

REVIEW ARTICLE

The Warburg effect and drug resistance

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The Warburg effect describes the increased utilization of glycolysis rather than oxidative phosphorylation by tumour cells for their energy requirements under physiological oxygen conditions. This effect has been the basis for much speculation on the survival advantage of tumour cells, tumourigenesis and the microenvironment of tumours. More recently, studies have begun to reveal how the Warburg effect could influence drug efficacy and how our understanding of tumour energetics could be exploited to improve drug development. In particular, evidence is emerging demonstrating how better modelling of the tumour metabolic microenvironment could lead to a better prediction of drug efficacy and the identification of new combination strategies. This review will provide details of the current understanding of the complex interplay between glucose metabolism and pharmacology and discuss opportunities for utilizing the Warburg effect in future drug development.

Abbreviations

AMPK, AMP-activated protein kinase; CSC, cancer stem cell; GLUTs, glucose transporters; HIF, hypoxia-inducible factor; HK2, hexokinase 2; HSF1, heat shock factor 1; LG, low glucose; mTOR, mammalian target of rapamycin; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PI3K, phosphoinositide 3-kinase; PKM2, pyruvate kinase M2; ROS, reactive oxygen species

Tables of Links

TARGETS		
Other protein targets ^a	Enzymes ^c	Transporters ^d
Bak	AMPK	ABC transporters
Bax	Akt (PKB)	GLUT1
Bcl-xL	c-Raf	GLUT2
Mcl-1	ERK	GLUT3
Notch receptors	Hexokinase	GLUT4
Catalytic receptors ^b	HIFs	Monocarboxylate transporters
EGFR	LKB1 (<i>STK11</i>)	
HER2	MEK	
Kit	mTOR	
MET	PDHK	
	PI3K	
	PIK3CA	

LIGANDS	
5-fluorouracil	Gemcitabine
3-bromopyruvate	Lactate
Acetyl-CoA	LY294002
ADP	Paclitaxel
ATP	Phosphoenolpyruvate
β-catenin	Pyruvate
Carboplatin	Ritonavir
Cisplatin	Sorafenib
Daunorubicin	Temozolomide
Etoposide	Trastuzumab

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (^{a,b,c,d}Alexander *et al.*, 2015a,b,c,d).

Introduction

In 1924, Otto Warburg reported the observation that cancer cells used glycolysis more than mitochondrial oxidative phosphorylation (OXPHOS) for their energy requirements (Diaz-Ruiz *et al.*, 2011; Mathupala *et al.*, 2010; Vander Heiden *et al.*, 2009). Now termed the 'Warburg effect', the increased aerobic glycolysis in many tumours has been extensively scrutinized and is accepted as a feature of tumours (Ganapathy *et al.*, 2009; Gatenby and Gillies, 2004; Gatenby and Gillies, 2007). Over the years, a better understanding of why tumours bear these characteristics has developed. More recently, data have emerged indicating that the Warburg effect could also influence drug efficacy. This review will summarize current knowledge on the role of the Warburg effect in tumourigenesis and drug efficacy and how this information could be exploited to optimize cancer drug testing and cancer treatment.

The Warburg effect and cancer

The key biochemical process promoting the Warburg effect is glucose metabolism. Glucose metabolism is a complex process involving glycogenolysis and gluconeogenesis, both of which regulate blood glucose levels.

An essential function of glucose is to provide cellular energy for supporting various biochemical reactions. Glycolysis and OXPHOS are the two major metabolic pathways responsible for generating ATP, a coenzyme responsible for generating energy for cellular processes (Icard *et al.*, 2014; Solaini *et al.*, 2011; Villalba *et al.*, 2013). OXPHOS, which is a mitochondrial reaction, is a more efficient metabolic process, generating more ATP than glycolysis and is, therefore, preferentially utilized by normal cells for their energy requirements. The rate-limiting requirement for OXPHOS is oxygen and almost every aerobic organism utilizes this metabolic pathway. OXPHOS also leads to the generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (Solaini *et al.*, 2011). This leads to the production of free radicals, which by themselves can catalyse a variety of processes including DNA damage and cellular signalling. Glycolysis is an anaerobic fermentation process and is less efficient for ATP generation. The process also generates NADH and results in the accumulation of pyruvate and lactate. The utilization of glycolysis is employed by cancer cells even in the presence of oxygen and is therefore also known as 'aerobic glycolysis'.

