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SYMPOSIUM REVIEW

Revisiting the Warburg effect: historical dogma *versus* current understanding

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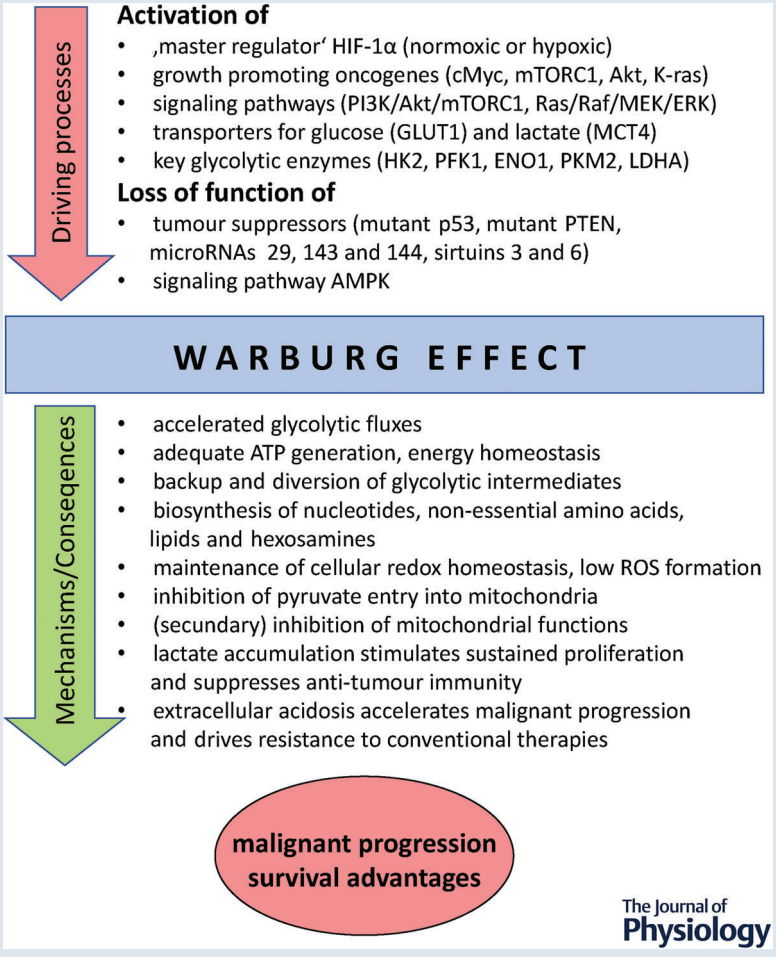

Abstract Contrary to Warburg's original thesis, accelerated aerobic glycolysis is not a primary, permanent and universal consequence of dysfunctional or impaired mitochondria compensating for poor ATP yield per mole of glucose. Instead, in *most tumours* the Warburg effect is an

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essential part of a 'selfish' metabolic reprogramming, which results from the interplay between (normoxic/hypoxic) hypoxia-inducible factor-1 (HIF-1) overexpression, oncogene activation (cMyc, Ras), loss of function of tumour suppressors (mutant p53, mutant phosphatase and tensin homologue (PTEN), microRNAs and sirtuins with suppressor functions), activated (PI3K–Akt–mTORC1, Ras–Raf–MEK–ERK–cMyc, Jak–Stat3) or deactivated (LKB1–AMPK) signalling pathways, components of the tumour microenvironment, and HIF-1 cooperation with epigenetic mechanisms. Molecular and functional processes of the Warburg effect include: (a) considerable acceleration of glycolytic fluxes; (b) adequate ATP generation per unit time to maintain energy homeostasis and electrochemical gradients; (c) backup and diversion of glycolytic intermediates facilitating the biosynthesis of nucleotides, non-essential amino acids, lipids and hexosamines; (d) inhibition of pyruvate entry into mitochondria; (e) excessive formation and accumulation of lactate, which stimulates tumour growth and suppression of anti-tumour immunity – in addition, lactate can serve as an energy source for normoxic cancer cells and drives malignant progression and resistances to conventional therapies; (f) cytosolic lactate being mainly exported through upregulated lactate–proton symporters (MCT4), working together with other H^+ transporters, and carbonic anhydrases (CAII, CAIX), which hydrate CO_2 from oxidative metabolism to form H^+ and bicarbonate; (g) these proton export mechanisms, in concert with poor vascular drainage, being responsible for extracellular acidification, driving malignant progression and resistance to conventional therapies; (h) maintenance of the cellular redox homeostasis and low reactive oxygen species (ROS) formation; and (i) HIF-1 overexpression, mutant p53 and mutant PTEN, which inhibit mitochondrial biogenesis and functions, negatively impacting cellular respiration rate. The glycolytic switch is an early event in oncogenesis and primarily supports cell survival. All in all, the Warburg effect, i.e. aerobic glycolysis in the presence of oxygen and – in principle – functioning mitochondria, constitutes a major driver of the cancer progression machinery, resistance to conventional therapies, and poor patient outcome. However, as evidenced during the last two decades, in a *minority* of tumours primary mitochondrial defects can play a key role promoting the Warburg effect and tumour progression due to mutations in some Krebs cycle enzymes and mitochondrial ROS overproduction.

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Abstract figure legend Driving processes causing the Warburg effect during carcinogenesis (upper part), and mechanisms/consequences of metabolic reprogramming in Warburg phenotypes (lower part) leading to survival advantages, malignant progression and, ultimately, poor patient outcome.


