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Enzymatic features of the glucose metabolism in tumor cells

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Many tumor types exhibit an impaired Pasteur effect, i.e. despite the presence of oxygen, glucose is consumed at an extraordinarily high rate compared with the tissue from which they originate – the so-called 'Warburg effect'. Glucose has to serve as the source for a diverse array of cellular functions, including energy production, synthesis of nucleotides and lipids, membrane synthesis and generation of redox equivalents for antioxidative defense. Tumor cells acquire specific enzyme-regulatory mechanisms to direct the main flux of glucose carbons to those pathways most urgently required under challenging external conditions such as varying substrate availability, presence of anti-cancer drugs or different phases of the cell cycle. In this review we summarize the currently available information on tumor-specific expression, activity and kinetic properties of enzymes involved in the main pathways of glucose metabolism with due regard to the explanation of the regulatory basis and physiological significance of the Warburg effect. We conclude that, besides the expression level of the metabolic enzymes involved in the glucose metabolism of tumor cells, the unique tumor-specific pattern of isozymes and accompanying changes in the metabolic regulation below the translation level enable tumor cells to drain selfishly the blood glucose pool that non-transformed cells use as sparingly as possible.

Glucose metabolism in tumor cells – an overview

Glucose is a treasured metabolic substrate for all human cells and is utilized for numerous metabolic functions (Fig. 1).

1 Formation and degradation of glycogen serves as a means of internal glucose buffering.
2 The synthesis of ribose phosphates along the oxidative (OPPPW) and non-oxidative pentose phosphate pathway (NOPPPW) is essential for the synthesis of

Abbreviations
ALD, aldolase; AMF, autocrine motility factor; BGP, brain-type glycogen phosphorylase; DHAP, dihydroxyacetone phosphate; EN, enolase; FASN, fatty acid synthetase; FH, fumarate hydratase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDPH, α-glycerophosphate dehydrogenase; GLUT, glucose transporter; GP, glycogen phosphorylase; G6PD, glucose 6-phosphate dehydrogenase; GPI, glucose 6-phosphate isomerase; 2HG, 2-hydroxyglutarate; HIF-1, hypoxia-inducible transcription factor; HK, hexokinase; IDH, isocitrate dehydrogenase; αKG, α-ketoglutarate; LDH, lactate dehydrogenase; MCT, monocarboxylate transporters; MPT, mitochondrial pyruvate transporter; NOPPPW, non-oxidative pentose phosphate pathway; OPPPW, oxidative pentose phosphate pathway; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PDHK-1, pyruvate dehydrogenase kinase; PFK-1, phosphofructokinase-1; PFK-2, phosphofructokinase-2; PFKFB, fructose 2,6-bisphosphatase; 6PGD, 6-phosphogluconate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PHD, prolyl hydroxylase; PK, pyruvate kinase; PRPPS, phosphoribosyl pyrophosphate synthetase; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SMCT1, Na^+-coupled lactate transporter; TCA, tricarboxylic acid; TIGAR, TP53-induced glycolysis and apoptosis regulator; TKT, transketolase; TPI, triosephosphate isomerase; VDAC, voltage-dependent anion channel.

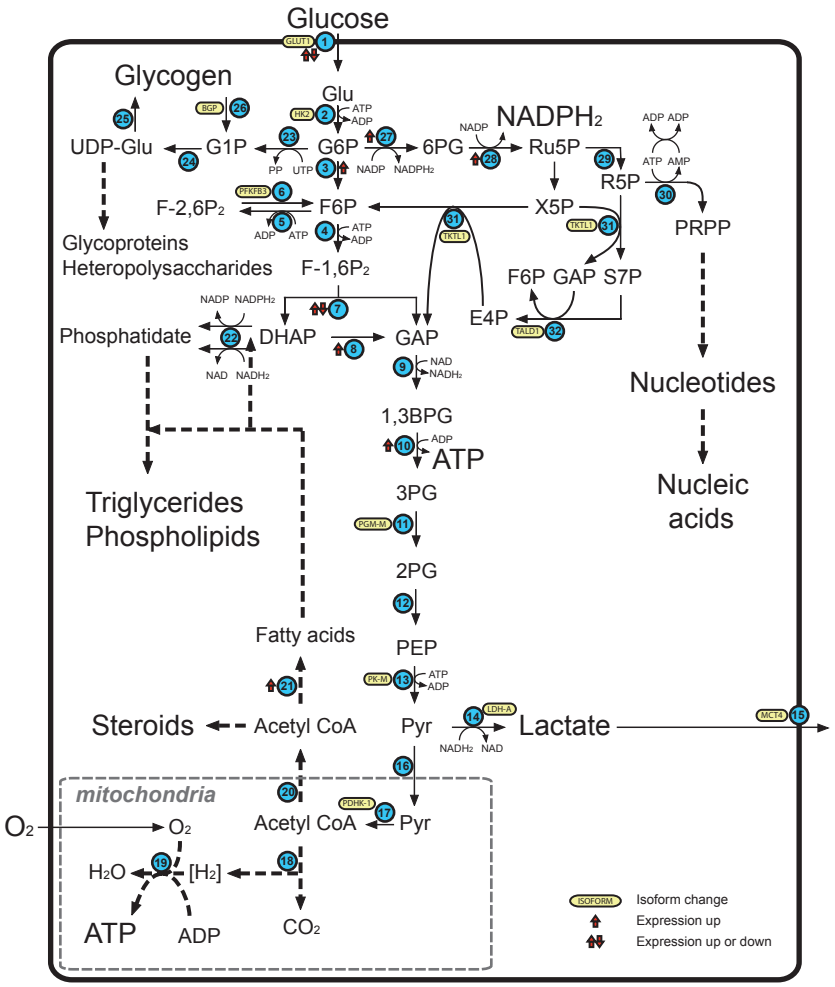

Fig. 1. Glucose metabolism in cancer cells. Main glucose metabolism consisting of glycolysis (1–15), mitochondrial pyruvate metabolism, synthesis of fatty acids (21), lipid synthesis (21–22), glycogen metabolism (23–26) and pentose phosphate pathway (27–31). Reaction numbers correspond to numbers in the text. Characteristic isoforms occurring in cancer cells are marked by yellow boxes, characteristic gene expression changes by red arrows (see Table 1 for summary information on gene expression and isoforms).

nucleotides, which serve as co-factors in phosphorylation reactions as well as building blocks of nucleic acids.

3 The OPPPW is also the major source of NADPH H^+ required as co-factor for reductive biosyntheses as well as for antioxidative enzymatic reactions such as the glutathione reductase reaction.

4 Reduction and acylation of the glycolytic intermediate dihydroxyacetonphosphate delivers the phosphatidic acid required for the synthesis of triglycerides and membrane lipids.

5 Acetyl-CoA produced from the glycolytic end product pyruvate may either enter the tricarboxylic acid (TCA) cycle, the main hydrogen supplier of oxidative energy production, or serve as a precursor for the synthesis of fatty acids, cholesterol and some non-essential amino acids.

6 The carbon skeleton of all monosaccharides used in the synthesis of heteroglycans and glycoproteins may derive from glucose.

All these metabolic objectives of glucose utilization are present in normal cells as well as in tumor cells. However, in tumor cells the importance of the objectives and thus their relative share in total glucose utilization varies during different stages of tumor development. For example, progressive impairment of mitochondrial respiration or administration of anti-cancer drugs may result in higher production rates of reactive oxygen species (ROS). This requires tumor cells to direct an increasing fraction of glucose to the $NADPH_2$ delivering oxidative pentose pathway, an important switch in glucose utilization which has recently been shown to be promoted by deficient p53 [1].