As a result of increased glycolysis, one of the key metabolic features of cancer cells is rapid utilization of glucose to fuel glycolysis. Indeed, this increased utilization of glucose by tumours forms the basis for using the glucose analogue tracer ¹⁸fluorodeoxyglucose in PET imaging for tumours in the body (Kelloff *et al.*, 2005). The metabolic reactions of glycolysis are catalysed by numerous enzymes, of which enzymes such as hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2) are implicated in cancer (Hirschhaeuser *et al.*, 2011). In addition, the excessive lactate produced as an end product creates an acidic tumour microenvironment, facilitating tumour migration and invasion (Gatenby and Gillies, 2004; Hirschhaeuser *et al.*, 2011).

As discussed previously, glycolysis is essentially an anaerobic reaction and the fact that cancer cells utilize it in the presence of oxygen affords them a survival advantage. This is because the tumour microenvironment is characterized by areas of hypoxia (low oxygen) (Kim *et al.*, 2009), which ultimately compromises OXPHOS. Therefore, in cancers, the hypoxic microenvironment may lead to enhanced glycolysis, rather than defects in mitochondrial OXPHOS. Furthermore, lowered OXPHOS leading to reduced ROS may protect cancer cells from the cytotoxic effects of oxidative damage (Denko, 2008; Nogueira *et al.*, 2008). The dependence of cancer tissues on glycolysis over OXPHOS may also help in the generation of biosynthetic precursors that are required for nucleic acid, amino acid and lipid synthesis to fuel proliferation (Vander Heiden *et al.*, 2009). Furthermore, mitochondrial membrane permeabilization by Bax and Bak is a critical step in the induction of the apoptotic cascade and OXPHOS is known to activate Bax and Bak (Harris *et al.*, 2000). The propensity of tumour cells to rely on glycolysis over OXPHOS serves as a potential mechanism to circumvent apoptosis and hence promote tumourigenesis (Tomiyama *et al.*, 2006).

The Warburg effect and oncogenes

Over the years, many oncogenes and pathways have become linked to glucose metabolism, providing further evidence for the role of glucose metabolism in tumourigenesis (Figure 1). The RAS oncogene activates the MEK and the phosphoinositide 3-kinase (PI3K) pathways that regulate essential proliferative and survival cascades. These pathways are frequently activated in cancer, and mutant RAS has been reported to induce mitochondrial dysfunction and confer a glycolytic phenotype (Hu *et al.*, 2012). In addition, when colorectal cancer cells with wild-type RAS were cultured in a glucose-deprived environment, they acquired RAS mutations (Yun *et al.*, 2009). Hence, an auto-regulatory loop could be envisaged, in which the deprivation of glucose induces mutant RAS, which further promotes glycolysis.

Through the PI3K pathway, RAS can also activate Akt and the mammalian target of rapamycin (mTOR), both of which have been reported to activate hypoxia-inducible factors (HIFs) (Abraham, 2004; Pore *et al.*, 2006). HIFs are transcription factors critical for regulating the transcription of numerous oncogenes and glucose transporters (GLUTs), which all contribute to tumour progression and survival (Rohwer and Cramer, 2011). AKT has also been reported to directly stimulate aerobic glycolysis in both solid tumours and cancers of haematopoietic origin (Elstrom *et al.*, 2004), although the precise mechanism is unclear. In addition to PI3K/mTOR, MEK activation of ERK has also been known to activate HIFs and in turn be reactivated by HIFs (Minet *et al.*, 2000; Mottet *et al.*, 2002). Furthermore, activated MEK and PI3K/mTOR pathways are also known to up-regulate HK and PKM2 (Ru *et al.*, 2013; Sun *et al.*, 2011).

