Clinical impact of the Warburg effect in gastrointestinal cancer (Review)

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Received May 24, 2014; Accepted July 9, 2014

DOI: 10.3892/ijo.2014.2563

Abstract. Cancer cells exhibit altered glucose metabolism, termed the Warburg effect, which is described by the increased uptake of glucose and the conversion of glucose to lactate in cancer cells under adequate oxygen tension. Recent genetic and metabolic analyses have provided insights into the molecular mechanisms of genes that are involved in the Warburg effect and tumorigenesis. The aim of this review was to discuss significant molecular insights into clinical impacts of the Warburg effect such as oncogenic alterations and overexpression of transcriptional factors (c-Myc and hypoxia-inducible factor), metabolite transporters (glucose transporters) and glycolytic enzymes (hexokinases 2, pyruvate kinase M2, pyruvate dehydrogenase kinase, isozyme 1, lactate dehydrogenase A). Overexpression of transcriptional factors, metabolite transporters and glycolytic enzymes was associated with poor prognosis and may be associated with chemoradiotherapy resistance in multiple gastrointestinal cancer cell types. Novel small molecules targeting these enzymes or transporters exert anti-proliferative effects. Glycolytic enzymes and metabolite transporters may be significant biomarkers for predicting cancer prognosis and may be therapeutic targets in gastrointestinal cancer.

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Key words: Warburg effect, small molecule, inhibitor, gastrointestinal cancer, prognosis

1. Introduction

Cancers exhibit altered glucose metabolism, defined as the Warburg effect (1), which is characterized by an increased uptake of glucose (2) and the conversion of glucose to lactate in cancer cells, rather than catabolizing glucose via the TCA cycle under adequate oxygen tension (3). While the electron transfer system generates 36 ATP molecules per glucose molecule across the mitochondrial inner membrane, glycolysis metabolizes glucose to pyruvate in the cytoplasm to produce a net 2 ATP molecules from each glucose. The Warburg effect represents high levels of glycolysis and thus enables the clinical application of metabolic imaging, such as ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), which is a non-invasive imaging technique that allows quantification of tumor activity on the basis of altered tissue glucose metabolism (4). Small molecule inhibitors targeting the enzymes that function in the Warburg effect have been identified and pursued in preclinical studies.

The direct mechanistic link between an activated oncogene and altered glucose metabolism is regulated by phosphoinositide 3-kinase (PI3K) (5), Akt (6), p53 (7,8), AMP-activated protein kinase (AMPK) (9,10), c-Myc and hypoxia-inducible factor (HIF). c-Myc and HIF1A transcription factors target many of the same glycolytic enzyme genes, including hexokinase 2 (HK2), pyruvate kinase type M2 (PKM2), lactate dehydrogenase A (LDHA), and pyruvate dehydrogenase kinase, isozyme 1 (PDK1). Recent investigations using genetic and metabolic analyses have provided insights into the molecular mechanisms of these genes that contribute to the Warburg effect and tumorigenesis (Fig. 1).

In this review, significant molecular insights into clinical impacts of the Warburg effect, such as oncogenic alterations and overexpression of glycolytic enzymes and metabolite transporters, will be discussed.

2. HIF-1A and c-Myc transcription factors and the Warburg effect

HIF-1A and c-Myc cooperatively induce a transcriptional program for glycolysis. HIF plays a crucial role in cellular