Peter Vaupel obtained his Dr. med. degree in 1969 at the University of Mainz (Germany) followed by postdoctoral research in Physiology. He was appointed Professor of Physiology in 1975. In 1987 he took up the Andrew Werk Cook Professorship in Radiation Biology/Tumour Biology/Physiology at Harvard Medical School. In 1990–2008 he was Professor and Chairman of the Institute of Physiology & Pathophysiology, University of Mainz. His research interests include the oxygenation of tumours, pathophysiology of tumour hypoxia and glucose supply. In 1993 he (in a pioneering observation together with Michael Höckel) elucidated the hypoxia-triggered pathogenesis of malignant progression of cancers. Since 2008 he has been Professor Emeritus and Senior scientist at the University Medical Centers in Mainz and Freiburg (Germany). In 1966 he had the unique opportunity to discuss oxygen supply problems in normal and tumour tissues with the Nobel laureate Otto H. Warburg. **Gabriele Multhoff** obtained her Dr. rer. nat. degree in 1991 at the Ludwig-Maximilians-University Munich (Germany) followed by postdoctoral research in Immunology. In 2002 she was appointed Professor of Molecular Oncology (University of Regensburg, Germany). Since 2007 she has held a Professorship for Radiooncology/Radiobiology at the Technical University Munich. Her major interests include the role of heat shock proteins in the induction of anti-tumour responses, the identification of tumour-specific markers for multimodal imaging, and the analysis of hypoxia-triggered immunosuppressive mechanisms.

Introduction

We are approaching the 100th anniversary of Otto H. Warburg's first description of the enhanced, accelerated conversion of glucose to lactate in malignant tumours even in the presence of abundant oxygen (Warburg, 1923; Warburg *et al.* 1924). He attributed this metabolic trait to an (primary) 'irreversible respiratory injury not only characterizing cancer but causing it' (Warburg, 1956*a*) (Fig. 1), and considered this a universal metabolic alteration in carcinogenesis (Warburg, 1956*b*). This interpretation of the data has been questioned since the early 1950s by Chance and coworkers (Chance & Castor, 1952; Chance, 1953; Chance & Hess, 1956, 1959; Weinhouse, 1956, 1976; Aisenberg, 1961) showing that cytochromes of tumour cells were intact and functional, which clearly argues against a general mitochondrial dysfunction. There is clear indication that glycolysis in *most* tumours is upregulated without mitochondrial dysfunction. In these cancers, oxidative phosphorylation (OxPhos) continues normally, producing as much ATP as OxPhos in normal tissues under the same oxygen partial pressure ('damage to the regulation of glycolysis' instead of damage to respiration; Koppenol *et al.* 2011). Yet misconceptions persisted about the causes of the Warburg effect and its relationship to oxidative metabolism in the mitochondria even in the early 2010s (Gaude & Frezza, 2014). Additional arguments speaking against Warburg's initial 'respiration injury theory' include a methodological problem, i.e. tumour tissue sections used in Warburg's *ex vivo* experiments were definitely thicker than critical O_2 diffusion distances and, thus, tissue slices were at least partially hypoxic (Vaupel *et al.* 2019). Furthermore, there is clear indication that the O_2 uptake rates of most malignant tumours *in vivo* are determined by O_2 availabilities, not by sequelae to damaged mitochondria (Vaupel *et al.* 1971; Vaupel, 1974; Vaupel & Mayer, 2012).

Against these arguments, during the last two decades evidence has accumulated that in some tumours the Warburg effect can – as initially postulated – be triggered

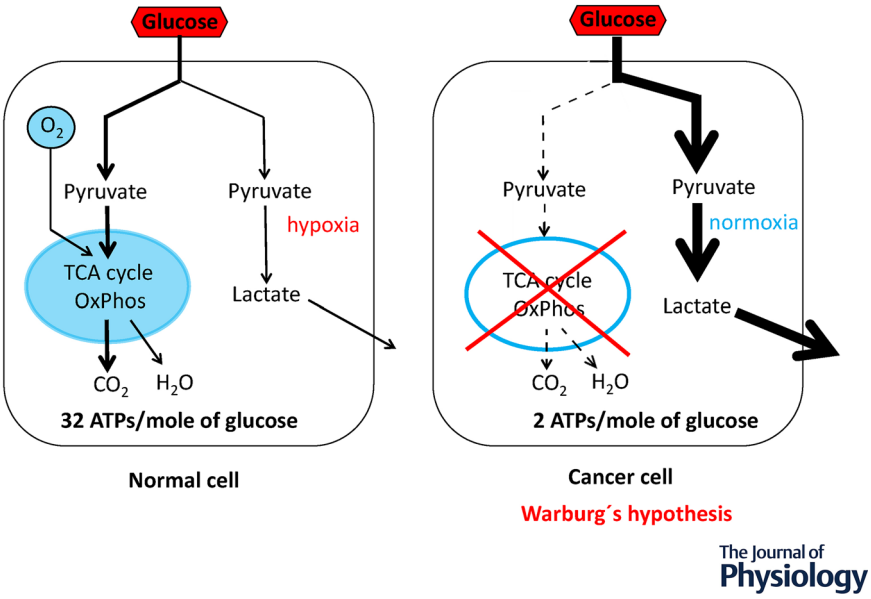

Figure 1. Warburg's historical postulate
Cancer cells – as a sequela to (primarily) damaged mitochondria – have a 10- to 40-fold higher glucose uptake rate, and a lactate production which is 10–100 times faster than the complete oxidation of glucose in mitochondria ('facilitated glycolytic flux') in order to maintain energy homeostasis. Lactate is accumulated in the tumour extracellular space upon export from cancer cells (right panel). In normal cells, lactate is produced only under hypoxic/anoxic conditions (anaerobic or hypoxic glycolysis, e.g. during strenuously exercising muscle, left panel). Abbreviations: ATP, adenosine triphosphate; OxPhos, oxidative phosphorylation; TCA, tricarboxylic acid. (Modified from Vaupel & Multhoff, 2020.) Lactate formation is not restricted to hypoxic tumour areas (at a certain distance away from tumour microvessels), but also occurs in normoxic ('aerobic') tumour tissue volumes. Of note, the absolute values of O_2 radial diffusion distances in tumour tissues decrease from the arterial to the venous end of the tumour microvessels, depend on the vessel arrangement and blood flow directions (e.g. centripetal diffusion from vessels surrounding tumour cords (Hill model) *vs.* centrifugal diffusion from a central vessel within a cord (Krogh model) *vs.* irregular vascular networks, existence of concurrent *vs.* countercurrent flow in neighbouring vessels), and vary between different tumour types with different blood flow and respiration rates (Vaupel, 1990, 2004). In addition, O_2 diffusion shunts, shunting of microcirculatory blood flow, and varying O_2 transport capacities of the blood can substantially impact critical diffusion distances.