An outstanding biochemical characteristic of neoplastic tissue is that despite the presence of sufficiently high levels of oxygen tension a substantial part of ATP is delivered by glycolytic substrate-chain phosphorylation, a phenomenon that is referred to as aerobic glycolysis or the 'Warburg effect' [2]. The share of aerobic glycolysis in the total ATP production of a

tissue can be roughly estimated from the ratio between lactate formation and glucose uptake: if lactate is exclusively formed via glycolysis this ratio is two; if glucose is fully oxidized to carbon dioxide and water the ratio is zero. Based on mitochondrial P/O ratios of 2.5 or 1.5 with NADH H^+ or $FADH_2$, respectively, glycolysis generates approximately 15-fold less ATP per mole of glucose as the free energy contained in the glycolytic end product lactate is not exploited [3,4]. Hence, in conditions where the ATP demand of the tumor is exclusively covered by glycolysis [2,5], the utilization rate of glucose has to be increased 15-fold compared with conditions of complete glucose oxidation via oxidative phosphorylation. The 'glucose addiction' of tumors exhibiting the Warburg effect implies that dietary restriction can effectively reduce the growth rate of tumors unless they have acquired mutations that confer resistance to it [6,7].

Why aerobic glycolysis in tumors?

Various explanations have been offered to account for the occurrence of aerobic glycolysis in tumors, all of them having some pros and cons.

(a) Zonated energy metabolism in massive tumors In a massive tumor with poor or even non-existent vascularization the oxygen concentration decreases sharply from the periphery to the center of the tumor [8]. It is conceivable that cells located nearest to the blood supply exhibit predominantly oxidative phosphorylation whereas cells further away will generate their ATP predominantly by anaerobic glycolysis (the Pasteur effect) [9]. Taking these two spatially distinct modes of energy production together the tumor as whole will appear to rely on aerobic glycolysis.

(b) Aggressive lactate production Accumulation of lactate in the tumor's microenvironment is accompanied by a local acidosis that facilitates tumor invasion through both destruction of adjacent normal cell populations and acid-induced degradation of the extracellular matrix and promotion of angiogenesis [10]. According to this view, aerobic lactate production is used by tumors to gain a selective advantage over adjacent normal cells. The existence of specific proton pumps in the plasma membrane of tumor cells that expel protons into the external space, thereby contributing to cellular alkalinization and extracellular acidosis [11], support this interpretation.

Arguments (a) and (b) fail, however, to explain the presence of aerobic glycolysis in leukemia cells [12] that do not form massive tumors, which have free access to oxygen and which cannot form an acidic microenvironment.

(c) Attenuation of ROS production Reduction of mitochondrial ATP production can diminish the production rate of ROS as the respiratory chain is a major producer of ROS [13]. Indeed, enforcing a higher rate of oxidative phosphorylation either by restricted substrate supply of tumors [14] or inhibition of the glycolytic enzyme lactate dehydrogenase A (LDH-A) [15] leads to a higher production of ROS and a significant reduction in tumor growth. However, forcing tumors to increase the rate of oxidative phosphorylation does not necessarily lead to higher ROS production. For example, reactivating mitochondrial ATP production of colon cancer cells by overexpression of the mitochondrial protein frataxin [14] was not accompanied by a significant increase in ROS production.

(d) Enforced pyruvate production An increase of lactate concentration through enhanced aerobic glycolysis is paralleled by an increase of pyruvate concentration as both metabolites are directly coupled by an equilibrium reaction catalyzed by LDH (see reaction 14 in Fig. 1). Pyruvate and other ketoacids have been shown to act as efficient antioxidants by converting hydrogen peroxide to water in a non-enzymatic chemical reaction [16]. Thus, increased pyruvate levels could contribute to diminishing the otherwise high vulnerability of tumors to ROS.

Finally, it has to be noted that a switch from oxidative to glycolytic ATP production in the presence of sufficiently high oxygen levels also occurs in normal human cells such as lymphocytes or thrombocytes [17,18], which are able to abruptly augment their energy production upon activation. To make sense of this phenomenon one has to distinguish the thermodynamic efficiency of a biochemical process from its absolute capacity and flexible control according to the physiological needs of a cell [19]. From our own model-based studies on the regulation of glycolysis [20,21] we speculate that its high kinetic elasticity, i.e. the ability to change the flux rate instantaneously by more than one order of magnitude due to allosteric regulation and reversible phosphorylation of key glycolytic enzymes [22], may compensate for the lower ATP yield of this pathway. This regulatory feature of glycolysis might be of particular significance for tumors experiencing large variations in their environment and internal cell composition during development and differentiation.

As the focus of this review is on tumor-specific enzyme variants in glucose metabolism we will also discuss some recent findings on mutated enzyme variants in the TCA cycle which have been implicated in tumorigenesis.

In the following we will review current knowledge on tumor-specific expression and regulation of the individual enzymes catalyzing the reactions shown in Fig. 1. Quantitative assessment of the regulatory relevance of an enzyme for flux control in a specific metabolic pathway is the topic of metabolic control theory [23–25]. Rate limitation (or rate control) by an enzyme means that changing the activity of the enzyme by x% results in a significant change of the pathway flux by at least 0.5x% (whether 0.5x% or higher is a matter of convention). The way that the change of enzyme activity is brought about is important: increasing the amount of the enzyme through a higher rate of gene expression or increasing the concentration of an allosteric activator by the same percentage may have completely different impacts on the pathway flux. Moreover, the degree of rate limitation exerted by an enzyme depends upon the metabolic state of the cell. For example, in intact mitochondria and with sufficient availability of oxygen the rate of oxidative phosphorylation is determined by the ATP/ADP ratio, not by the capacity of the respiratory chain. However, under hypoxic conditions rate limitation through the respiratory chain becomes significant [26]. We will use the term 'control enzyme' to designate the property of an enzyme to become rate limiting under certain physiological conditions and to be subject to several modes of regulation such as, for example, binding of allosteric effectors, reversible phosphorylation or variable gene expression of its subunits.

Tumor-specific expression and regulation of enzymes involved in glucose metabolism

Glycolysis (reactions 1–15)

The pathway termed glycolysis commonly refers to the sequence of reactions that convert glucose into pyruvate or lactate, respectively (Fig. 1).

(1) Glucose transporter (GLUT) (TCDB 2.A.1.1)

Multiple isoforms of GLUT exist, all of them being 12-helix transmembrane proteins but differing in their kinetic properties. GLUT1, a high affinity glucose transporter ($K_m \sim 2$ mM), is overexpressed in a significant proportion of human carcinomas [27–29]. By contrast, the insulin-sensitive transporter GLUT4 tends to be downregulated [30], thus rendering glucose uptake into tumor cells largely insulin-insensitive. Abundance of GLUT1 correlates with aggressive tumor behavior such as high grade (poorly differentiated) invasion and metastasis [31–33]. Transcription of the *GLUT1* gene has been demonstrated to be under multiple control by the hypoxia-inducible transcription factor HIF-1 [34], transcription factor c-myc [35] and the serine/threonine kinase Akt (PKB) [36,37]. The hypoxia response element, an enhancer sequence found in the promoter regions of hypoxia-regulated genes, has been found for GLUT1 and GLUT3 [38]. Stimulation of GLUT1-mediated glucose transport by hypoxia occurs in three stages (reviewed by Behrooz and Ismail-Beigi [39] and Zhang *et al.* [40]). Initially, acute hypoxia stimulates the 'unmasking' of glucose transporters pre-existing on the plasma membrane. A more prolonged exposure to hypoxia results in enhanced transcription of the *GLUT1* gene. Finally, hypoxia as well as hypoglycemia lead to increased GLUT1 protein synthesis due to negative regulation of the RNA binding proteins hnRNP A2 and hnRNP L, which bind an AU-rich response element in the GLUT1/3 UTR under normoxic and normoglycemic conditions, leading to translational repression of the glucose transporter [41].