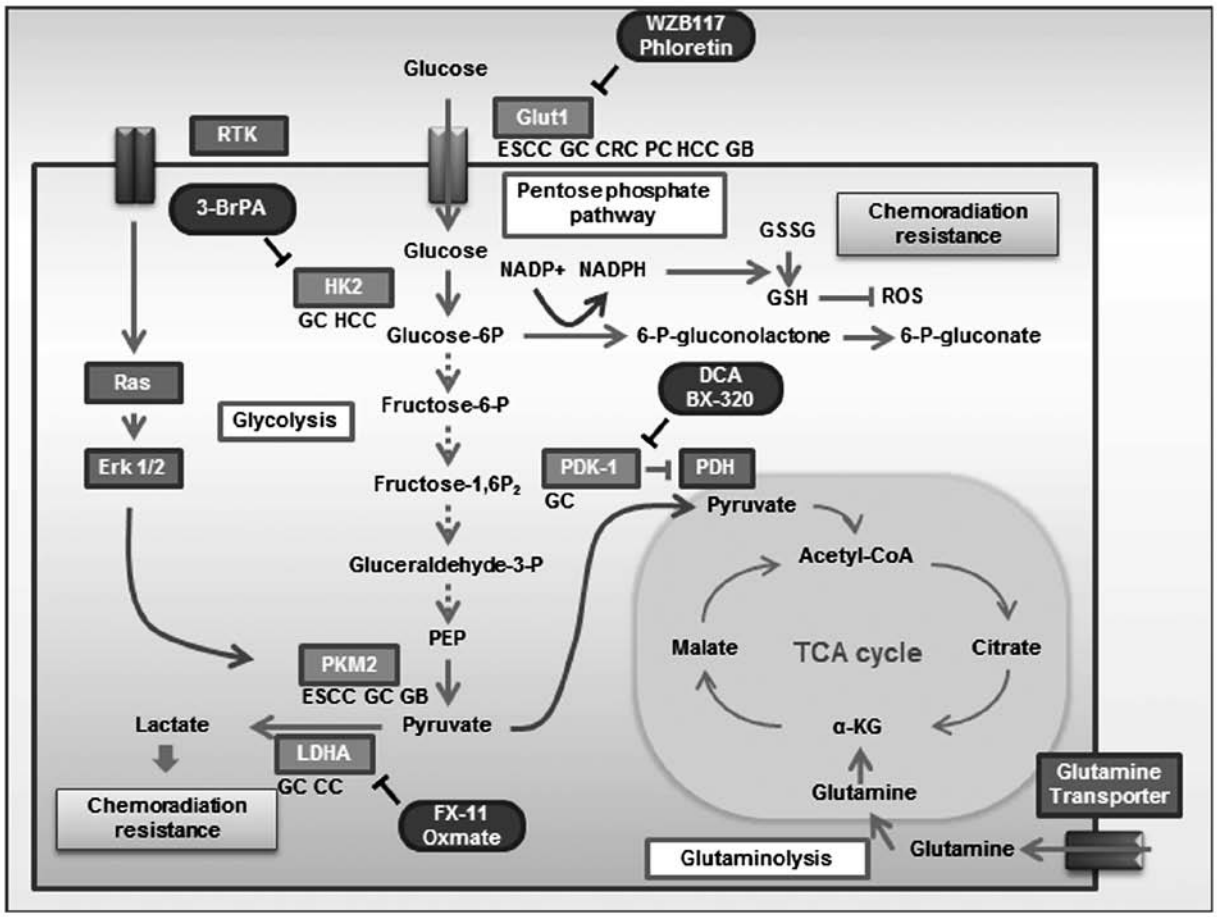

Figure 1. Glycolytic enzymes, metabolite transporters and small molecule inhibitors in the Warburg effect.

adaptation to hypoxia and regulates the expression of genes responsible for glucose metabolism, angiogenesis, and cell survival (11). Cellular HIF levels are regulated by both an oxygen-dependent pathway and an oxygen-independent pathway. HIF contains two key regulatory subunits, HIF1A and endothelial PAS domain protein 1 (EPAS1; HIF-2), and the genes encoding these proteins are overexpressed in human cancers (12,13). Many studies have assessed the significance of HIF-1A positive expression in the prediction of clinical outcome of gastrointestinal cancer. HIF-1A expression is associated with poor prognosis in esophageal squamous cell carcinoma (ESCC) (14,15), gastric cancer (16,17), colorectal cancer (CRC) (18) and hepatocellular carcinoma (HCC) (19). Low expression of HIF1A may be associated with a favorable effect of 5-FU-based adjuvant chemotherapy in gastric cancer patients (20,21). HIF-2A is associated with poor survival in gastric cancer patients (22) but not CRC patients (18,23).

The *c-Myc* oncogene, a member of the MYC family, encodes the transcription factor c-Myc and is upregulated in many human cancers, linking altered cellular metabolism to tumorigenesis (24). *MYC* gene expressions are often elevated or deregulated in human neoplasms, and c-Myc seems to be at the crossroads of several important pathways and processes involved in carcinogenesis. *MYC* deregulation due to gene amplification (25), chromosomal translocation or insertion (26), mutations (27), and epigenetic modifications (28) has been reported in different types of cancers. The number of studies of *MYC* expression as detected by immunohistochemistry (IHC) is less than that of HIF1A. *c-Myc* overexpression and promoter hypomethylation may have a role in the gastric carcinogenesis process and *c-Myc* deregulation was associated mainly with poor prognosis (29). *c-Myc* expression detected by IHC was associated with poor prognosis in pancreatic cancer (30), but its expression was not associated with poor prognosis in CRC patients (18,23) (Table I).