by mutations ('damage') in the mitochondrial enzymes fumarate hydratase, succinate dehydrogenase and isocitrate dehydrogenase (King *et al.* 2006; Schmidt *et al.* 2020; Ratcliffe, 2007; Rasheed & Tarjan, 2018), and by overproduction of mitochondrial reactive oxygen species (ROS).

Considering contributions by modern genomic and ^{13}C mass spectrometry tracing experiments, realistic causative molecular mechanisms and consequences of the Warburg effect have been described only during recent years and are summarized in this review.

The Warburg effect: essential part of metabolic reprogramming and favouring biosynthesis pathways

The Warburg effect, i.e. the conversion of glucose to lactate in the presence of oxygen and functioning mitochondria, is certainly more than a simple adaptation to hypoxia (Gatenby & Gillies, 2004). It is instead a crucial component of the malignant phenotype and a central feature of the 'selfish' metabolic reprogramming of cancer cells, which is considered a 'hallmark of cancer' (updated list: Hanahan & Weinberg, 2011). The switch to aerobic glycolysis (i.e. the conversion of glucose to pyruvate) followed by lactate formation is acquired very early in carcinogenesis (oncogenesis), even before tumour cells are exposed to hypoxic conditions (Vander Heiden *et al.* 2009). The Warburg phenotype constitutes a metabolic signature of 70–80% of human cancers, which results from the interplay between the normoxic/hypoxic activation of the transcription factor hypoxia-inducible factor-1 (HIF-1), oncogene activation, loss of function of tumour suppressors, altered signalling pathways and interaction with components of the tumour microenvironment (TME), sometimes working in concert with epigenetic mechanisms. The Warburg effect reflects a metabolic programme of cancer cells driving sustained proliferation and accelerating malignant progression (Vaupel *et al.* 2019).

Excursus. Beyond cancer cells, rapidly proliferating mammalian cells, acutely regenerating tissues and mature human red blood cells also show a switch to a Warburg-type glucose metabolism (e.g. Ghashghaeinia *et al.* 2019; Sun *et al.* 2019). Rapidly dividing, proliferating cells include pluripotent stem cells, immune cells and endothelial cells (the Warburg effect as a 'hallmark of rapid proliferation'; Abdel-Haleem *et al.* 2017).

Mature, mitochondria-free, oxygen-carrying erythrocytes (approx. 44 nl O_2 per corpuscle in arterialized blood) also rely on a Warburg-type glucose metabolism. The oxidative branch of the pentose-phosphate pathway emanating from the glycolytic intermediate glucose-6-phosphate (see section below) produces NADPH used for reduction of glutathione (GSH). The latter maintains redox homeostasis and protects haemoglobin against oxidation at high oxygen partial pressures.

A Warburg-type glucose metabolism has also been described in early mammalian embryos to support rapid cell proliferation and growth, with similarities and differences in the underlying regulatory mechanisms (e.g. Redel *et al.* 2012; Krisher & Prather, 2012; Smith & Sturmey, 2013; Miyazawa & Aulehla, 2018; Manzo, 2019).

Major biochemical steps of aerobic glycolysis. The glycolytic pathway involves the *catabolism of glucose to pyruvate* in 9–10 biochemical steps, which are schematically shown in Fig. 2. Noted since the late 1990s, key transcriptional activations include the 'high-affinity' glucose transporter GLUT1 and lactate exit transporter ('exporter') monocarboxylate transporter 4 (MCT4), and key ('crucial') glycolytic enzymes: hexokinase 2, phosphofructokinase 1, enolase 1, the low-activity pyruvate kinase M2 (PKM2) and the strongly overexpressed lactate dehydrogenase A (LDHA). Transcriptional activation also includes the mitochondrial pyruvate dehydrogenase kinase 1, which inactivates the 'bottleneck' or 'gatekeeper' enzyme pyruvate dehydrogenase and thus impedes the intra-mitochondrial conversion of pyruvate to acetyl-CoA. As a result, pyruvate is shunted from the mitochondria and is converted to lactate in the cytosol. Lactate is exported into the extracellular space, where it accumulates (up to 40 mM).

The overexpression of the poorly active/nearly inactive (dimeric) pyruvate kinase M2 (PKM2), another 'gatekeeper' enzyme, limits the phosphoenolpyruvate-to-pyruvate conversion (i.e. the final, irreversible step of glycolysis, providing ATP) and thus leads to a backup of upstream glycolytic phospho-intermediates ('glucose carbons above pyruvate kinase; Mazurek *et al.* 2002), which are shuttled into biosynthesis pathways allowing for sustained proliferation and unlimited growth. Beyond this metabolic role in glycolysis, PKM2 regulates gene expression in the nucleus, and phosphorylates several proteins that regulate key signalling pathways and contribute to the redox homeostasis of cancer cells (for a recent review see Alquraishi *et al.* 2019). Further insights into the metabolic and non-metabolic roles of PKM2, and apparently discrepant functions, have been highlighted in several reports (e.g. Iqbal *et al.* 2014; Amim *et al.* 2019; Zahra *et al.* 2020; Wang *et al.* 2020a; Pascale *et al.* 2020).