Intriguingly, to further increase the transport capacity for glucose, epithelial cancer cells additionally express SGLT1 [42,43], an Na^+-coupled active transporter which is normally only expressed in intestinal and renal epithelial cells and endothelial cells at the blood–brain barrier.

Metabolic control analysis of glycolysis in AS-30D carcinoma and HeLa cells provided evidence that GLUT and the enzyme hexokinase (see below) exert the main control (71%) of glycolytic flux [44]. Evidence for the regulatory importance of the two isoforms GLUT1 and GLUT3 typically overexpressed in tumor cells is also provided by the fact that these transporters are upregulated in cells and tissues with high glucose requirements such as erythrocytes, endothelial cells and the brain [45].

(2) Hexokinase (HK) (EC 2.7.1.1)

There are four important mammalian HK isoforms. Besides HK-1, an isoenzyme found in all mammalian cells, tumor cells predominantly express HK-2 [46]. Expression studies revealed an approximately 100-fold increase in the mRNA levels for HK-2 [47–51]. The prominent role of HK-2 for the accomplishment of the Warburg effect has been demonstrated by Wolf *et al.* who found that inhibition of HK-2, but not HK-1, in a human glioblastoma multiforme resulted in the restoration of normal oxidative glucose metabolism with decreased extracellular lactate and increased O_2 consumption [51]. Both HK-1 and HK-2 are high affinity enzymes with K_m values for glucose of about 0.1 mM.

Thus, the flux through these enzymes becomes limited by the availability of glucose only in the case of extreme hypoglycemia.

The main allosteric regulators of HK-1 and HK-2 are ATP, inorganic phosphate and the reaction product glucose 6-phosphate. Inorganic phosphate antagonizes glucose 6-phosphate inhibition of HK-1 but adds to glucose 6-phosphate inhibition of HK-2. This remarkable difference has led to the suggestion that HK-1 is the dominant isoform in tissues with high catabolic (=glycolytic) activity whereas HK-2 is better suited for anabolic tasks, i.e. re-synthesis of glycogen [52] and provision of glucose 6-phosphate for the OPPPW [53].

HK-2 has been shown to be attached to the outer membrane of mitochondria where it interacts via its hydrophobic N-terminus (15 amino acids) with the voltage-dependent anion channel (VDAC) [54]. Akt stimulates mitochondrial HK-2 association whereas high cellular concentrations of the reaction product glucose 6-phosphate cause a conformational change of the enzyme resulting in its detachment from the VDAC. HK-2 bound to mitochondria occupies a preferred site to which ATP from oxidative phosphorylation is directly channeled, thus rendering this 'sparking' reaction of glycolysis independent of glycolytic ATP delivery [55,56]. However, experiments with isolated hepatoma mitochondria demonstrated that adenylate kinase (used as extra-mitochondrial ATP regenerating reaction) and oxidative phosphorylation contributed equally to the production of ATP used by HK-2 [57]. Apparently, the results of *in vitro* experiments with HK-2 bound to isolated mitochondria depend on the specific assay conditions (e.g. ADP concentration, type of ATP regenerating system used), so that the degree of coupling between the rate of oxidative phosphorylation and HK-2 activity and the physiological implications of such a coupling remain elusive. For neuronal cells, expressing predominantly the HK-2 isoform, it has been proposed that direct coupling of HK-2 activity to the rate of oxidative phosphorylation may ensure introduction of glucose into the glycolytic metabolism at a rate commensurate with terminal oxidative stages, thus avoiding production of (neurotoxic) lactate [58]. Such a hypothetical function of HK-2 can hardly be reconciled with the notion of excessive lactate production being the ultimate goal of the Warburg effect (see above). Furthermore, attachment of HK-2 to the VDAC is thought to be anti-apoptotic by hindering the transport of the pro-apoptotic protein BAX to the outer mitochondrial membrane. This prevents the formation of the mitochondrial permeability pore and hence the mitochondrial release of cytochrome c and APAF-1, an initial event in the activation of the proteolytic cascade leading to cell destruction [54]. However, a recent genetic study indicated that a mitochondrial VDAC is dispensable for induction of the mitochondrial permeability pore and apoptotic cell death [59].

(3) Glucose 6-phosphate isomerase (GPI/AMF) (EC 5.3.1.9)

GPI can occur as alternatively monomer, homodimer or tetramer, with the monomer showing the highest and the tetramer showing the lowest activity. Phosphorylation of Ser185 by protein kinase CK2 facilitates homo-dimerization and thus diminishes the activity of the enzyme [60]. Studies in eight different human cancer cell lines have consistently revealed 2- to 10-fold elevated mRNA levels of GPI. Both HIF-1 and vascular endothelial growth factor have been shown to induce enhanced expression of GPI [61].

GPI can be excreted by tumor cells in detectable amounts thus serving as a tumor marker. Extracellular GPI acts as an autocrine motility factor (AMF) eliciting mitogenic, motogenic and differentiation functions implicated in tumor progression and metastasis [62]. The exact mechanism responsible for the conversion of the cytosolic enzyme into a secretory cytokine has not yet been fully elucidated [63]. It has been proposed that GPI/AMF phosphorylation is a potential regulator of its secretion and enzymatic activity [60,64].

(4) Phosphofructokinase-1 (PFK-1) (EC 2.7.1.11)

PFK-1 catalyzes a rate-controlling reaction step of glycolysis. Although the enzyme level has little effect on glycolytic flux in yeast [65], the activity of this enzyme is subject to multiple allosteric regulators, which considerably change the rate of glycolysis. Allosteric activation is mainly exerted by fructose 2,6-P_2 [66]. PFK of tumor cells is less sensitive to allosteric inhibition by citrate and ATP [67], important for two regulatory phenomena: the Pasteur effect, i.e. the increase of glucose utilization in response to a reduced oxygen supply; and the so-called Randle effect, i.e. reduced utilization of glucose in heart and resting skeletal muscle with increased availability of fatty acids [68,69]. Hence, alterations in the allosteric regulation of tumor PFK by ATP and citrate may be crucial for partially decoupling glycolysis from oxidative phosphorylation and fatty acid utilization. This change in allosteric inhibition is probably due to the simultaneous presence of various isoforms of PFK subunits which may associate with different types of oligomers showing altered allosteric

properties compared with the 'classical' homomeric tetramers in normal cells [70]. In melanoma cells, elevation of the cellular Ca^{2+} concentration leads to detachment of PFK from the cytoskeleton and thus diminishes the provision of local ATP in the vicinity of the cytoskeleton [71]. The expression of PFK in tumor cells can be enhanced by Ras and src [72].