The oncogene MYC is overexpressed in many cancers and is also known to positively regulate the Warburg effect by up-regulating the expression of GLUTs, LDH and stimulating increased glutaminolysis (Dang *et al.*, 2009; Osthus *et al.*, 2000; Wise *et al.*, 2008). The p53 tumour suppressor can inhibit

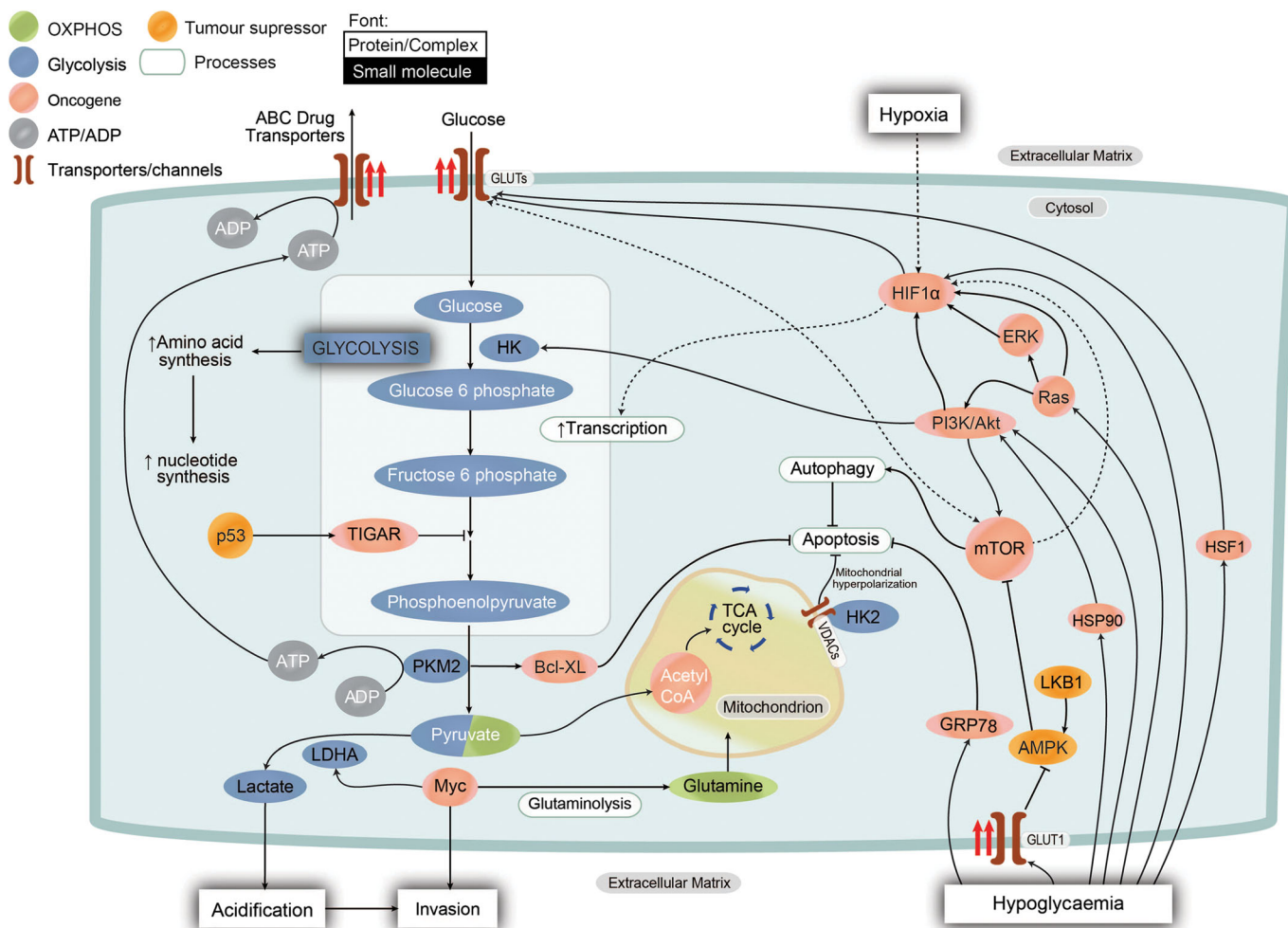


Figure 1
The role of glucose metabolism in tumourigenesis.

glycolysis through activation of TIGAR (*TP53*-induced glycolytic and apoptotic regulator) (Xie *et al.*, 2014). TIGAR inhibits GLUTs as well as fructose-2,6-biphosphate, a key glycolytic enzyme. Through loss of TIGAR, loss of p53 function through its frequent inactivation in cancer, could conceivably be a major contributor to the glycolytic phenotype of tumours (Ma *et al.*, 2007). The Bcr–Abl oncogene that drives chronic myelogenous leukaemia has also been reported to stimulate glucose uptake and increase glycolysis (Gottschalk *et al.*, 2004). There is also evidence indicating that other key oncogenic drivers positively regulate a Warburg phenotype in cancer cells, including components of the Wnt, Hedgehog and PI3K signalling pathways (Blouin *et al.*, 2010; Foster *et al.*, 2012; Pate *et al.*, 2014; Teperino *et al.*, 2012).