These pathways can satisfy the anabolic demands of the macromolecular biosynthesis of nucleotides, lipids, proteins and hexosamine (Hay, 2016). (a) To generate pentose phosphates for ribonucleotide synthesis (and

NADPH production), glucose-6-phosphate is diverted via the pentose phosphate pathway (PPP). The key enzyme facilitating the entry at the branching point into the oxidative PPP is glucose-6-phosphate dehydrogenase (e.g. activated by phosphoinositide 3-kinase (PI3K), Akt–mechanistic target of rapamycin (mTOR), cMyc, mutant p53; Jiang *et al.* 2011; Hoxhaj & Manning, 2020).

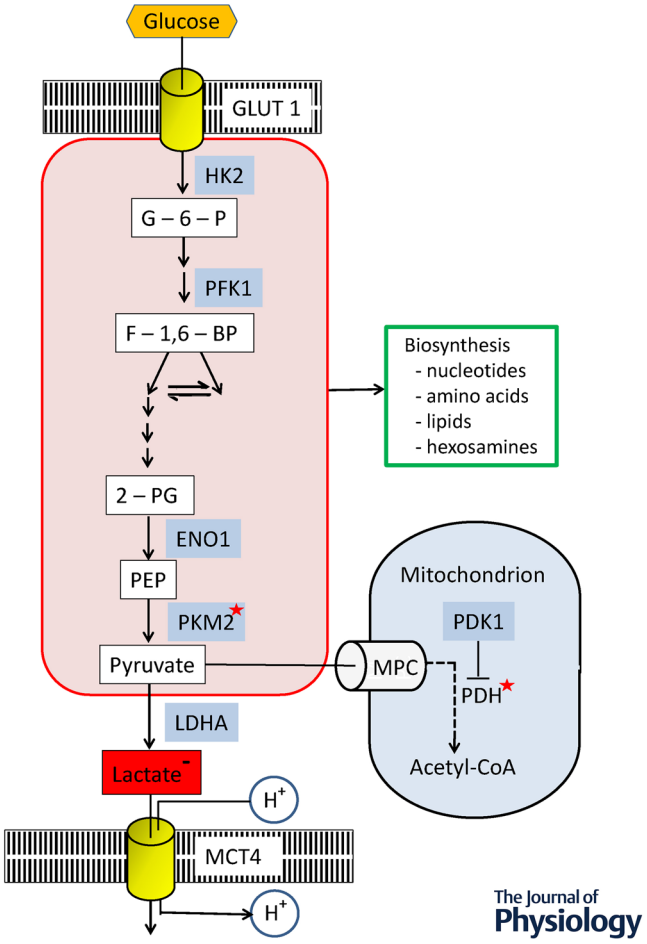

Figure 2. Key biochemical steps of the Warburg phenotype
Upon enhanced import through overexpressed GLUT1 transporters, glucose is converted into 2 pyruvate molecules, the end product of aerobic glycolysis. In a following step, pyruvate is converted into lactate, which is exported via upregulated MCT4 transporters into the extracellular space, where it contributes to acidification (pH <6.8). 'Gatekeeper' enzymes (marked with a red star) lead (a) to an accumulation of upstream glycolytic intermediates (PKM2∗) utilized for the biosynthesis of nucleic acids, non-essential amino acids, lipids and hexosamines, and (b) to a diversion of pyruvate away from the mitochondria (TCA cycle, OxPhos) by encoding PDK1, which in turn activates the 'gatekeeper' enzyme PDH∗. Abbreviations: 2-PG, 2-phosphoglycerate; acetyl-CoA, acetyl-coenzyme A; F-1,6-BP, fructose-1,6-bisphosphate; G-6-P, glucose-6-phosphate; MPC, mitochondrial pyruvate carrier; PEP, phosphoenolpyruvate; PDK1, pyruvate dehydrogenase kinase 1. Red Box: 9(−10) biochemical steps of aerobic glycolysis in the cytosol. (Modified from Vaupel & Multhoff, 2020.)

(b) For biosynthesis of cell membrane components, phospholipids and triglycerols, the intermediates glyceraldehyde-3-phosphate or dihydroxyacetone phosphate are precursors. The flux-enhancing enzyme for this pathway is dihydroxyacetone-phosphatase. (c) The non-essential amino acids serine and glycine and the one-carbon metabolism pathway emanate from the intermediate 3-phosphoglycerate (Yang & Vousden, 2016). The first step in the *de novo* serine synthesis is catalysed by phosphoglycerate dehydrogenase, upregulated by Akt–mTOR complex 1 (mTORC1), cMyc and HIF-1a (Hoxhaj & Manning, 2020; Wang *et al.* 2020b). (d) Fructose-6-phosphate is the glycolytic intermediate for the hexosamine pathway; the rate-limiting, directing enzyme is glutamine:fructose-6-phosphate amidotransferase, e.g. activated by HIF-1 and mutant AMPK (Hay, 2016; Szymura *et al.* 2019; Akella *et al.* 2019).

Of note, normoxic cancer cells can utilize lactate produced by – and exported from – hypoxic cancer cells as an energy source for OxPhos following MCT1-mediated import from the TME ('metabolic symbiosis' between hypoxic and normoxic cancer cells) (Semenza, 2008; Sonveaux *et al.* 2008). In addition, normoxic cancer cells can utilize lactate produced by stressed stromal cells of the TME (e.g. cancer-associated fibroblasts). This phenomenon is called the 'reverse Warburg effect' (Pavlides *et al.* 2009; Fu *et al.* 2017).