(5), (6) Phosphofructokinase-2 (PFK-2), fructose 2,6-bisphosphatase (PFKFB) (EC 2.7.1.105)

Unlike yeast cells, human PFK-2 and PFKFB represent one and the same bifunctional protein (PFK-2/FBPase) that upon phosphorylation/dephosphorylation may function as either phosphatase or kinase, respectively, and control the concentration of the allosteric PFK-1 activator fructose 2,6-P_2. Four genes encoding PFK-2/FBPase have been identified and termed PFKFB1 to PFKFB4. The PFKFB3 protein (also named iPFK-2) is expressed in high levels in human tumors *in situ*. Induction of this isoform is mediated by HIF-1, cMyc, Ras, src and loss of function of p53 [73]. Rapidly proliferating cancer cells constitutively express the isoform iPFK-2 [74]. PFKFB3 comprises an additional phosphorylation site that can be phosphorylated by the regulatory kinases AMPK [75] and Akt [76]. This phosphorylation results in a stabilization of the kinase activity of the enzyme. Besides PFKFB3, tumor cells express the specific p53-inducible histidine phosphatase TIGAR (TP53-induced glycolysis and apoptosis regulator). This enzyme is capable of reducing the level of fructose 2,6-P_2 independent of the phosphorylation state of iPFK-2. Reducing the level of fructose 2,6-P_2 and thus the activity of PFK-1 improves the supply of glucose 6-phosphate for the OPPPW, the main supplier of NADPH H^+ required for antioxidative defense reactions. At a low consumption rate of NADPH H^+, the rate of glucose 6-phosphate dehydrogenase (G6PD) catalyzing the first step of the OPPPW is controlled by the level of $NADP^+$ while glucose 6-phosphate is almost saturating at this enzyme (K_m values lie in the range of 0.04–0.07 mM [77] whereas glucose 6-phosphate levels between 0.1 and 0.3 mM have been reported [78]). Enhanced NADPH H^+ consumption, e.g. due to higher activity of antioxidative defense reactions, may increase the flux through the G6PD and the OPPPW by more than one order of magnitude. Mathematical modeling suggests that the availability of glucose 6-phosphate may become rate limiting [79]. This may account for the observation that high activity levels of TIGAR result in decreased cellular ROS levels and lower sensitivity of cells to oxidative-stress-associated apoptosis [80]. Taken together, the simultaneous presence of iPFK-2 and TIGAR allows much higher variations in the level of fructose 2,6-P_2 and thus of PFK-1 activity compared with normal cells [81].

(7) Aldolase (ALD) (EC 4.1.2.13)

There are three tissue-specific isoforms (A, B, C) of ALD. Studies on representative tumors in the human nervous system revealed largely varying abundance of ALD C [82]. The ALD A enzyme has been demonstrated to be inducible by HIF-1 [83–85]. Expression of ALD isoforms in cancer cells can be either downregulated, as for example in glioblastoma multiform [86] or human hepatocellular carcinoma [87,88], or upregulated as in pancreatic ductal adenocarcinoma [89]. Serum content of ALD may become elevated in malignant tumors [90] with ALD A being the predominant isoform [91] and thus being a candidate for a tumor marker [92]. Intriguingly, glyceraldehyde 3-phosphate, the reaction product of ALD, has been characterized as an anti-apoptotic effector owing to its ability to directly suppress caspase-3 activity in a reversible noncompetitive manner [93].

The flux through the ALD reaction splits into fluxes towards pyruvate, phospatidic acid and nucleotides via the NOPPPW. Thus, larger differences in ALD expression may reflect tissue-specific differences in the relative activity of these pathways. For example, in pancreatic tumor cells changes of the lipid content induce a higher proliferation rate [94] so that a higher demand for the glycerol lipid precursor DHAP might necessitate higher activities of ALD and triosephosphate isomerase in this tumor type.

(8) Triosephosphate isomerase (TPI) (EC 5.3.1.1)

Early studies have shown that the concentration of TPI in the blood plasma of patients with diagnosed solid tumors is significantly enhanced [95]. This finding has recently been confirmed by detection of auto-antibodies against TPI in sera from breast cancer patients [96]. Expression of TPI seems to be downregulated in quiescent parts of the tumors as shown for drug-resistant SGC7901/VCR gastric cancer cells [97].

(9) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12)

GAPDH has been implicated in numerous non-glycolytic functions ranging from interaction with nucleic acids to a role in endocytosis and microtubular

transport (for a review see [98]). Expression of GAPDH is highly dependent on the proliferative state of the cell and can be regulated by the transcription factors HIF-1, p53 and c-jun/AP1 [99,100]. GAPDH is a key redox-sensitive protein, the activity of which is largely affected by covalent modifications by oxidants at its highly reactive Cys152 residue. These oxidative changes not only affect the glycolytic function but also stimulate the participation of GAPDH in cell death [101].

(10) Phosphoglycerate kinase (PGK) (EC 2.7.2.3)

As with most glycolytic enzymes, the level of PGK-1 in tumor cells is enhanced by hypoxia. Immuno-histochemical analysis of 63 pancreatic ductal adenocarcinoma specimens revealed moderate to strong expression of PGK-1 in about 70% of the tumors [102]. This enzyme can be secreted and facilitates cleavage of disulfide bonds in plasmin, which triggers proteolytic release of the angiogenesis inhibitor angiostatin [103]. PGK secretion is under the control of oxygen-sensing hydrolases; hypoxia inhibits its secretion [104].

(11) Phosphoglycerate mutase (PGM) (EC 5.4.2.1)

PGM exists in mammalian tissues as three isozymes that result from homodimeric and heterodimeric combinations of two subunit types (muscle M and brain B). The level of PGM-M is known to be largely upregulated in many cancers, including lung, colon, liver and breast [105,106]. In mouse embryonic fibroblasts, a 2-fold increase in PGM activity enhances glycolytic flux, allows indefinite proliferation and renders cells resistant to ras-induced arrest [107]. More recent evidence indicates that p53 is capable of downregulating the expression of PGM. This finding is consistent with the notion that p53 would negatively regulate glycolysis.

(12) Enolase (EN) (EC 4.2.1.11)

The α-enolase gene encodes both a glycolytic enzyme (α-enolase) and a shorter translation product, the c-myc binding protein (MBP-1) lacking enzymatic activity. These divergent α-enolase gene products are interlinked: expression of the glycolytic enzyme α-enolase is upregulated by c-myc, a transcription factor that is known to be overexpressed in approximately 70% of all human tumors [35]. On the other hand, the alternative gene product MBP-1 negatively regulates c-myc transcription by binding to the P2 promotor [108].

(13) Pyruvate kinase (PK) (EC 2.7.1.40)

PK has two isoforms, PK-M and PK-L. In contrast to differentiated cells, proliferating cells selectively express the M2 isoform (PK-M2) [109]. During tumorigenesis, the tissue-specific isoenzymes of PK (PK-L in the liver or PK-M1 in the brain) are replaced by the PK-M2 isoenzyme [110]. Unlike other PK isoforms, PK-M2 is regulated by tyrosine-phosphorylated proteins [111]. Phosphorylation of the enzyme at serine and tyrosine residues induces the breakdown of the tetrameric PK to the trimeric and dimeric forms. Compared with the tetramer, the dimer has a lower affinity for phosphoenolpyruvate [112]. This regulation of enzyme activity in the presence of growth signals may constitute a molecular switch that allows proliferating cells to redirect the flux of glucose carbons from the formation of pyruvate and subsequent oxidative formation of ATP to biosynthetic pathways branching in the upper part of glycolysis and yielding essential precursors of cell components [113].

The regulation of PK by HIF-1 is not fully understood [114]. Discher *et al*. [115] reported the finding of two potential binding sites for HIF-1 in the first intron of the *PK-M* gene. On the other hand, Yamada and Noguchi [116] reported that there is no HIF-1 binding sequence 5′-ACGTGC-3′ in the promoter of the *PK-M2* gene and suggest that the interaction of SP1 and HIF-1 with CREB binding protein/p300 might account for the stimulation of *PK-M* gene transcription by hypoxia.