The Warburg effect and drug resistance

In addition to promoting tumourigenesis, the metabolic differences of cancer cells provide an environment that often increases drug resistance or provides an opportunity for

intervention. The potential mechanisms by which components of the glycolytic pathway may confer a chemoresistant phenotype are discussed in the following section.

Glucose transport

The entry of glucose inside the cell is facilitated by a family of 14 transporters known as the GLUTs (Thorens and Mueckler, 2010), of which GLUT1, GLUT3 and GLUT4 have been the most widely studied in cancer. It has been observed that cancer cells can oppose the activation of AMP-activated protein kinase (AMPK) evoked by increased glucose and ATP by up-regulating GLUT1. Indeed, GLUT1 up-regulation has been reported in numerous malignancies (Wang *et al.*, 2013). Increased GLUT1 is associated with the activation of mTOR leading to an increase in glycolysis and reduction in autophagy (Bhattacharya *et al.*, 2014; Buller *et al.*, 2011). Increased mTOR activation may also lead to increased GLUT1 expression, forming a positive feedback loop (Buller *et al.*, 2008). A study using lung and breast cancer models has demonstrated a potent antitumour effect of the GLUT1 inhibitor, WZB117, linked to a decrease in glycolysis and consequent reduction

in intracellular ATP (Liu *et al.*, 2012). The compound interacted synergistically with paclitaxel or carboplatin in these cancer models. Similar reports of synergistic interactions between WZB117 and daunorubicin have been reported in colorectal cancer and leukaemic cells *in vitro* (Cao *et al.*, 2007).

Multiple myeloma has been shown to be dependent on GLUT4 for glucose consumption, maintenance of Mcl-1 levels and growth and survival (McBrayer *et al.*, 2012). The antiviral drug, ritonavir, is known to inhibit GLUT4 (Vyas *et al.*, 2010) and inhibits the proliferation of primary multiple myeloma cells in addition to enhancing the effect of doxorubicin (McBrayer *et al.*, 2012). GLUT3 is the major neuronal glucose transporter, and its expression is significantly increased in brain tumours (Flavahan *et al.*, 2013). In addition, prolonged exposure of glioblastoma cells to temozolomide leads to acquired resistance to the drug, which is associated with an increase in GLUT3, and selectively targeting GLUT3 delayed the onset of such acquired resistance (Le Calve *et al.*, 2010).

Glycolytic enzymes

The enzymes directly regulating glycolysis have also been implicated in promoting a drug-resistant phenotype. HK2, the first rate-limiting enzyme in the glycolytic pathway, is known to be up-regulated in many cancers and inhibits mitochondrial apoptosis by direct insertion in the mitochondrial outer membrane (Jang *et al.*, 2013; Mathupala *et al.*, 2006). The direct interaction of the voltage-dependent anion channel with mitochondrial bound HK2 inhibits cytochrome c release and subsequent apoptosis (Pastorino *et al.*, 2002). Furthermore, survival pathways such as the PI3K/Akt/mTOR pathway can also activate HK in cancer cells and induce drug resistance (Min *et al.*, 2013).

Due to its contribution to regulating apoptosis and cellular bioenergetics, HK2 is considered to be an important anticancer drug target. The HK2 inhibitor, 3-bromopyruvate, reduces ATP reserves, which is often considered a significant factor in promoting a chemoresistant phenotype (Geschwind *et al.*, 2004; Ko *et al.*, 2001). This is because raised ATP levels directly influence the activity of ATP-binding cassette transporters, which contribute to drug efflux and promote chemoresistance (Ruetz and Gros, 1994). Furthermore, elevated ATP levels, as a result of increased glycolysis, have been known to activate HIF1 α , which in turn can lead to drug resistance (Morten *et al.*, 2013).