Metabolic network of the Warburg phenotype. A recent review emphasizes the interplay of metabolic reprogramming (Warburg effect) with the metabolism of 'geographically' separated cancer tissues or systemic metabolic changes induced by cancer, e.g. the oncogenic Cori cycle, in which metabolic parasitism forced production of energy-rich nutrients by stromal cells (e.g. lactate, ketones) to feed cancer cells (Paul, 2020). On the other hand, metabolic stresses imposed by the microenvironment and the metabolism of the patient (e.g. obesity, diabetes) can contribute to the metabolic phenotypes in cancers (Faubert *et al.* 2020).

The Warburg effect and energy homeostasis. In order to maintain energy homeostasis, cancer cells must consume large amounts of glucose (and glutamine, the second principal growth supporting substrate). This can lead to glucose deprivation in the extracellular space, as illustrated in Fig. 3 (upper panel). Low-glucose conditions in the TME in turn can cause a loss of stromal caveolin-1, yielding an oxidative stress which mimics hypoxia ('pseudohypoxia') through activation of HIF-1 and nuclear factor-κB (see 'reverse Warburg effect', above).

Aerobic glycolysis has been described as an inefficient means of energy metabolism, since the net production is

only 2 moles of ATP per glucose molecule, whereas the total yield is 32(−33) ATPs (Mookerjee *et al*. 2017) from the complete oxidation of 1 glucose molecule. However, the speed of the cytosolic ATP generation is approx. 100 times faster (range: 20–300 times) than in mitochondria ('low yield, but high-speed ATP production'). ATP provision *per unit time* is higher than in oxidative glucose metabolism as long as an adequate glucose supply is maintained in the extracellular compartment (Vaupel *et al*. 2019). In cases of greatly increased ATP demand by cancer cells, aerobic glycolysis can rapidly increase while OxPhos remains quite constant due to the much faster ATP production through the Warburg effect. Representative ATP levels in cancer tissue are depicted in Fig. 3 (central panel).

The Warburg effect ensures redox homeostasis and reduces ROS production. The Warburg effect provides reducing equivalents benefitting cancer cell proliferation. The oxidative branch of the PPP provides 2 NADPHs per mole glucose, which maintains the antioxidative power of GSH, thus increasing the radioresistance of cancer cells, and can act as a directly operating antioxidant in the mitochondrial compartment (Kirsch & De Groot, 2001). Furthermore, the activated PPP can minimize the production of ROS, which, at lower concentrations, can promote increased survival of cancer cells. In addition, NADPH is used in reductive biosynthesis in the *de novo* synthesis of fatty acids which are needed to provide membrane lipids.

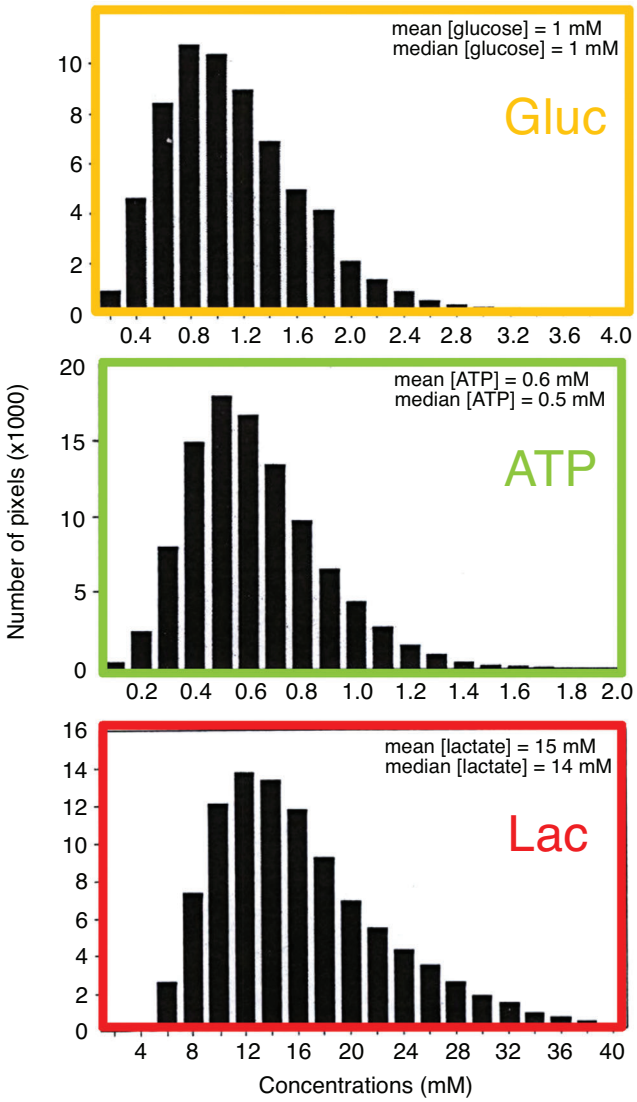

Figure 3. Histograms showing glucose (upper panel), ATP (central panel) and lactate concentrations (lower panel) in cancers of the uterine cervix
Concentrations of glucose, ATP and lactate in cervix cancers assessed by quantitative bioluminescence technique. (Modified from Vaupel, 1992.)