(14) Lactate dehydrogenase (LDH) (EC 1.1.1.27)

Tumor cells specifically express the isoform LDH-A, which is encoded by a target gene of c-Myc and HIF-1 [15,99]. The branch of pyruvate to either lactate or acetyl-CoA is controlled by the cytosolic LDH and the mitochondrial pyruvate dehydrogenase (see reaction 16 in Fig. 1). Reducing the activity of either reaction will cause an accumulation of pyruvate and hence promote its utilization through the complementary reaction. Indeed, reducing the LDH-A level of human Panc (P) 493 B-lymphoid cells by siRNA or inhibition of the enzyme by the inhibitor FX11 reduced ATP levels and induced significant oxidative stress and subsequent cell death that could be partially reversed by the antioxidant *N*-acetylcysteine [15].

(15) Plasma membrane lactate transport (LACT)

Lactate is transported over the plasma membrane by facilitated diffusion either by the family of proton-linked

monocarboxylate transporters (MCTs) (TCDB 2.A.1.13.1) or by SMCT1 (TCDB 2.A.21.5.4), an Na^+-coupled lactate transporter. Multiple MCT isoforms with different kinetic properties and tissue distribution exist [117]. The MCT4 isoform is upregulated in many cancer types [42,118,119]. However, some studies could not show an increased expression of MCT4 in cancer [120,121].

Increased expression of MCT1, the isoform found in most cell types, has been demonstrated in some studies [119,120,122], whereas other groups found a decreased expression [121,123]. The expression of MCT2, a high affinity isoform mainly implicated in the import of lactate [42], is decreased in tumor cell lines [119,120].

SMCT1, the Na^+-coupled lactate transporter with high affinity for lactate and implicated in lactate import [42], is downregulated in a variety of cancer tissue, including colon [124,125], thyroid [126,127], stomach [128], brain [129], prostate [130] and pancreas [131]. Re-expression of SMCT1 in cancer cell lines results in growth arrest and apoptosis in the presence of butyrate or pyruvate [42].

Mitochondrial pyruvate metabolism (reactions 16–20)

(16) Mitochondrial pyruvate transporter (MPT) (EC 3.A.8)

Current knowledge of the structural and kinetic features of MPT is limited. No tumor-specific MPT is currently known, as indicated by the practically identical K_m values for pyruvate determined for transporters isolated from mitochondria of several types of tumor cells and normal cells [132]. A comparative study of the transport of pyruvate in mitochondria isolated from normal rat liver and from three hepatomas revealed consistently diminished transport capacity in the tumors [133]. The activity of the MPT in Ehrlich ascites tumor cells was found to be 40% lower than in rat liver mitochondria [132]. A lower activity of MPT in conjunction with a significantly reduced activity of pyruvate dehydrogenase (reaction 17, see below) favors branching of pyruvate to lactate and thus aerobic glycolysis.

(17) Pyruvate dehydrogenase (PDH) (EC 1.2.4.1)

PDH is a multi-catalytic mitochondrial enzyme complex that catalyses the conversion of pyruvate to acetyl-CoA, a central metabolite of the intermediary metabolism. Acetyl-CoA can be oxidized in the citric acid cycle for aerobic energy production, serve as a building block for the synthesis of lipids, cholesterol and ketone bodies and provide the acetyl group for numerous post-translational acetylation reactions. The activity of PDH is mainly controlled by reversible phosphorylation that renders the enzyme inactive. One of the four known mammalian isoforms of the pyruvate dehydrogenase kinase (PDHK-1) (EC 2.7.11.2) has been shown to be inducible by HIF-1 in renal carcinoma cells and in a human lymphoma cell line [134,135], consistent with a reduction of glucose-derived carbons into the TCA cycle. However, overexpression of PDHK-1 and thus inhibition of PDH is not a common feature of all tumor cells. Oxidation of exogenous pyruvate by PDH was found to be enhanced in mitochondria isolated from AS-30D hepatoma cells in comparison with their normal counterpart [136].

(18) Citric acid cycle

Mutations in TCA cycle enzymes can lead to tumorigenesis [137–139]. Mutations of the succhinate dehydrogenase (SDH) (EC 1.3.5.1) and the fumarate hydratase (FH) (EC 4.2.1.2) have been shown to result in paragangliomas and pheochromocytomas. The succinate dehydrogenase complex assembly factor 2 (SDHAF2/SDH5), responsible for the incorporation of the co-factor FAD into the functional active SDH, was recently shown to be a paraganglioma-related tumor suppressor gene [137,140].

FH mutations have been found in cutaneous and uterine leiomyomas, leiomyosarcomas and renal cell cancer [137,141–146].

Two mechanisms have been suggested to account for the connection between loss of function of SDH or FH and tumorigenesis. (a) Redox stress due to generation of ROS by mutant SDH proteins [147,148] causes an inhibition of HIF-dependent prolyl hydroxylase (PHD) (EC 1.14.11.2) [149,150], an enzyme targeting under normoxic conditions the α-subunit of HIF for degradation. According to this explanation ROS can lead to pseudo-hypoxia in tumors with SDH mutations via stabilization of HIF [151]. (b) Metabolic signaling in SDH-deficient tumors via increased succinate levels inhibits the PHD and therefore leads to stabilization of the HIF-1α subunit at normal oxygen levels [141,151,152]. A similar mechanism was proposed for the consequences of FH deficiency: accumulating fumarate can act as a competitive inhibitor of PHD leading to a stabilization of HIF-1 [138,152,153].

Another enzyme of the TCA cycle that is frequently mutated specifically in some gliomas, glioblastomas and in acute myeloid leukemias with normal karyotype is the $NADP^+$-dependent isocitrate dehydrogenase

(IDH) (EC 1.1.1.42) 1 and 2 (for a recent review see [154]). Mutant forms of the brain IDH1 acquired a new catalytic ability to reduce α-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG) [155]. Elevated levels of 2HG are supposed to promote carcinogenesis [156]. However, the molecular mode of action of this compound has not yet been established. It can be speculated that owing to chemical similarity 2HG acts as a competitive inhibitor in αKG-dependent oxygenation reactions, in particular those catalyzed by PHD. If this were true, increased levels of 2HG could mimic hypoxic conditions.

The impact of the discovered enzyme mutants for flux control of the TCA cycle has not been studied so far. Labeling studies of TCA cycle intermediates using $^{[1-14C]}$acetate as substrate yielded consistently lower fluxes in cells from Ehrlich mouse ascites tumors, Walker carcinoma and LC-18 carcinoma [157]. The authors of this very old study attributed their finding to some defect in an intra-Krebs-cycle reaction which, however, has not been identified so far. As the TCA cycle is the main supplier of redox equivalents for the respiratory chain, a reduction of its turnover rate lowers the mitochondrial transmembrane potential, the formation rate of ROS and the rate of oxidative phosphorylation and thus promotes the tumor to switch to aerobic glycolysis.

(19) Respiratory chain and F0F1-ATPase (EC 3.6.3.14)

Recent observations suggest a wide spectrum of oxidative phosphorylation (OXPHOS) deficits and decreased availability of ATP associated with malignancies and tumor cell expansion [158]. Expression levels of OXPHOS enzymes and distribution patterns, most importantly the b-F1 subunit of ATPsynthetase, are downregulated in a variety of cancers [159–161], including colon, esophagus, kidney, liver, mammary gland and stomach [162–164]. This is probably one reason for the tumor's switch to aerobic glycolysis, which can also be induced by incubating cancer cells with oligomycin, an inhibitor of mitochondrial ATP synthetase [159,160]. Similarly, reduction of OXPHOS by targeted disruption of frataxin, a protein involved in the synthesis of mitochondrial Fe/S enzymes, leads to tumor formation in mice [165].