PKM2 is the final rate-limiting enzyme of the glycolytic pathway and is involved in the conversion of phosphoenolpyruvate and ADP into pyruvate and ATP. This enzyme is represented by four isoforms (M1, M2, L and R) and is expressed on different cell types. PKM2 is highly expressed in cancers (Seton-Rogers, 2011) and maintains high lactate levels, which is potentially oncogenic on its own (Gatenby and Gillies, 2007). Indeed, a recent study has demonstrated the role of lactate as a signalling intermediate in hypoxic conditions leading to activation of survival pathways (Lee *et al.*, 2015). The authors demonstrated lactate-dependent stabilization of a protein, NDRG3, which binds to c-Raf and promotes neovascularization and survival. Apart from promoting cell survival, lactate can also attenuate immune signalling, for example, tumour-derived lactate can prevent human T-cell response (Fischer *et al.*, 2007). Additionally,

inhibiting the monocarboxylate family of transporters that include lactate transporters is being considered as a potential therapeutic option for cancer treatment (Porporato *et al.*, 2011).

In addition to its role in regulating glycolysis, PKM2 has also been reported to promote cell survival and prevent apoptosis by increasing the expression of Bcl-xL (Kwon *et al.*, 2012) and activating transcription factors such as β -catenin, STAT3 and HIF (Gatenby and Gillies, 2007; Jang *et al.*, 2013; Vander Heiden *et al.*, 2009).

Clinically, an association between PKM2 expression levels and drug resistance has been reported. Increased PKM2 levels have been linked with resistance to 5-fluorouracil in patients with colorectal cancer (Shin *et al.*, 2009). Also, genetic silencing of PKM2 potentiates the effects of docetaxel and cisplatin in *in vitro* and lung cancer xenograft models (Guo *et al.*, 2011; Shi *et al.*, 2010). In contrast, other studies have demonstrated a negative correlation between PKM2 levels and resistance to drugs such as cisplatin (Martinez-Balibrea *et al.*, 2009; Yoo *et al.*, 2004). These conflicting reports and the absence of a clear consensus on the actual role of PKM2 in the development of drug resistance have raised concerns on the potential of PKM2 as a valid cancer drug target.

Pyruvate, an essential molecule of the metabolic machinery, can feed into either glycolysis or OXPHOS. The enzyme pyruvate dehydrogenase (PDH) catalyses the reaction that converts pyruvate into acetyl-CoA, thereby promoting mitochondrial respiration (Tomiya *et al.*, 2006). PDH kinase inactivates PDH leading to inhibition of OXPHOS and formation of lactate. Lower levels of PDH were associated with sorafenib-acquired resistance in hepatocellular carcinoma cells, which was reversed using a PDH kinase inhibitor (Shen *et al.*, 2013). Another enzyme, LDH, converts pyruvate into lactate that is the end product of glycolysis (Vander Heiden *et al.*, 2009). Increased levels of LDHA have been demonstrated to confer resistance to trastuzumab in breast cancer (Zhao *et al.*, 2011a).

Glycolysis and cancer stem cells

Amongst a heterogeneous population of tumour cells, some are believed to be rapidly proliferating but unable to generate tumours, while a fraction of cells with a slow rate of division generate new tumour-initiating cells. The latter type of cells are known as cancer stem cells (CSCs) and were initially identified in acute myeloid leukaemia (Lapidot *et al.*, 1994). Since then, the presence of these CSCs has been observed in both solid and haematological malignancies (Lobo *et al.*, 2007). Due to their slow rate of division, over expression of drug efflux pumps, detoxification enzymes and anti-apoptotic proteins, CSCs are believed to possess a drug-resistant phenotype (Vinogradov and Wei, 2012). Similar to normal stem cells, the CSCs reside in niches, a microenvironment that is capable of maintaining a balance between self-renewal and differentiation. The major difference between the niches of normal and CSCs is the predominance of proliferative signals in the latter. The tumour niche has been characterized to involve hypoxia and hypoglycaemia as a result of glycolysis. Indeed, hypoxia has been reported to generate CSCs in tumours, which can lead to a drug-resistant phenotype (Heddleston *et al.*, 2010).

In addition to hypoglycaemia, the Notch signalling pathway is typically associated with 'stemness' and is also affected by glucose. Breast cancer xenografts with high Notch activity have been demonstrated to grow better in glucose-deprived conditions compared with tumours with low Notch activity (Goodman, 2012). However, this study did not determine whether the survival advantage of the tumours was due to the initiation of CSCs. Further evidence supporting the role of glycolysis in generating CSCs comes from the observation that PKM2 via TIGAR and p53 interact with 'stemness' transcription factors, for example, Oct4 (Bensaad *et al.*, 2006; Green and Chipuk, 2006). The acidic microenvironment created by increased glycolysis in tumours is also considered to provide a favourable niche for CSCs (Martinez-Outschoorn *et al.*, 2011).