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Regulatory network of the Warburg effect

As schematically illustrated in Fig. 4, major mechanisms acting independently or in cooperation to sustain aerobic glycolysis in cancer cells are: (a) hypoxic/normoxic activation of HIF-1 ('master regulator', which can also work in concert with epigenetic mechanisms), (b) activation (gain of function) of oncogenes (e.g. cMyc, mTORC1, Akt, K-Ras), (c) inactivation (loss of function) of tumour suppressors (e.g. mutant p53, mutant phosphatase and tensin homologue), (d) activation of receptor-tyrosine kinase–PI3K–Akt–mTORC1 signalling pathway, (e) inactivation of AMP-activated protein kinase (AMPK) signalling pathway, (f) downregulation/loss of function of several microRNAs (miRs) and sirtuins 3 and 6 (SIRT3, SIRT6), (g) allosteric regulation by glycolytic intermediates, (h) interactions with the hostile TME and cancer-associated stromal cells, and (i) maintenance of the pH_i–pH_e gradient (Semenza, 2010; Cantor & Sabatini, 2012; Martinez-Pastor & Mostolavsky, 2012; Faubert *et al.* 2013; Lu *et al.* 2015; Tran *et al.* 2016; He *et al.* 2017; Yu *et al.* 2017; Parks *et al.* 2017; Kato *et al.* 2018; Counihan *et al.* 2018; Tameemi *et al.* 2019; Nagao *et al.* 2019; Ganapathy-Kanniappan, 2019; Faubert *et al.* 2020; Yang *et al.* 2020).

As mentioned above, downregulation or loss of several miRs and SIRT6 with tumour suppressor functions can create a tumour-permissive phenotype via activation of the Warburg effect (not shown in Fig. 4) (Martinez-Pastor & Mostolavsky, 2012; Yu *et al.* 2017; Pedroza-Torres *et al.* 2019). These include miR-29,

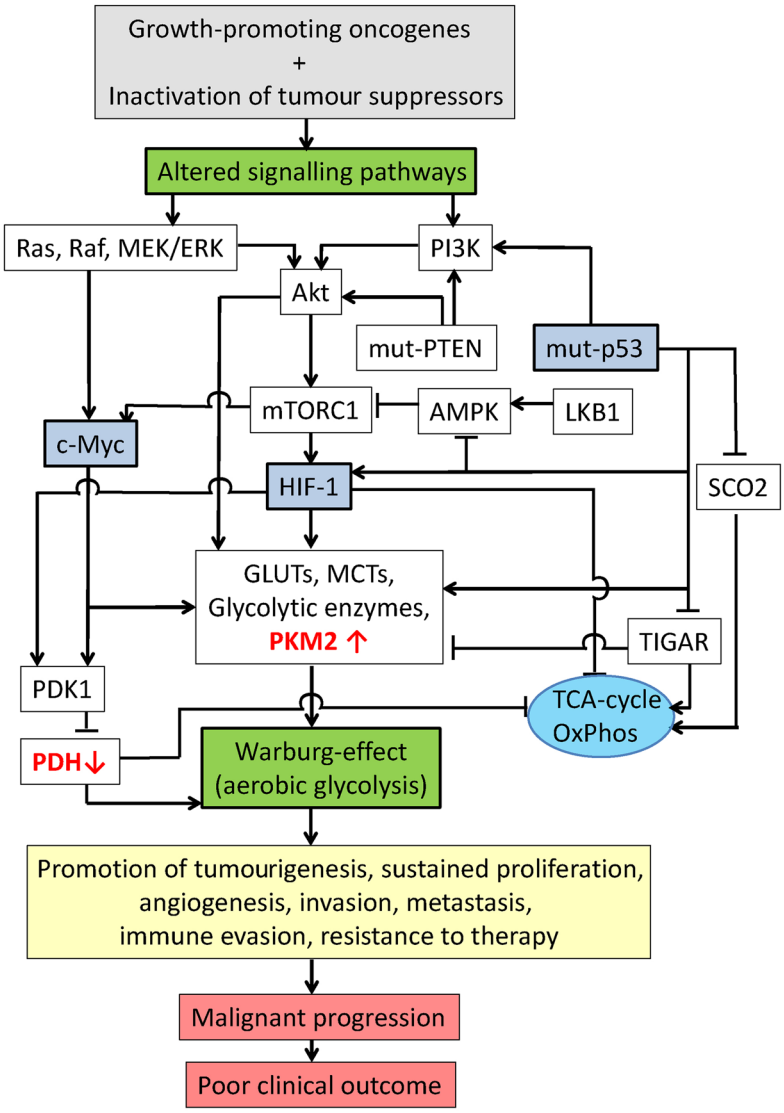

Figure 4. Regulatory network of the Warburg effect, mitochondrial contributions to sustained glycolysis and major mechanisms leading to cancer progression
Green boxes: 'landmarks'; grey boxes: key regulators; enzymes in red: gatekeeper/bottleneck enzymes (upregulated low-activity PKM2, downregulated PDH-complex). Arrows represent positive impacts/activations, T-bars represent negative impacts/inhibitions. Jak/Stat3 signalling pathway not shown in this flowchart. Abbreviations: Akt, protein kinase B ('Warburg kinase'); AMPK, AMP-activated protein kinase; cMyc, cellular Myc oncogene; ERK, extracellular signal-regulated kinase; LKB1, liver kinase B1 (tumour suppressor); MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic ('mammalian') target of rapamycin; mut-p53, mutant p53; mut-PTEN, mutant phosphatase and tensin homologue; PDH, pyruvate dehydrogenase (complex); PI3K, phosphatidylinositol-3-kinase; SCO2, synthesis of cytochrome oxidase factor 2; TIGAR, tp53-inducible glycolysis and apoptosis regulator. (Modified from Vaupel & Multhoff, 2020.)

miR-143 and miR-144. Loss of these miRs and of the tumour suppressors SIRT3 and SIRT6 can – *inter alia* – activate HIF-1, cMyc, Akt (= 'Warburg kinase') and key glycolytic enzymes, expedite PI3K–Akt–mTOR and Ras–Raf–mitogen-activated protein kinase (MAPK) kinase–extracellular signal-regulated kinase pathways, and can activate GLUT1 and MCT4 transporters or inactivate the MPC carrier.