3. Glucose transporters (Gluts)

Glut1 is composed of 492 amino acid residues and possesses a single site of N-linked glycosylation at N45 (31). Multiple glucose transporter-like proteins have been identified and characterized (32) with sequence similarity to Glut1, and these genes appear to belong to the family of solute carriers 2A (SLC2A, protein symbol Glut). The 14 Gluts are categorized into three classes based on sequence similarity: Class 1 (Gluts 1-4 and 14), Class 2 (Gluts 5, 7, 9 and 11), and Class 3 (Gluts 6, 8, 10, 12, and HMIT) (32). Glut families were evaluated with the GEO data set *in silico* (http://www.ncbi.nlm.nih.gov/gds/). Glut1 mRNA levels were remarkably upregulated in tumor lesions compared with normal lesions in CRC (GDS 4382), ESCC (GDS 3838) and pancreatic cancer (GDS 4336) (Table II). Several studies have been published on Glut family members, especially Glut3 (33-35), but Glut1 has been the main focus of investigation. A previous study evaluating Glut1

Table I. Impact of HIF and MYC on cancer prognosis and correlation with clinicopathological features.

Total					Prognosis		
Organ	N	%	Cut-offs	Expression correlated with: (condition)	Univariate	Multivariate	(Ref.)
HIF-1A							
ESCC	1,261			Depth of invasion, N^+, stage, VEGF (meta-analysis-2011)	NA	NA	(16)
ESCC	215	68	Scores 3-4	VEGF	DFS: poor OS: NS	DFS: NS OS: NS	(14)
ESCC	96	68	Score 4-6	N^+ (T1b patients)	DSS: poor DFS: poor	Poor	(15)
GC				NA (meta-analysis 2003-2012)	DFS: NS OS: poor	NA	(16)
GC	1103			Differentiation, T-stage N^+, ly^+, v^+, stage (meta-analysis 2003-2013)	OS: poor	NA	(17)
GC	216	39	>10%	$HIF1A^+$-$p53^+$ cases undifferentiated, ly^+, N^+	OS: poor	OS: poor	(84)
GC	193	52		N^+	DFS: NS OS: NS	DFS: poor OS: poor	(45)
GC	128	66	>5%	Histology, depth of invasion VEGF expression, MVD	DFS: poor OS: poor	DFS: poor OS: poor	(85)
GC	64	58		No correlation [adjuvant CT S-1 (77%)]	DFS: poor DSS: poor	DFS: poor DSS: NS	(21)
GC	44	57	>10%	No correlation (adjuvant CT 5-FU based)	DSS: poor DFS: poor	NA	(20)
CRC	731	19	>50%	COX-2, CIMP-high LINE1 hypomethylation	CSS: poor OS: poor	CSS: poor OS: poor	(18)
RC	90	54		N^+, v^+, stage	DFS: poor CSS: poor	OS: poor	(23)
RC	92	55	Scaling system	pT4, N^+, v^+ ($T3,4/N^{+/-}$)	DFS: poor OS: poor	DFS: poor OS: poor	(86)
HCC	953			Tumor grade, N^+, v^+ (meta-analysis-2013)	DFS: poor OS: poor		(19)
HCC	110			Male, LC, COX-2, PDGFRA MMP7, MMP9, MYC	DFS: poor OS: poor	DFS: poor OS: poor	(87)
HCC	200	63		Intrahepatic metastasis	DFS: poor OS: poor	DFS: poor OS: poor	(88)
PC	50	66	>5%	VEGF	DFS: NS OS: NS	NA	(89)
HIF-2A							
GC	80	38	>Score 0	Diffuse type	DFS: poor OS: poor	CSS: NS OS: NS	(22)
CRC	731	19	>50%	Low tumor grade, male, BMI<30	CSS: NS OS: NS	CSS: NS OS: NS	(18)
RC	90	64		No correlation	DFS: NS CSS: NS		(23)

Table I. Continued.

