL cell lines. Moreover, NVP-BEZ235 can sensitize vincristine-resistant Jurkat cells, indicating that inhibition of mTORC1 activity may revert chemoresistance.[103] Glutaminolysis activates mTORC1 signaling and inhibition of glutaminolysis via GLS inhibitors (DON and BPTES) or siRNA-targeting GLS or GDH, prevents mTORC1 activation.[97] It is reasonable to predict that targeting glutaminolysis or GLS may sensitize cancer cells to common chemotherapeutic agents by reducing mTORC1 activity.

Conclusions

Cancer cells reprogram their metabolism in order to satisfy their bioenergetic and biosynthetic requirements. Increased aerobic glycolysis, fatty acid synthesis and glutamine metabolism has been linked to therapeutic resistance in cancer. We speculate that deregulated cancer metabolism promotes cell proliferation because of increased energy production and metabolite synthesis, which decreases drug-induced apoptosis, conferring therapeutic resistance. Molecular mechanisms of drug resistance are complex and include increased drug efflux, drug inactivation, enhanced DNA damage repair and activation of pro-survival signaling (Figure 1). Increased glycolysis produces higher ATP and NADPH levels. Chemotherapeutic drugs display antitumor effects in part by inducing oxidative damage. NADPH is a critical antioxidant and high levels maintained through increased glycolysis in

cancer cells may contribute to chemoresistance. ATP exerts two effects on drug resistance: elevated ATP levels can activate ABC transporters leading to increased drug efflux and upregulate HIF-1α signaling inducing hypoxia-associated drug resistance. Both increased drug efflux and upregulation of HIF-1α signaling result in therapeutic resistance.

HIF-1α-mediated resistance occurs through a variety of mechanisms. Upregulation of the enzymes necessary for glycolysis facilitates a metabolic shift that enhances non-mitochondrial mechanisms of ATP production.[104] Reduced reliance on mitochondria results in less reactive oxygen species, which prevents the DNA damage that would activate both DNA repair and stress response pathways, steps that help set the stage for the induction of apoptotic pathways.[105,106]

The increase in glycolytic metabolism also results in the production of lactate, whose export results in the acidification of the extracellular environment. The resulting extracellular acidification coupled with HIF-1α-induced expression of carbonic anhydrases causes a significant change in the pH ratio between the intracellular and extracellular environments.[107–109] This pH shift decreases the passive absorption of many drugs that would otherwise accumulate at a greater concentration within the cell. Active drug efflux is also fueled by glycolytic ATP production and HIF-1α-induced transporter overexpression resulting in a significant decrease in the cytoplasmic retention of many anticancer agents.[110,111]

HIF-1α overexpression also induces cellular compensations that can bypass the mechanisms on which common drugs rely. Inhibitors of EGFR family signaling may demonstrate reduced effect under high HIF-1α expression because of an upregulation of c-MET, which allows alternative signaling networks to produce similar phenotypic effects in the presence of reduced EGFR family signaling.[112,113] In addition, HIF-1α induces a shift in β-tubulin isoform expression, undermining the effect of microtubule destabilizing agents.[114,115] Downregulation of other drug targets, such as topoisomerase II or estrogen receptor α (ERα), can occur when HIF-1α expression is high and reduces the effect of drugs such as tamoxifen and etoposide.[116–118]

Finally, HIF-1α induces expression of genes that promote survival through anti-apoptotic signaling (survivin, Bcl-X$_L$, Mcl-1) or other survival mechanisms such as autophagy (BNIP3, BNIP3L).[119–123] HIF-1α expression also decreases pro-apoptotic signaling by inducing the expression of decoy receptors (such as DcR2) that compete for pro-apoptotic signaling factors like tumor necrosis factor-related apoptosis-inducing ligand, thereby decreasing productive signaling through apoptosis inducing receptors including DR4 and DR5.[124,125] Attenuation of pro-apoptotic signaling allows cells to tolerate a higher level of chemotherapeutic insult before inducing cellular death pathways. HIF-1α signaling works with glycolytic metabolism to trigger a variety of anti-drug mechanisms that generate *in vitro* and clinical resistance (Figure 1). We have provided examples of how disrupting the cancer metabolism can short circuit the feedback loops that provide protection from anticancer agents.

Targeting key metabolic enzymes enhances therapeutic efficacy or combats drug resistance by promoting drug-induced apoptosis of cancer cells. ATP depletion by glycolytic inhibitors promotes intracellular drug accumulation, leading to increased drug sensitization. However, the molecular mechanisms by which targeting metabolism could impair chemoresistance is not fully understood and deserves further investigation. Combining chemotherapeutic agents with targeted disruption of dysregulated cellular metabolism represents a promising strategy to overcome drug resistance and improve the efficacy of current chemotherapeutic agents in cancer patients. Although therapeutic resistance can arise by multiple mechanisms, the examples listed above demonstrate that targeting a common feature across multiple types of cancer – dysregulated metabolism – can result in reduction of chemoresistance in a wide array of cancer types. Further investigation into the workings of cancer metabolism and resistance will help us to design more selective metabolic inhibitors allowing for a wide array of options and a more individually tailored response to chemoresistance.

Conflict of Interest

The authors declare no conflict of interest.

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