Mitochondrial contributions may also drive maintenance of aerobic glycolysis. They include (a) inhibition of OxPhos by mutant p53 (through regulators tp53-inducible glycolysis and apoptosis regulator and synthesis of cytochrome oxidase factor 2), (b) inhibition of mitochondrial biogenesis (by deactivated AMPK pathway and HIF-1), (c) miR-210-triggered inhibition of the electron transport chain, (d) B-cell lymphoma 2-interacting protein 3-induced mitochondrial autophagy (mitophagy), and (e) mitochondrial DNA (mtDNA) mutations.

Of note, in a recent comprehensive review on the mitochondrial functions and their contribution to carcinogenesis and cancer progression, Grasso *et al.* (2020) aimed to re-centre mitochondria in the overall metabolic map of cancers. They clearly illustrate, using data published in the last decade, that (a) aerobic glycolysis does not predict loss of oxidative metabolism as initially postulated by Warburg, i.e. cancer cells are not constitutively glycolytic, and (b) mitochondria are capable of adapting to cancerous conditions ('mitochondrial plasticity'). Cancer cells to a great extent rely on both cytosolic glycolysis *and* mitochondrial respiration. An additional argument for intact mitochondria in aerobic glycolysis may be the connection between complex I and HIF stabilization (e.g. Chandel *et al.* 1998; Agani *et al.* 2000).

Attempts to quantify substrate uptake and release rates of tumours *in vivo*

In xenograft breast cancers uptake rate of glucose, the major source for lactate generation, was 430 nmol/g/min. The respective extraction from the blood was 34% (Kallinowski *et al.* 1987).

Cancer cells are also addicted to glutamine, mainly driven by the Myc oncogene (Altman *et al.* 2016). In addition to aerobic glycolysis, cancer cells derive energy, carbon and nitrogen to support biosyntheses from substantially increased glutaminolysis, a pathway which greatly relies on intact mitochondria (e.g. Yang *et al.* 2017). Glutamine uptake was 16 nmol/g/min in xenograft human breast cancers, and the corresponding extraction was 24% (Kallinowski *et al.* 1987). The glutaminolysis-derived lactate adds to lactate emanating from glycolysis. This has been substantiated using *in vivo* studies by judging lactate output/glucose uptake ratios. Based on *in vivo* data from xenograft human tumours with different histologies, in 83 out of 110 malignant tumours investigated, the lactate release was higher than the glucose uptake (ratio: 1.5; Kallinowski *et al.* 1989*b*). In well-perfused xenograft lung cancers the ratio was 1.7 (Kallinowski *et al.* 1989*a*). In another series of experiments using 67 breast cancer xenografts, the ratio lactate output/glucose uptake was approx. 1 (Kallinowski *et al.* 1988). On average, these data may be explained by (a) an additional lactate output from glutaminolysis, or (b) by a diversion of glycolytic intermediates for the biosynthetic pathways mentioned above, and/or (c) a combination thereof. In isotransplant rat sarcomas the ratio was 1.8 (Kallinowski *et al.* 1989*b*), also accounting for additional lactate output from glutaminolysis. In contrast, in head and neck cancers of patients, the lactate output/glucose uptake ratio was 0.26 (Richtsmeier *et al.* 1987) and 0.78 in colon cancers *in situ* (Holm *et al.* 1995).

In the *in situ* study conducted by Richtsmeyer *et al.* the uptake rate of ketones (β-hydroxybutyrate, acetoacetate) was 7 nmol/g/min, the extraction being 33% (glucose: 15%). In the xenograft human cancers the respective data were 4 nmol/g/min, and 24% (glucose: 34%). On average, in xenograft human tumours the relative uptake rates for the key substrates were: 1 (glucose) *vs.* 0.04 (glutamine) *vs.* 0.02 (ketones). The relative availabilities were as follows: 1 (glucose) *vs.* 0.05 (glutamine) *vs.* 0.02 (ketones). The relative extraction rates were: 1 (glucose) *vs.* 0.7 (glutamine) *vs.* 0.7 (ketones). For *in vivo* situations, the key information for these major substrates may be as follows: (a) the uptake rates are related to the individual availabilities (blood flow rate $\times$ arterial concentration), and (b) the extraction rate drops with increasing availability. It is concluded that in xenograft human tumours the metabolic rate is perfusion-limited (Kallinowski *et al.* 1989*a*). This finding is supported by *in vivo* data assessed in locally advanced human cancers (head and neck cancers, Richtsmeier *et al.* 1987; colon cancers, Holm *et al.* 1995). Thus, uptake rates in these human tumours *in situ* are barely governed by their nutrient demand. Large inter-tumour heterogeneity may be caused – inter alia – by variations in tumour histologies, tumour sizes, cell densities, vascular density, interstitial fluid pressures, volumes and compositions of stromal compartments, traits of the TME and shunt perfusion.

An alternative (respiratory) substrate is circulating lactate (serum level: 1–2 mmol/l) as evidenced by Hui *et al.* (2017) and Faubert *et al.* (2017). In these reports the turnover of lactate is even higher than that of glucose (serum level: 4–6 mmol/l), After uptake of lactate through MCT1 transporters into the lung cancer cells its contribution to the TCA cycle predominated. This recent information makes 'quantification' of the individual contributions of the major substrates in

cancer metabolism even more complex than anticipated before.

Tumour cells have the enzymatic and metabolic capacity for *de novo* synthesis of fatty acids (FAs), whereby saturated and monosaturated FAs are preferentially formed and released. This has also been substantiated for xenograft human breast cancers, the rate of FA-release being linearly correlated with the respective glucose uptake rate (Mazurek *et al.* 2002). Besides FAs, a series of amino acids, preferentially glycine, proline, alanine and lysine, are released into the tumour venous blood (discussed in detail in Eigenbrodt *et al.* 1998).