Organ	Total N	Total %	Cut-offs	Expression correlated with: (condition)	Prognosis Univariate	Prognosis Multivariate	(Ref.)
MYC							
GC	125	77	>10%	Intestinal-type, late-onset deeper tumor extension, M^+	NA	NA	(29)
PC	70	52	Score 5-9	Perineural invasion, stage	OS: poor	OS: poor	(30)
CRC	731	19	>50%	Low tumor grade male, BMI<30	CSS: NS OS: NS	CSS: NS OS: NS	(18)
RC	90	64		No correlation	DFS: NS CSS: NS		(23)

ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; CRC, colorectal cancer; RC, rectal cancer; HCC, hepatocellular carcinoma; PC, pancreatic cancer; NA, no assessment; NS, not significant.

Table II. Overexpression of metabolite transporters and glycolytic enzymes in the Warburg effect.

	Colorectal cancer (N=17) GDS4382			ESCC (N=17) GDS3838			Panctreatic cancer (N=45) GDS4336		
	T/N ratio	95% CI	P-value	T/N ratio	95% CI	P-value	T/N ratio	95% CI	P-value
Glut1	1.90	(1.16-3.09)	0.01	2.44	(1.78-3.34)	<0.001	3.58	(2.74-4.67)	<0.001
Glut2	0.92	(0.87-0.98)	0.01	0.97	(0.93-1.01)	NS	0.60	(0.46-0.80)	<0.001
Glut3	1.55	(0.72-3.31)	NS	1.96	(1.23-3.13)	0.01	1.54	(1.18-2.01)	<0.001
Glut4	1.18	(0.99-1.40)	NS	0.97	(0.83-1.13)	NS	0.89	(0.83-0.97)	0.01
Glut5	0.52	(0.37-0.72)	<0.001	1.08	(0.80-1.44)	NS	1.10	(0.91-1.31)	NS
Glut6	0.84	(0.71-1.00)	0.05	1.31	(1.12-1.54)	<0.001	0.95	(0.88-1.02)	NS
Glut8	0.59	(0.10-3.60)	NS	1.17	(1.00-1.36)	0.04	0.93	(0.88-0.98)	0.01
Glut9	1.13	(1.01-1.26)	0.03	1.21	(1.01-1.45)	0.04	1.07	(0.98-1.17)	NS
Glut10	0.65	(0.35-1.19)	NS	0.89	(0.66-1.18)	NS	1.13	(1.02-1.25)	0.02
Glut11	1.33	(0.96-1.85)	NS	0.96	(0.88-1.05)	NS	0.85	(0.78-0.93)	<0.001
Glut14	1.72	(1.08-2.72)	0.03	1.45	(1.04-2.02)	0.03	1.10	(0.98-1.23)	NS
HK2	0.46	(0.26-0.79)	0.009	1.53	(1.16-2.03)	0.005	2.55	(1.97-3.30)	<0.001
LDHA	1.05	(0.92-1.19)	NS	0.92	(0.78-1.10)	NS	1.89	(1.57-2.28)	<0.001
PMK2	0.80	(0.65-0.98)	0.033	1.41	(1.02-1.95)	0.04	2.03	(1.72-2.40)	<0.001
PDK1	1.18	(0.85-1.63)	NS	1.37	(1.08-1.74)	0.012	1.38	(1.18-1.61)	<0.001

by IHC in tissue microarray slides comprising 1,955 samples detected Glut1 positivity in 47% prostate adenocarcinomas, 29% thyroid cancer, 10% gastric cancer, 5% breast adenocarcinomas, 36% head and neck SCC, 42% uterine cervix SCC, 18.6% glioblastomas and 9.4% retinoblastomas (36).

Glut1 is transcriptionally regulated by c-Myc (24) and HIF1A (37). A recent study demonstrated that Glut1 was one of three genes consistently upregulated in cells with KRAS or BRAF mutations (38). Glut1 expression in CRC cells was positively correlated with FDG accumulation and KRAS/BRAF mutation (39). EGFR and ERK1/2 correlate with levels of PKM2 Ser 37 phosphorylation, and nuclear PKM2 induces c-Myc expression, resulting in the upregulation of Glut1 (40). In a recent study using xenografts, overexpression of Glut1 in a mammary tumor cell lines with low levels of endogenous Glut1 results from both a decrease in apoptosis and an increase in proliferation (41).

Glut1 expression is generally absent in normal tissue, but in multiple gastrointestinal cancer cell types, Glut1 expression is remarkably enhanced. Glut1 positivity is associated with poor

prognosis in diverse gastrointestinal cancers, ESCC (15,42,43), gastric cancer (44,45), CRC (46,47), pancreatic cancer, HCC (48), and gallbladder cancer (49,50) (Table III).

