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Energy metabolism in tumor cells

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In early studies on energy metabolism of tumor cells, it was proposed that the enhanced glycolysis was induced by a decreased oxidative phosphorylation. Since then it has been indiscriminately applied to all types of tumor cells that the ATP supply is mainly or only provided by glycolysis, without an appropriate experimental evaluation. In this review, the different genetic and biochemical mechanisms by which tumor cells achieve an enhanced glycolytic flux are analyzed. Furthermore, the proposed mechanisms that arguably lead to a decreased oxidative phosphorylation in tumor cells are discussed. As the O_2 concentration in hypoxic regions of tumors seems not to be limiting for the functioning of oxidative phosphorylation, this pathway is re-evaluated regarding oxidizable substrate utilization and its contribution to ATP supply versus glycolysis. In the tumor cell lines where the oxidative metabolism prevails over the glycolytic metabolism for ATP supply, the flux control distribution of both pathways is described. The effect of glycolytic and mitochondrial drugs on tumor energy metabolism and cellular proliferation is described and discussed. Similarly, the energy metabolic changes associated with inherent and acquired resistance to radiotherapy and chemotherapy of tumor cells, and those determined by positron emission tomography, are revised. It is proposed that energy metabolism may be an alternative therapeutic target for both hypoxic (glycolytic) and oxidative tumors.

In biochemical and physiological studies, tumor cells are usually classified according to their rate of growth: low; intermediate; or fast [1]. For tumors in experimental animals, the growth rate is determined by size and volume, mitotic count, degree of differentiation and thymidine incorporation [2]. Examples of fast-growth tumors in mice include several experimental cancers, such as Ehrlich ascites tumor, fibrosarcoma 1929 and lymphocytic leukemia L1210; and in rats, fast-growth tumors include the hepatomas of Morris (3924A, 7793, 7795, 7800, 7288C, 7316B, 3683), Reuber H-35, Novikoff, AH130 and AS-30D, breast carcinosarcoma Walker 256, hepatocellular carcinoma HC-252, hepatoma induced by dimethylazobenzene and DS-carcinosarcoma [1].

In human tumors, classification is based on their histological characteristics and stage of clinical progression. By their advanced developmental stage and metastatic properties, some human tumors considered to be of fast growth are breast carcinoma, ovarian carcinoma, melanoma, thyroid carcinoma, uterine carcinoma and lung carcinoma [1,3]. Human primary brain tumors, such as gliomas, glioblastomas and medulloblastomas, are also considered as fast-growth tumors because of their high rate of proliferation (average transfer in days or weeks) and their conversion to a poorly differentiated status [4,5].

Tumor cells exhibit profound genetic, biochemical and histological differences with respect to the original,

Abbreviations
ALD, aldolase; ANT, adenine nucleotide translocase; COX, cyclooxygenase; CT, computed tomography; F2,6BP, fructose-2,6-bisphosphate; FDG, ^{18}fluoro-deoxyglucose; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; G6P, glucose-6-phosphate; HIF, hypoxia inducible factor; HK, hexokinase; LDH, lactate dehydrogenase; NSAID, nonsteroidal anti-inflammatory drug; PDH, pyruvate dehydrogenase complex; PET, positron emission tomography; PFK-1, phosphofructokinase type 1; PFK-2, phosphofructokinase type 2; Pyr, pyruvate.