Glycolysis and hypoglycaemia

An obvious consequence of increased glycolysis is an increased utilization of glucose by tumour cells. The increased glucose consumption in turn creates a hypoglycaemic microenvironment or a nutrient-deprived state. This relationship of the tumour with its microenvironment can be envisaged as being almost parasitic. Consequently, a nutrient-deprived state can be perceived as a stress signal by tumour cells, which may lead to the activation of stress signalling pathways to support proliferation and survival (Altman and Rathmell, 2012). Furthermore, the tumour microenvironment is also characterized by hypoxia and poor leaky vasculature and lactic acidosis, which may be associated with extreme metabolic stress, thereby potentiating stress-activated signalling (Schneider *et al.*, 2008). These stress-related signalling components are intricately linked with survival signalling and are discussed in the following sections.

Stress response elements

Studies have indicated that many stress response elements are activated as a result of the altered microenvironment associated with the Warburg effect. Heat shock protein 90 (HSP90), a molecular chaperone evoked during stress reactions, has been reported to be activated by hypoglycaemic stress (Saito *et al.*, 2009). HSP90 has been found to provide a cytoprotective response to drug treatment by preventing the degradation of client proteins such as Akt, EGFR, HER2, Kit, MET and Bcr-Abl (Clarke *et al.*, 2000; Martins *et al.*, 2012; Sain *et al.*, 2006; Workman, 2004). Glucose-regulated protein 78, a molecular chaperone in the endoplasmic reticulum, which confers cytoprotective effects from apoptosis signals, has also been known to be activated by hypoglycaemia (Fu and Lee, 2006). Colon carcinoma cells cultured in hypoglycaemic medium were rendered resistant to etoposide treatment and were resensitized by glucose-regulated protein (GRP) 78 inhibitors (Hwang *et al.*, 2008).

Heat shock factor 1 (HSF1) has been known to regulate heat shock responses, and glucose deprivation has been reported to activate HSF1 (Eickelberg *et al.*, 2002). HSF1 has also been reported to increase glucose uptake and promote glycolysis (Dai *et al.*, 2007), and down-regulation of HSF1 decreases glycolysis and hence may be involved in a feedback loop. HSF1 signalling also promotes cellular adaptation to stress by activating survival signals, which can lead to drug resistance (Page *et al.*, 2006).

Studies have shown that trastuzumab-resistant cells have higher levels of HSF1, and inhibition of HSF1 sensitizes cells to trastuzumab. This was attributed to an increase in glycolysis by HSF1, and the combination of oxamate (glycolysis inhibitor) and trastuzumab interacted synergistically in the resistant cells (Zhao *et al.*, 2011b).

Energy sensors

The tumour suppressor *STK11* encodes LKB1, a serine threonine kinase regarded as a 'master kinase' for regulating essential cellular functions. Such functions include energy conservation, cell polarity and various metabolic processes (Lizcano *et al.*, 2004). AMPK is one of the major substrates of LKB1 (Shackelford and Shaw, 2009) and is also a central energy-sensing kinase. A reduction in ATP and increase in AMP activate AMPK. Activated AMPK in turn inhibits mTOR signalling and protein translation for conserving energy (Jeon *et al.*, 2012). Furthermore, AMPK activation leads to cell cycle arrest as a result of p53 stabilization and activation of cyclin-dependent kinase inhibitors p21 and p27 (Jones *et al.*, 2005; Liang *et al.*, 2007).

During nutrient-deprived states, cancer cells, by initiating AMPK signalling, halt energy-consuming processes. Under normal physiological conditions, such cells undergo apoptotic cell death. However, cancer cells under nutrient deprivation or hypoglycaemic conditions avoid cell death through the induction of autophagy. A key regulator of autophagy is mTOR, while apoptosis is controlled intrinsically by the mitochondrial machinery. Inhibition of mTOR as a result of AMPK activation activates autophagy, thus promoting cell survival (Jung *et al.*, 2010). It is believed that autophagy promotes cell survival by recycling intracellular organelles to produce energy. Autophagic cells are highly resistant to treatment with both conventional and targeted agents, therefore providing a connection between glycolysis and drug resistance (Duffy *et al.*, 2014; Martinez Marignac *et al.*, 2013; Nihira *et al.*, 2014; Sui *et al.*, 2013). Nonetheless, prolonged autophagy and AMPK activation can also lead to additional signalling changes, which ultimately will lead to cell death.