Warburg effect: the role of lactate accumulation and extracellular acidosis

High lactate levels in the TME (up to 40 mM) primarily result from the upregulation of aerobic glycolysis. As mentioned above, anaerobic glycolysis and (strongly) enhanced glutaminolysis, to a certain extent, contribute to lactate accumulation since lactate is the ultimate degradation product of glutamine in the glutaminolytic pathway.

Lactate (lactate$^-$ anion) probably is the only *oncometabolite* involved and necessary in nearly all main sequelae for malignant progression. A series of these pathophysiological conditions results after binding of the ligand lactate to the GPR81 receptor and activation of the PI3K–Akt–mTOR pathway and HIF-1 stabilization. Major sequelae driving malignant progression have been described in detail recently (e.g. Mayer & Vaupel, 2013; San-Millan & Brooks, 2017; Kato *et al.* 2018; Vaupel & Multhoff, 2018; Vaupel *et al.* 2019; Pereira-Nunes *et al.* 2020). The use of lactate to fuel OxPhos in normoxic cancer cells has been described above.

Cytosolic lactate is mainly exported through upregulated lactate–proton symporters (MCT4) working together with Na^+/H^+ exchangers (NHE1), H^+-V-ATPases, and carbonic anhydrases (CAII, CAIX) which hydrate CO_2 from oxidative metabolism to form H^+ and bicarbonate. In concert with poor vascular drainage these protons are responsible for extracellular acidification.

Extracellular acidosis (pH_e <6.8) is another detrimental trait arising from aerobic (and anaerobic) glycolysis. Its key role in driving malignant progression and resistances to conventional therapies has been comprehensively reviewed recently (e.g. Riemann *et al.* 2016; Corbet & Feron, 2017; Thews & Riemann, 2019; Ippolito *et al.* 2019). There is ample information that lactate$^-$ anions, H^+ (acidosis) and hypoxia are independent parameters (i.e. they can act independently) promoting malignant phenotypes in many instances, but may also interact in regulatory systems (e.g. hypoxia-inducible pH-regulation).

As a footnote, diagnostic aspects of the Warburg effect (e.g. [^{18}F]fluorodeoxyglucose positron emission tomography (FDG-PET), ^{13}C magnetic resonance spectroscopy, ^{13}C magnetic resonance imaging, use of PKM2 and LDHA as biomarkers) and metabolic targeting in cancer therapy (e.g. inhibition or activation of key elements of the Warburg effect) are intentionally not discussed in this review. The clinical use of FDG-PET contributes to the perception of the Warburg effect. However, this tracer only reports *glucose uptake* and represents *phosphorylation of glucose by hexokinase* (when glucose uptake is limited) without providing any information about the subsequent intracellular *glucose metabolism*. In addition, the FDG signal neither indicates suppressed glucose oxidation nor gives any relevant information about the status of the mitochondria (DeBeradinis & Chandel, 2020).

Warburg effect and mitochondrial dysfunction

Multiple observations outlined during the last two decades suggest that the Warburg effect could also result from mitochondrial dysfunctions (for reviews see Senyilmaz & Teleman, 2015; Srinivasan *et al.* 2016; Zhang *et al.* 2019; Cassim *et al.* 2020; Grasso *et al.* 2020). Examples are (a) mutations in the Krebs (TCA) cycle enzymes succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH). Loss of function of SDH and FH lead to accumulation of fumarate and succinate, inducing a state of 'pseudo-hypoxia' through stabilization of HIF-1α. Gain of function of IDH leads to 2-hydroxyglutarate. All three metabolites act as oncometabolites, i.e. they enhance oncogenesis and cancer progression, the Warburg effect included. And (b) mtDNA mutations in complex I and III result in defective electron transport chain complexes. ROS over-produced by defective mitochondria drive mechanisms involved in oncogenesis and lead (through stabilization of HIF-1α, and activation of PI3K–Akt–mTOR and MAPK pathways) to an intensification of the Warburg effect.

Warburg-effect: timetable, cornerstones

1923	Warburg's hypothesis: enhanced conversion of glucose to lactate even in the presence of abundant oxygen as a consequence of irreversible respiratory injury in tumours.
1956	Warburg's claim that 'irreversible respiratory injury is a universal cause for oncogenesis'.
Since 1950s	Interpretation of the data is questionable.
Mid-1970s	E. Racker coined the term 'Warburg effect'.

Mid-1990s	Increasing evidence that mitochondrial dysfunctions contribute to the Warburg effect.
Late-1990s	Re-interpretation of the Warburg effect: central feature of metabolic reprogramming and crucial component of the malignant phenotype, regulated by activated oncogenes, deactivated tumour suppressors, activated or deactivated signalling pathways.
At present	Partial revival of Warburg's initial theory: Warburg effect can also be caused by mitochondrial dysfunctions in *some* tumours.
Conclusion	There are some cases where Warburg was not completely wrong!

Summary

Genetic instability, mutagenesis, aberrant gene expression and altered signalling pathways cause a glycolytic switch in 70–80% of human cancers leading to aerobic glycolysis (the Warburg effect). The glycolytic phenotype constitutes an essential component of the metabolic reprogramming of cancer cells and occurs early in oncogenesis, i.e. before tissue hypoxia develops. A series of molecular intricacies allows cancer cells to escape from typical regulatory constraints ensuring sustained, uncontrolled growth, invasion and metastasis. Survival advantages and malignant progression, resistance to radio-/chemotherapy and other conventional cancer therapies, and escape from anti-tumour immune responses ultimately lead to poor patient outcome.

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Additional information

Competing interests

The authors confirm that no competing interests and conflict of interests exist regarding the content of the article.

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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