Deficiencies of electron carriers of the respiratory chain implicated in tumor growth have also been identified in complex I (EC 1.6.5.3) [144,166].

A key component determining the balance between the glycolytic pathway and mitochondrial OXPHOS is the p53-dependent regulation of the gene encoding cytochrome c oxidase 2 (SCO2) (EC 1.9.3.1) [167] which, in conjunction with the SCO1 protein, is required for the assembly of cytochrome c oxidase [168]. SCO2, but not SCO1, is induced in a p53-dependent manner as demonstrated by a 9-fold increase in transcripts. Thus, mutations of p53 cause impairment of OXPHOS due to COX deficiency and a shift of cellular energy metabolism towards aerobic glycolysis [167].

(20) Transport of mitochondrial acetyl-CoA to the cytosol

Formation of acetyl-CoA from the degradation of glucose and fatty acids occurs in the mitochondrial matrix whereas synthesis of fatty acids and cholesterol requires cytosolic acetyl-CoA. Hence, the efficiency of acetyl-CoA export from the mitochondrion to the cytosol is critical for the synthesis of membrane lipids and cholesterol needed for the rapid size gain of tumor cells. Mitochondrial acetyl-CoA condenses with oxaloacetate to citrate that can be transported to the cytosol [169]. Tumor mitochondria export comparably large amounts of citrate [161,170,171]. In the cytosol, citrate is split again into oxaloacetate and acetyl-CoA by the ATP citrate lyase (EC 2.3.3.8). Inhibition of ATP citrate lyase was reported to suppress tumor cell proliferation and survival *in vitro* and also to reduce *in vivo* tumor growth [172]. The activity of ATP citrate lyase is under the control of the Akt signaling pathway [173].

Lipid synthesis (21, 22)

(21) Fatty acid synthetase (FASN) (EC 2.3.1.85)

In cancer cells, *de novo* fatty acid synthesis is commonly elevated and the supply of cellular fatty acids is highly dependent on *de novo* synthesis. Numerous studies have shown overexpression of FASN in various human epithelial cancers, including prostate, ovary, colon, lung, endometrium and stomach cancers [174]. FASN expression is regulated by signaling pathways associated with growth factor receptors such as epidermal growth factor receptor, estrogen receptor, androgen receptor and progesterone receptor. Downstream of the receptors, the phosphatidylinositol-3-kinase Akt and mitogen-activated protein kinase are candidate signaling pathways that mediate FASN expression through the sterol regulatory element binding protein 1c. In breast cancer BT-474 cells that overexpress HER2, the expression of FASN and acetyl-CoA carboxylase (ACC) are not mediated by sterol regulatory element binding protein 1 but by a mammalian target of rapamycin dependent selective translational induction [175].

Apart from the transcriptional regulation, the activity of FASN is also controlled at post-translational

levels. Graner *et al.* showed that the isopeptidase ubiquitin-specific protease 2a (EC 3.4.19.12) interacts with and stabilizes FASN protein in prostate cancer [176]. Finally, a significant gene copy number gain of FASN has been observed in prostate adenocarcinoma [177]. Taken together, these observations suggest that tumor-related increase of FASN activity could be regulated at multiple levels [178].

(22) Formation of 1,2-diacyl glycerol phosphate (phosphatidate)

There are two alternative pathways leading from the glycolytic intermediate dihydroxyacetone phosphate (DHAP) to 1,2-diacyl glycerol phosphate, the precursor of both triglycerides and phospholipids: (a) initial NADH H^+-dependent reduction of DHAP to glycerol phosphate by α-glycerophosphate dehydrogenase (EC 1.1.1.8) (GDPH) and subsequent attachment of two fatty acid moieties, and (b) acylation of DHAP to acyl-DHAP followed by an NADPH H^+-dependent reduction to 1-acyl glycerol phosphate and attachment of the second fatty acid. Notably, GDPH competes with the LDH reaction 14 for cytosolic NADH H^+. There is also a membrane-bound mitochondrial form of this enzyme that works with the redox couples FAD/$FADH_2$ and Q/QH_2. The redox shuttle constituted by the cytosolic and mitochondrial enzyme species enables electron transfer from cytosolic NADH H^+ to complex II (EC 1.3.5.1) of the respiratory chain. Whereas in a wide variety of normal tissues the ratio of LDH/GPDH varies between the extremes of 0.5 and 7.0, this ratio in tumors ranges from 10 to several hundred [179] enabling preferential utilization of glycolytically formed NADH H^+ for lactate production. The increase in ratio is primarily due to reduced GPDH activity in the presence of normal or slightly increased LDH activity. In order to assure a sufficiently high rate of lipid synthesis, conversion of DHAP to phosphatidic acid has to proceed predominantly via the acyl-DHAP branch, as has been demonstrated in homogenates of 13 different tumor tissues [180].

Glycogen metabolism (reactions 23–26)

Glycogen is the main cellular glucose storage. Large variations in glycogen content have been reported in various tumor tissues [181]. While human cervix [182] tumor tissue exhibits decreased glycogen levels, in colon tumor tissue [183] and lung carcinoma [181] increased glycogen levels can be observed. Studies in three different human tumor cell lines have provided evidence that these tumor-specific differences in glycogen content are due to growth-dependent regulation of the glycogen synthase (reaction 25) and glycogen phosphorylase (reaction 26) [184]. These observations together with the findings reported below for some key enzymes of the glycogen metabolism suggest large variations in the ability of individual tumors to store and utilize glycogen.

(23) Phosphoglucomutase (EC 5.4.2.2)

Phosphoglucomutase catalyses the reversible interconversion of glucose 1-phosphate and glucose 6-phosphate into each other. Early studies in five different solid tumors (hepatoma, carcinosarcoma, sarcoma, leukemia and melanoma) showed significantly reduced activity of phosphoglucomutase [185]. Gururaj *et al.* [186] discovered that signaling kinase p21-activated kinase 1 binds to phosphorylates and enhances the enzymatic activity of phosphoglucomutase 1 in tumors. The increase of activity of the phosphorylated enzyme was only about 2-fold so that the implications of this activation for metabolic regulation remain unclear as the phosphoglucomutase reaction is not considered a rate limiting step in glucose metabolism [187].

(24) UTP-glucose-1-phosphate uridylyltransferase (UGPUT) (EC 2.7.7.9)

UGPUT catalyses the irreversible reaction of glucose 1-phosphate to UDP-glucose, a central metabolite of glucose metabolism that is indispensable for the synthesis not only of glycogen but also of glycoproteins and heteropolysaccharides. Therefore, we were surprised that a literature search did not provide any information on the expression and regulation of this enzyme in tumor cells. According to a proteome analysis of human liver tumor tissue there is no evidence for a significant tumor-related change of the protein level of this enzyme [188]. On the other hand, enzymatic assays showed – with the exception of melanoma – a significant decrease of activity of about 50% in the several tumors also tested for the activity of phosphoglucomutase (see above).