Glut1 expression has the potential to serve as a biomarker for cancer. Anticancer therapies, such as radiation and several chemotherapeutic drugs, induce oxidative stress in targeted cells. Reactive oxygen species (ROS) are required for the fixation of radiation-induced DNA damage (51). Therefore, an accumulation of antioxidants (e.g., lactate) may induce or enhance resistance to radiation and may cause chemoresistance (52). Glut1 positivity was associated with tumor regression grade (TRG) and may be a useful predictive marker of response to chemoradiotherapy in rectal cancer (47,53).

Phloretin, a natural product found in apples and pears with Glut inhibitory activity, exerts antitumor effects in HCC and color cancer cell lines (54,55). The WZB117 small molecule inhibitor of Glut1 was effective in inhibiting cancer cell growth both *in vitro* and *in vivo* (56) (Table IV).

4. Glycolytic enzymes (HK2, PKM2, PDK1 and LDHA)

Hexokinases catalyze the phosphorylation of glucose to glucose-6-phosphate. This is the first and rate-limiting step in glucose metabolism. HK2 is one of four members of the hexokinase family. The four isoenzymes (HK1, HK2, HK3, and glucokinase) are structurally similar, but only HK1 and HK2 are functionally similar. HK2, but not HK1, is overexpressed in several cancer types compared with normal tissue. HK2 localizes to the outer membrane of the mitochondria and is the major hexokinase isoform expressed in cancer cells (57). High expression of HK2 confers a poor prognosis in HCC and gastric cancer (Table II), and HK2 positivity was associated with poor differentiation and advanced stage in HCC (58,59). Tumor differentiation in HCC correlated with FDG uptake (60), and the cellular retention of FDG may be mediated by HK2 (58).

The widely used 3-bromopyruvate (3-BrPA) (61) depletes cellular ATP. A previous study showed that 3-BrPA inhibits HK2 expression and exhibits anti-proliferative effects combined with daunorubicin in CRC cell lines (54) and combined with protein disulfide isomerase in HCC cell lines *in vivo* (55).

Pyruvate kinase (PK) is a glycolytic enzyme that catalyzes a reaction generating pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP. Four isoforms of PK (L, R, M1, and M2) have been identified in mammals. Splicing of PKM is controlled by splicing repressors, and the expression of the repressors is upregulated by c-Myc oncoprotein (62,63). M2 is expressed in embryonic cells, adult stem cells, and cancer cells and is necessary for aerobic glycolysis. This metabolic phenotype provides a selective growth advantage for tumor cells *in vivo* (64,65). PKM2 expression is associated with poor prognosis in ESCC, gallbladder cancer and signet ring cell carcinoma of gastric cancer (Table III). Small molecule inhibitors that selectively target PKM2 have been identified, suggesting that inhibition of PKM2 could be synergistic with other targeted therapies, including gefitinib. However, small molecule activation of PKM2 that promotes PKM2 tetramer formation interferes with anabolic metabolism and suppresses tumorigenesis (66). Mutation of the ERK-phosphorylation site S37 in PKM2 blocked translocation of PKM2 to the nucleus (40), suggesting that PKM2 moves into the nucleus as a monomer. Tumor cells have multiple ways to regulate PKM2 that are favorable to cell growth and survival, including PKM2 expression, localization, post-translational modification, and allosteric regulation. PKM2 also regulates non-metabolic functions as a transcriptional coactivator and protein kinase. PKM2 is considered an attractive target for cancer treatment (67). Further studies are needed before inhibitors and activators of PKM2 can be used as therapeutic interventions (68).

PDK regulates the mitochondrial gatekeeper pyruvate dehydrogenase (PDH), which links glycolysis to the TCA cycle by reversible phosphorylation. Phosphorylation of PDH by PDK inhibits the action of PDH and halts pyruvate use in the TCA cycle (69). Four PDK isoforms have been verified in human tissue, and the expressions of the isoforms are organ specific. PDK-1 positivity was associated with poor prognosis in gastric cancer (70), but expression of PDK-1 was decreased in colon cancer compared with normal tissue. PDK-3 expression was detected in colon cancer, and PDK-3 positivity was associated with poor prognosis (71). Several studies reported the relationship between PDK positivity and prognosis in gastrointestinal cancer, but the clinical significance of PDK expression has remained unclear. Many small molecule PDK-1 inhibitors have been identified (72). DCA, a PDK-1 inhibitor, reduced lactate production and increased responsiveness to 5-FU in MKN45 cells (70) and CRC cell lines (73). DCA treatment exerts anti-proliferative effects and sorafenib resistance in HCC cell lines *in vivo* (74).