The informativeness of supraphysiological glucose levels in preclinical drug testing

Recently, our laboratory tested the hypothesis that a reduced glucose microenvironment can increase chemoresistance due to its activation of survival pathways (Bhattacharya *et al.*, 2014). The basis for this study was the observation that traditional drug testing was routinely performed on cells grown in glucose-rich (25 mM) medium, a concentration that is approximately five times higher than normal physiological glucose levels (5.6 mM) (Kleman *et al.*, 2008). We surmised that culturing cells in conditions closer to its 'native' environment may generate results that would more closely reflect physiological efficacy. We assessed the sensitivity of two *PIK3CA* mutant and two *PIK3CA* wild-type gastric cancer cells grown in routine high glucose (25 mM, HG) or low glucose (5 mM, LG) media to two standard-of-care cytotoxic agents (5-fluorouracil and carboplatin), a PI3K/mTOR inhibitor

(PI103) and a mTOR inhibitor (Ku-0063794). In support of our hypothesis, resistance to the cytotoxic agents was significantly increased in all cell lines in LG conditions, despite a lack of difference in growth characteristics between HG and LG cells. LG-associated resistance to PI3K/mTOR inhibitors was also observed but only in *PIK3CA* mutant cells. Further investigation revealed the class-specific resistance to be accompanied by selectively increased GLUT1, mTOR activation, increased lactate and reduced ROS, highlighting increased glycolysis as a feature of cells susceptible to LG-associated chemoresistance. We further demonstrated that this resistance can be selectively attenuated by a combination of PI103 and Ku-0063794 treatment. These data were consistent with a 'synthetic lethal' model that explained why synergy only occurred in cells with mutant *PIK3CA*, increased glycolysis and low extracellular glucose but not in cells with only some of these features.

Another study employing pancreatic cancer cell lines and culturing them in LG and hypoxia demonstrated emergence of resistance to gemcitabine, which was partially sensitized by IY294002 (PI3K inhibitor) and UCN-01 (cell cycle inhibitor) (Onozuka *et al.*, 2011). The mechanism of resistance was attributed to a reduction in the proliferation rate of the cells in LG and hypoxic conditions. However, sorafenib cytotoxicity was significantly increased in rat liver stem cells upon glucose withdrawal and inhibition of glycolysis by 2-deoxyglucose (Tesori *et al.*, 2015). This result suggests that a reduction of glucose in the culture medium does not

necessarily have to be associated with drug resistance. It may also lead to an enhancement of drug activity particularly if the cells or tumour are dependent on glycolysis and mitochondrial damage.

Conclusion and perspectives

Attrition rates for anticancer drugs are high compared with other therapeutic areas (Hoelder *et al.*, 2012). While the introduction of high-throughput technologies has enhanced the understanding of cancer biology, it has not been very successful in improving the dismal success rates in transitioning anticancer agents from the laboratory to the clinic. This is in part due to the multifaceted nature of the disease itself, with factors that include genetic and clonal heterogeneity and the complexity of the tumour environment.

The fact that the effectiveness of drugs is tested in models that poorly simulate the tumour microenvironment should be considered as it is also likely to have a role in the attrition (Figure 2). It can be presumed that Warburg's observations of the high glucose requirement of cells had a role in the practice of supplementing cells with extremely high glucose concentration (25 or 11 mM). However, we and others have now begun to demonstrate that cancer cells can be cultured in normoglycaemic and hypoglycaemic conditions, and a different drug response can be expected. In addition, the blood