(25) Glycogen synthase (EC 2.4.1.11)

Glycogen synthase has long been considered the rate limiting step of glycogen synthesis. However, glucose transport and glycogen phosphorylase activity have been shown to exert considerable control on glycogen synthesis [189–191]. The enzyme becomes inactive upon phosphorylation either by the cAMP-dependent protein kinase A or by the insulin-dependent glycogen

synthase kinase 3β, a multifunctional serine/threonine kinase that functions in diverse cellular processes including proliferation, differentiation, motility and survival [192]. In particular, glycogen synthase kinase 3β plays an important role in the canonical Wnt signaling pathway, which is critical for embryonic development [193,194]. Defects in Wnt signaling have been reported in a wide range of cancers [193,195,196]. Nevertheless, the role of glycogen synthase kinase 3β in tumorigenesis is still elusive [197].

(26) Glycogen phosphorylase (GP) (EC 2.4.1.1)

GP is the rate limiting enzyme in glycogenolysis. Reciprocally regulated as the glycogen synthase, it becomes active upon phosphorylation by the cAMP-dependent PKA [198]. Brain-type glycogen phosphorylase (BGP) is suggested to be the major isoform in tumor and fetal tissues [199–203]. Elevated levels of BGP have been detected in renal cell carcinoma [203], colorectal carcinomas [204], the glycogen-poor Morris hepatoma 3924A [205] and non-small-cell lung carcinoma where high BGP expression was associated with poorer survival [206]. The expression of BGP has been proposed to be a potential early biomarker for human colorectal carcinomas [204]. By contrast, in brain tumor tissues (astrocytoma and glioblastoma) the activity of GP was found to be practically zero. Interestingly, glycogen present in detectable amounts in these tumors is hydrolytically degraded by upregulated α-1,4-glucosidases [207]. The physiological role of BGP is not well understood, but it seems to be involved in the induction of an emergency glucose supply during stressful periods such as anoxia and hypoglycaemia.

The pentose phosphate cycle (reactions 27–32)

The pentose phosphate cycle is composed of two branches: the OPPPW irreversibly converts glucose 6-phosphate to ribose phosphates thereby yielding 2 moles NADPH H^+ per mole glucose, and the NOPPPW reversibly converts three pentose phosphates into two hexose phosphates (fructose 6-phosphate) and one triose phosphate (GAP). In contrast to non-transformed cells which produce most of the ribose 5-phosphate for nucleotide biosynthesis through the OPPPW, the NOPPPW has been suggested to be the main source for ribose 5-phosphate synthesis in tumor cells [208–210]. However, there are major differences in the relative share of these two pathways in the delivery of pentose phosphates when comparing slow and fast growing carcinoma [211].

(27) Glucose 6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49)

The activity of NADPH H^+-related dehydrogenases is generally increased in tumor cells [212]. The central importance of the redox couple $NADP^+$/NADPH H^+ for tumor cells has been attributed, amongst other possible reasons, to their role in the control of the activity of redox-sensitive transcription factors such as nuclear factor κB, activator protein 1 and HIF-1 and the need for NADPH H^+ as fuel for antioxidative defense reactions. Overexpression of G6PD in NIH3T3 cells resulted in altered cell morphology and tumorigenic properties that could be mitigated by glutathione depletion [213], whereas knockdown of the G6PD in a stable line of A375 melanoma cells decreased their proliferative capacity and colony-forming efficiency [214]. In line with the potential role of G6PD as an oncogene, its activity was found to be upregulated in virtually all cancer cells. There is evidence that the increased activity of G6PD in neoplastic tissues can be attributed to post-transcriptional activation, probably by attenuation of the inhibition by glucose 1,6-P_2 [215], as in neoplastic lesions of rat liver a 150-fold higher v_{max} value was determined although the amount of the enzyme was not significantly higher than in extra-lesional liver parenchyma [216].

(28) 6-Phosphogluconate dehydrogenase (6PGD) (EC 1.1.1.44)

Early biochemical and histological studies [217] revealed the level of 6PGD to be significantly increased in cervical cancer which led to the proposal to use this enzyme as a screen test for cervical carcinoma in women [218]. Later studies in tumors of canine mammary glands [219] and in human colon tumors [215] also showed an increased level of 6PGD. As 6PGD catalyzes the second NADPH H^+ delivering reaction of the OPPPW, its higher activity in tumors can be reasoned along the same line of arguments as outlined above for the higher tumor levels of G6PD. Indeed, the two OPPPW dehydrogenases essentially act as a single unit because the lactonase reaction (not shown in Fig. 1) very rapidly converts the product of G6PD into the substrate of 6PGD.

(29) Ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase (EC 5.3.1.6)

These two enzymes interconverting the three pentose phosphate species ribose 5-phosphate, ribulose

5-phosphate and xylulose 5-phosphate into each other have not attracted the attention of cancer enzymologists so far. This is strange as from a regulatory point of view high flux rates through the OPPPW as the main source of NADPH H^+ production in normal as well as in neoplastic tissues inevitably result in a high production rate of ribulose 5-phosphate which, if not used for nucleotide biosynthesis, has to be recycled back to intermediates of the glycolytic pathway via the NOPPPW, and this should require a correspondingly high activity of ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase linking the OPPPW and NOPPPW.

(30) Phosphoribosyl pyrophosphate synthetase (PRPPS) (EC 2.7.6.1)

The formation of phosphoribosyl pyrophosphate by PRPPS represents the first step in the *de novo* synthesis of purines, pyrimidines and pyridines. The activity of PRPPS was found to be about 4-fold augmented in rapidly growing human colon carcinoma compared with slowly growing xenografts [211]. This is not necessarily a tumor-specific feature as this enzyme is known to vary considerably in activity in different phases of the cell cycle. Remarkably, a super-active form of PRPPS has been identified in lymphoblast cell lines characterized by an increased v_{max} value, inhibitor resistance and increased substrate affinity [220]. Regulation of the PRPPS in tumor cells is yet poorly characterized.

(31) Transketolase (TKT) (EC 2.2.1.1)

Among the three members of the TKT gene family (TKT, TKTL1 and TKTL2), TKTL1 has been reported to be overexpressed in metastatic tumors and specific inhibition of TKTL1 mRNA can inhibit cell proliferation in several types of cancer cells [221–224]. However, direct determinations of TK activities in tumors are lacking so far [212]. Intriguingly, fructose induces thiamine-dependent TKT flux and is preferentially metabolized via the NOPPPW. Hence, cancer cells can readily metabolize fructose to increase proliferation [225].

(32) Transaldolase (TALD) (EC 2.2.1.2)

In liver tumors, TALD1 activity was increased 1.5- to 3.4-fold over the activities observed in normal control rat liver [222]. TALD1 was found to be extraordinarily highly expressed in a subgroup of squamous cell carcinoma tumors of the head and neck [226].

Concluding remarks

Rapid cell proliferation depends on both the permanent presence of growth stimuli and a sufficiently high metabolic capacity to produce all cell components needed in different phases of the cell cycle. After decades of predominantly genetic research on tumor cells, we are currently witnessing a renaissance of metabolic research. One central goal is to unravel the metabolic regulation underlying the ravenous appetite of most tumor types for glucose.