Lactate dehydrogenase is a tetrameric enzyme comprising two major subunits, A and/or B, resulting in five isozymes (A4, A3B1, A2B2, A1B3 and B4) that can catalyze the forward and backward conversion of pyruvate to lactate. LDHA (LDH-5, MLDH, or A4), which is the predominant form in skeletal muscle, kinetically favors the conversion of pyruvate to lactate, controlling the conversion of pyruvate to lactate in the cellular glycolytic process (75). Many human cancers have higher LDHA levels than normal tissues (76). LDHA is specifically phosphorylated at Y10 in various cancer cell lines, head and neck SCC, lung cancer, breast cancer, and prostate cancer cells and by diverse oncogenic tyrosine kinases, including FGFR1, ABL, JAK2, and FLT3 (77).

LDHA reduction can suppress the tumorigenicity of intestinal-type gastric cancer (ITGC) cells, colon cancer (78) and HCC (79). A previous study of 661 ITGC specimens showed that low LDHA expression exhibited better overall survival than high LDHA expression (80).

Similar to small interfering RNA (siRNA) reduction of LDHA expression, the FX11 small molecule inhibitor for LDHA could increase cellular oxygen consumption, increase ROS production, and induce cell death that could be partially rescued by the antioxidant NAC in a lymphoma cell line (81). Oxmate, a lactate dehydrogenase inhibitor, combined with phenformin exhibited cytotoxic effects in diverse cancer cell lines, including colon cancer (82).

5. Conclusions and future perspectives

This review describes the significance of protein expression of the transcriptional factors, glycolytic enzymes and metabo-

Table III. Impact of Glut1 and glycolytic enzymes on prognosis and correlation with clinicopathological features.

Total					Prognosis		
Organ	N	%	Cut-offs	Expression correlated with: (condition)	Univariate	Multivariate	(Ref.)
Glut1							
ESCC	145	43	>50%	pT3, v^+ MVD (no preoperative treatment)	DFS: poor CSS: poor	DFS: NS CCS: NS	(42)
ESCC	63	48	>30%	No correlation (curative operation)	OS: poor	OS: NS	(43)
ESCC	96	71	Score 4-6	N^+ (T1b patients)	DFS: poor CSS: poor	NS	(15)
GC	617	30	>1%	pap>por or tub, T-stage N^+, ly^+, v^+, H^+, stage	OS: poor	OS: poor	(44)
GC	152	24	>30%	T2-T4, N^+, diffuse type	DFS: NS OS: NS	DFS: NS OS: NS	(70)
GC	193	43		Age >65, T2-T4, N^+, stage, intestinal type	OS: poor	OS: NS	(45)
CRC	163			Poorly differentied higher in stage III + IV	OS: poor	OS: poor	(90)
CRC	112	18	>50%	N^+,	CSS: poor	CSS: poor	(46)
RC	46	48	>10%	No correlation	DFS: p=0.066	NA	(47)
PC	94	75	>50%	Historogical grade, MIB1 (ductal AC)	OS: poor	OS: poor	(91)
HCC	63	37	Scoring ≥Score 1	SUV, TNR, Ki67LI	DFS: poor OS: poor	NA	(48)
GB	56	34	>50%	Perinecrotic areas	OS: poor	NA	(49)
GB	71	52		Histologic tumor type tumor stage	OS: poor		(50)
HK2							
GC	257	17	>30%	No correlation	DFS: NS OS: NS	DFS: NS OS: NS	(92)
GC	152	5	>30%	No correlation	DFS: NS OS: NS	DFS: NS OS: NS	(70)
GC	188	21		Size, lower differentiation, stage, HIF1A	OS: poor	OS: poor	(93)
HCC	157	15	High mod.	Moderately and poorly, advanced stage	OS: poor	OS: poor	(58)
HCC	97	56		No correlation	OS: poor	NA	(94)
HCC	31	81		Moderately and poorly differentiated	OS: NS	OS: NS	(59)
PKM2							
ESCC	180	80	IRS strong mod.	Differntiation poorly tumor size, stage	OS: poor	OS: poor	(95)
GC	368	39	>25%	Age, t-stage, well differentiatied	OS: NS	OS: NS	(96)
GC	79	18	>25%	Subgroup analysis above study (signet cell)	OS: poor	OS: poor	(96)
GB	80	56	>25%	Differntiation poorly, tumor size, stage, N^+	OS: poor	OS: poor	(97)

Table III. Continued.