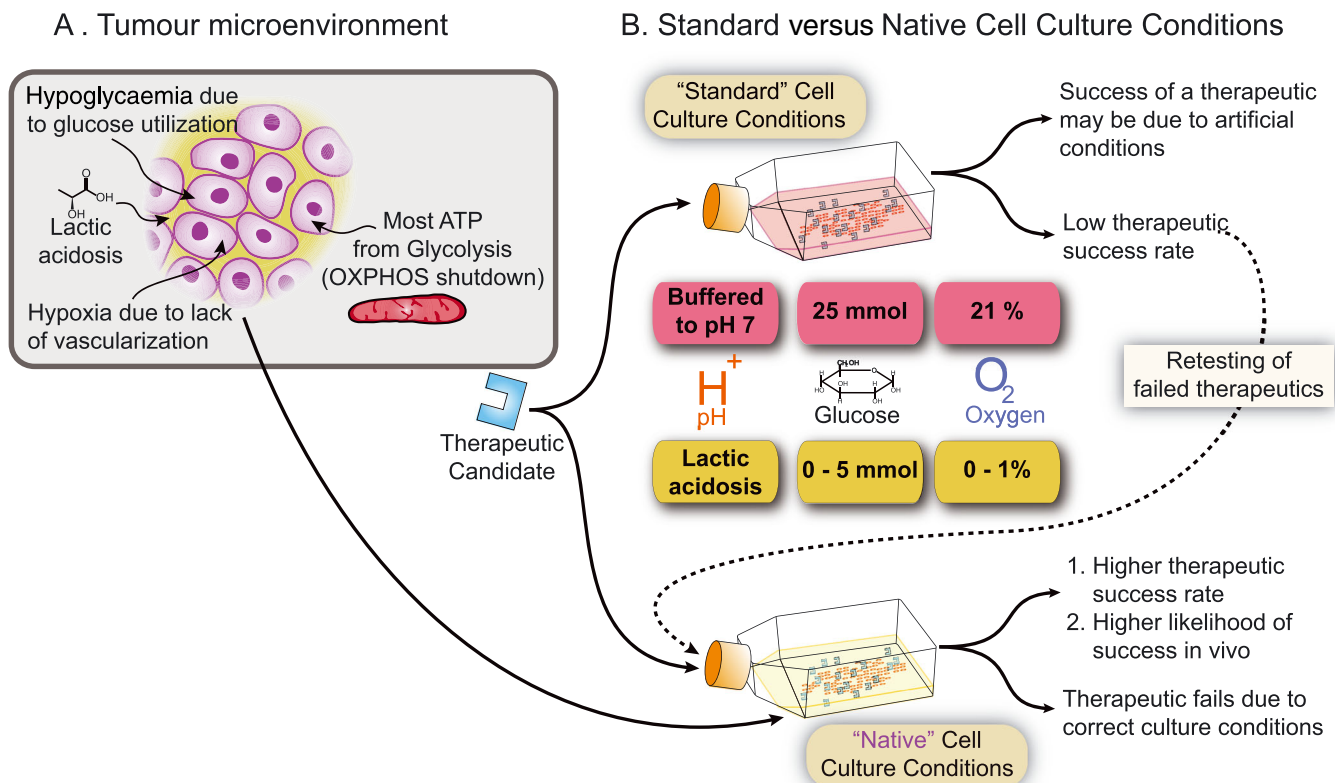


Figure 2

The concept of 'Native Culture' System.

glucose concentration of the mice used for *in vivo* testing also simulates an extreme diabetic state. In addition to better efficacy prediction, the testing of anticancer agents on cancer cells in models closer to their 'native' microenvironment may also help to define a new range of active compounds. The 'native' culture conditions should utilize physiological or hypoglycaemic levels of glucose (depending on metabolomic data of the cancer in question) in the culture medium along with the creation of a lactic acidosis like condition (by addition of lactic acid) and incubating cancer cells in hypoxic conditions. The combination of these three very important factors can therefore simulate the aspects of tumour microenvironment in an artificial model system. Understandably, many validation steps, for example, optimization of cell proliferation and other phenotypic characterization, need to be performed concurrently with standard culture conditions. However, once characterized, cells cultured in 'native' conditions retain the potential to be very informative in early-phase or even late-phase drug screens. Inclusion of other microenvironment components such as immune cells and fibroblasts can also be incorporated to study the immune response and therapeutic index of investigational agents. Furthermore, a drug repurposing screen can also be included in 'native' culture screens along with other agents that were considered inactive in standard culture conditions.

Author contributions

B. B. conceived the idea of 'native' environment and culture system, conducted background research and wrote the manuscript. M. H. M. O. created the illustrations used in this review. R. S. provided critical editing and advice and wrote specific sections of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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