While carefully reviewing the available literature on tumor-specific enzymes involved in the main pathways of glucose metabolism we observed a clear preponderance of gene expression studies compared with detailed enzyme-kinetic studies and metabolic flux determinations. Obviously, during the past decade, the application of high-throughput transcriptomics and proteomics has resulted in a huge set of data on gene expression of tumor-specific metabolic enzymes and of many other key proteins such as growth-related receptors, kinases and transcription factors. Taken together, these data reveal upregulation of most metabolic enzymes except the mitochondrial ones, a fact that does not come as a surprise for rapidly dividing cells exhibiting in most cases an accelerated aerobic glycolysis. Importantly, these high-throughput studies point to considerable differences in the level of specific metabolic enzymes observed in various tumor types and at different stages of tumor growth (see variations in the upregulation and downregulation of enzyme levels indicated in Fig. 1). It is important to refrain from the notion that there is a unique metabolic phenotype of tumor cells. Rather, tumor cells still exhibit specific metabolic functions accentuated in the normal tissue cells from which they derive. For example, HepG2 cells are still endowed with most reactions of the liver-specific bile acid synthesizing pathway [227] entailing a higher flux of glucose-derived carbons through this pathway compared with other tumor cells. We think that tumor-type-specific larger variations in the expression level of enzymes such as TIM or ALD situated at branching points within the metabolic network can be partially accounted for by differing capacities of the pathways that are required to pursue tissue-specific growth strategies (e.g. excretion of metabolites for extracellular signaling) and which tumor cells still maintain as heritage of their normal ancestor cells.

Notwithstanding, current knowledge of enzyme expression levels alone does not allow us to reconstruct the metabolic strategies pursued by a given tumor type in different stages of differentiation and in response to varying external conditions, e.g. drug therapy. This is

mainly because there appears to be a surprisingly weak concordance between the level of transcripts and proteins of metabolic enzymes [228] and the fluxes that they carry [229]. For example, the maximal rate of G6PD in hepatic tumor lesions can be increased by more than two orders of magnitude without significant changes in its expression level. Some tumor cells express specific GLUT mRNA but not the respective protein, indicating an important role of post-transcriptional regulation [230]. These examples illustrate striking discordances between transcript levels and enzymatic activities. Obviously, the importance of enzyme-regulatory mechanisms operating below the control of gene expression was dramatically underestimated in the past decade, which was characterized by the innovation and broad application of high-throughput technologies.

It is our conviction that the key for understanding the exceptional way of glucose utilization in tumors is less the expression level of metabolic enzymes as such, but rather the unique pattern of isoenzymes, the regulatory properties of which allow tumor cells to develop a selfish glucose-ravening phenotype (see Table 1). Therefore, we strongly suggest that large-scale expression studies should be complemented with hypothesis-driven experimentation [231] and mathematical modeling [232,233]. For example, while detailed kinetic models of glycolysis in red blood cells [21,234] and yeast [235] have provided valuable insights into the regulation of this important pathway in the respective cell type such models are not available for any tumor type. In order to obtain a consistent mechanistic and quantitative picture of metabolic regulation in tumor cells more experimentation is needed to determine, for example, the kinetic parameters of tumor-specific enzymes and membrane transporters, to identify novel allosteric effectors, to measure enzyme activities in various phosphorylation states and to measure the concentration of key metabolites in the cytosol and mitochondrial matrix. And we have to pay more attention to how key metabolites such as AMP, NADH H^+, NADPH H^+, glutathione, tetrahydrofolate and others feed back to the gene-regulatory level, e.g. by directly controlling the activity of transcription factors. Finally, carefully designed mathematical models are needed to bring together all these mechanistic details in a consistent manner. Once we are able to simulate the metabolism of tumors on the basis of reliable mathematical models it might be possible to identify 'Achilles' heels' in tumor metabolism that can be selectively targeted by specific drugs without globally impairing the metabolism of normal cells.

The debate whether metabolic changes such as the Warburg effect are a consequence or even the cause of tumorigenesis is currently starting again. Over decades, Otto Warburg's proposal that irreversible damage to mitochondrial respiration – a metabolic failure – is the primary cause of cancer has been criticized in that it does not account for the mutations and chromosomal abnormalities needed to disable surveillance systems and confer an uncontrolled growth potential to tumor cells. In our view there is dialectic interplay between genetic and metabolic alterations during tumorigenesis without a fixed cause–effect relationship. Exposure of cells to non-physiological challenges such as hypoxia, oxygen radicals produced endogenously or during inflammation, toxic drugs or radiation increases the risk of DNA damage. Notably, a pure metabolic perturbation such as the transition from normoxia to hypoxia may give rise to an increase of ROS production, probably at the level of complex III of the respiratory chain [236]. mtDNA is more vulnerable to damage than nDNA because it lacks protection by histones. Of mtDNAs analyzed in various tumor types 40%–80% display mutational changes [237], a remarkably high incidence for this tiny piece of DNA (16 569 bp coding for 13 polypeptides of the respiratory chain). Mutations of the nDNA hitting genes involved in mitochondria biogenesis such as, for example, enzymes of the cardiolipin synthesizing pathway, the TCA cycle or membrane transporters may additionally contribute to mitochondrial impairment. Mitochondrial defects have two important implications. First, lowered ATP production by oxidative phosphorylation is compensated through an increase in glycolytic ATP production by virtually the same regulatory mechanism as that underlying the 'classical' Pasteur effect that is usually elicited by reduced oxygen supply. Second, enhanced ROS production causes a higher rate of mutations in both nDNA and mtDNA, thereby stabilizing a high level of ROS in a vicious cycle. Trachootham *et al.* [238] have recently demonstrated that tumor cells experience more oxidative stress than normal cells. Long-term enhanced oxidative stress may drive the 'mutator' that is needed to generate the high number of mutations usually found in tumor cells. Once random nDNA mutations have hit a set of key proteins involved in the stabilization of the genome and the regulation of cell proliferation and apoptosis, the transformation into a malignant cell type is accomplished. A persistently high level of ROS is still beneficial for the tumor cell in that it enables Kras-induced anchorage-independent growth through regulation of the ERK MAPK signaling pathway [239]. In summary, there is accumulating evidence that a still ongoing but

Table 1. Expression changes and characteristic isoforms in cancer cells.

Name		Isoform	Expr. up	Reference	Expr. down	Reference	Isoform preference	Reference
1	GLUT		▲		▼		GLUT1	[27–29]
		GLUT1	▲	[27–29]				
		GLUT4			▼	[30]		
		SGLT1	▲	[42,43]				
2	HK						HK2	[46]
		HK2	▲	[47–51]				
3	GPI/AMF		▲	[61]				
4	PFK-1						Isoform change	[67]
5/6	PFK-2/PFKFB						PFKFB3	[73]
		PFKFB3 (iPFK-2)	▲	[73]				
		TIGAR	▲					
7	ALD		▲	[89]	▼	[86–88]		
		ALD A	▲	[91]				
8	TPI	TPI	(▲)	[95]				
9	GAPDH							
10	PGK		▲	[102]				
11	PGM						PGM-M	[105,106]
		PGM-M	▲	[105,106]				
13	PK						PK-M	[110]
14	LDH						LDH-A	[15,99]
		LDH-A	▲	[15,99]				
15	LACT						MCT4	[42,118,119]
		MCT4	▲	[42,118,119]	(▼)	[120,121]		
		MCT1	▲	[119,120,122]	▼	[121,123]		
		MCT2			▼	[119,120].		
		SMCT1			▼	[124–131]		
17	PDH						(PDHK-1)	
		PDHK-1	▲	[134,135]				
19	OXPHOS							
		b-F1 subunit ATPase			▼	[159–164]		
21	FASN		▲	[174]				
26	GP						BGP	[199–203]
		BGP	▲	[203–205]				
27	G6PD		(▲)	[213]				
28	6PGD		▲	[215,217,219]				
31	TK						(TKTL1)	[221–224]
		TKTL1	▲	[221–224]				
32	TALD						TALD1	[226]
		TALD1	▲	[226]				

functionally impaired mitochondrial metabolism is indeed essential for tumorigenesis.

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