Organ	Total N	Total %	Cut-offs	Expression correlated with: (condition)	Prognosis Univariate	Prognosis Multivariate	(Ref.)
PDK-1							
GC	152	12	>30%	T3-T4, N^+, tumor size HIF-1A	DFS: poor OS: poor	DFS: poor OS: poor	(70)
CC	74	-	Blot density	Expression PDK1 deceased in cancer tissue	NA	NA	(70)
PDK-3							
CC	206	86	$Stain^+$	Stage, HIF-1A	DFS: poor OS: poor	NA	(71)
LDH-5							
GC	94	62		Advanced tumor, v^+ HIF-1A, VEGF, COC-2	DFS: poor OS: poor	NA	(98)
CC	128	77		Poor differentiation HIF1A, pKDR	DFS: poor OS: poor	NA	(78)

ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; CRC, colorectal cancer; RC, rectal cancer; HCC, hepatocellular carcinoma; PC, pancreatic cancer; GB, gallbladder cancer; MVD, microvessel density; ductal AC, ductal adenocarcinoma; NA, no assessment; NS, not significant.

Table IV. Anti-proliferative effect of inhibitors of metabolite transporters and glycolytic enzymes.

Target	Inhibitor	Cancer type (cell lines)	Dose *in vitro*	Dose *in vivo*	Combination or drug resistance	(Ref.)
Glut1	WZB117	LC (A549)	10 μM	10 mg/kg (i.p.) daily	NA	(56)
	Phloretin	CRC (SW620)	50 μM	NA	DNR	(54)
Glut2	Phloretin	HCC (HepG2)	200 μM	10 mg/kg (i.p.) 3 times per week	DNR	(55)
HK-2	3-BrPA	HCC (Huh-7)	100 μM	1 mg/kg (i.p.)	PDI	(99)
	3-BrPA	CRC (HCT116, HT29)	30 μM	NA	Ox-resistant cells	(100)
PKM-2	Compound 3	LC (H1299), hematopoetic (FL5.12)	30 μM	NA	Gefitinib	(101)
PDK	DCA	HCC (Huh-7)	30 mM	100 mg per kg bw per day	Sorafinib-resistant cells	(74)
	DCA	GC (MKN45, AGS)	20 mM	NA	5-FU	(70)
	DCA	CRC (SW620, LoVo, LS174t, HT29)	10 mM	NA	5-FU	(73)
	BX-320	CRC (HCT116) PC (MiaPaCa)	0.28 μM 0.33 μM	NA	NA	(102)
LDHA	FX11	Lymphoma (P493)	9 μM	42 μg (i.p.) daily	FK866	(81)
	Oxmate	CRC (CT26)	9 μM	NA	Phenformin	(82)

DCA, dichloroacetate; 3-BrPA, 3-bromopyruvate; DNR, daunorubicin; PDI, protein disulfide isomerase; Ox, oxaliplatin; LC, lung cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; GC, gastric cancer; NA, no assessment; NS, not significant.

lite transporters involved in the Warburg effect as potential biomarkers. The functional and therapeutic importance of the Warburg effect is increasingly recognized, and glycolysis has become a target of anticancer strategies. Novel small molecule inhibitors targeting enzymes that function in the Warburg effect have been developed and anti-proliferative effects on diverse cancer cells have been demonstrated. The gene expressions of molecular factors involved in the Warburg effect are associated with poor prognosis and may be associated with chemoradiotherapy resistance in gastrointestinal cancers. Novel small molecules exert anti-proliferative effects and may reduce chemoradiotherapy resistance in gastrointestinal cancer, breast cancer (83) and lung cancer (56) (Table IV).

Future studies should examine whether inhibitors of glycolytic enzymes and metabolite transporters are useful in gastrointestinal cancer and evaluate adverse effect and feasibility for clinical practice. Furthermore, validation of imaging techniques, which establish glucose metabolism and predict response to drugs, is required for optimal patient selection.

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