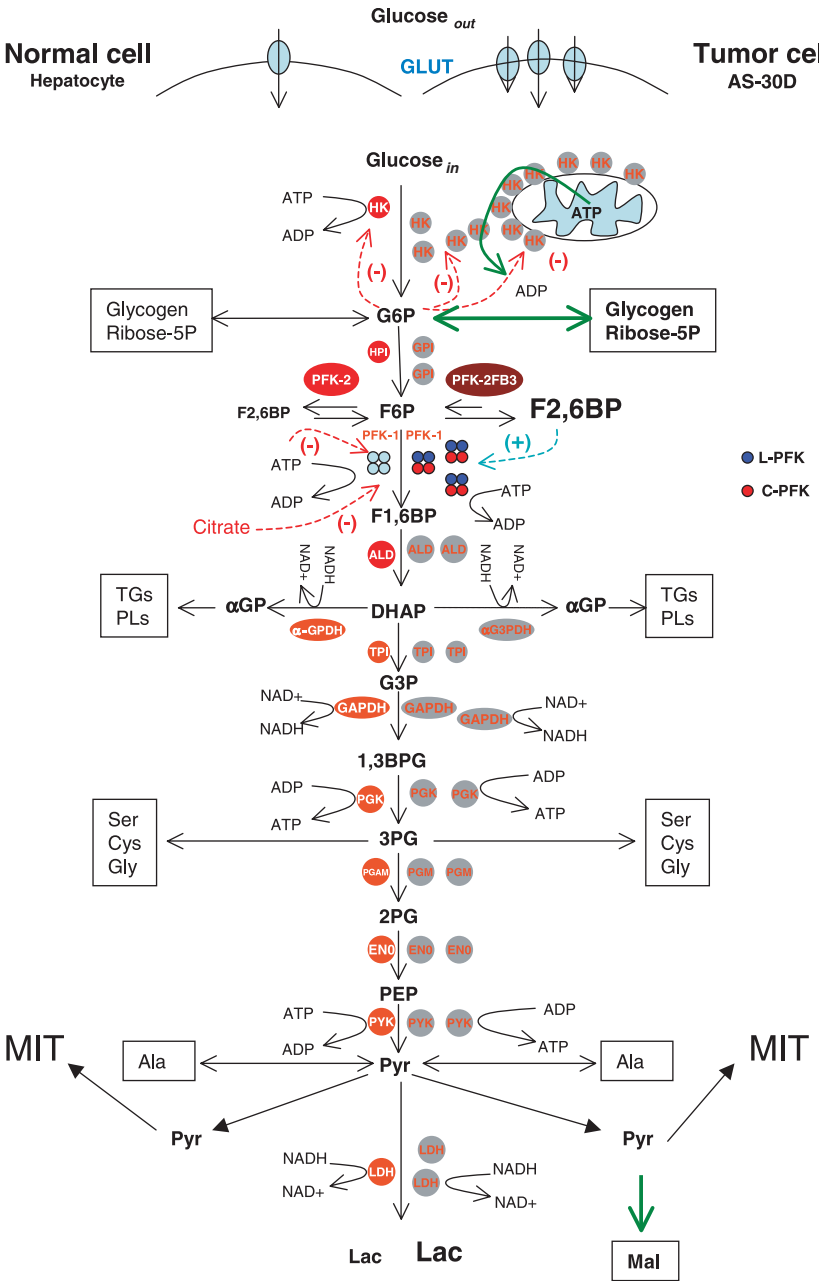

Fig. 1. The glycolytic pathway in normal cells (left) and tumor cells (right). In tumor cells, there is an increase of all enzymes and glucose transporters with respect to normal cells. In tumor cells hexokinase II (HK-II) is over-expressed, increasing both the activity and the binding to the outer mitochondrial membrane, which in turn increases the HK-II access to newly synthesized ATP by oxidative phosphorylation. An increased flux towards ribose-5-phosphate (and nucleotide) synthesis is documented for several tumor cells. Tumor phosphofructokinase type 1 (PFK-1) (C and L subunits) and the PFK-2FB3 isoform are also over-expressed. In some tumors, the amount of α-glycerol-3-phosphate dehydrogenase (αGPDH) decreases. In addition to be transformed in L-lactate, pyruvate may be oxidized by mitochondria (MIT), generate alanine (Ala) and, in tumor cells, synthesize malate in a reaction catalyzed by an over-expressed cytosolic malic enzyme. Other relevant branches of the glycolytic pathway are also indicated. The over-expressed HK is strongly inhibited by its product, glucose-6-phosphate (G6P), whereas PFK-1 activation by fructose-2,6-bisphosphate (F2,6BP) overcomes the citrate and ATP inhibition.

nontransformed cellular types. The vast majority of fast-growth tumor cell types display a markedly modified energy metabolism in comparison to the tissue of origin (Figs 1 and 2), which has been widely documented for human cervix (HeLa), pharynx and mammary gland (MCF-7, MDA-MB-453) tumors, as well as for astroblastomas, gliomas (U-251MG, D-54MG, U-87 and U118MG) and oligodendrogliomas. The same applies for tumors experimentally developed in rodents (hepatomas of Ehrlich, Ehrlich-Lettré, Morris and AS-30D; Walker 256 carcinoma; C6 glioma) [1,5–9].

The most notorious and well-known energy metabolism alteration in tumor cells is an increased glycolytic capacity, even in the presence of a high O_2 concentration [1,6–11]. For instance, the glycolytic flux is 2–17 times higher in rat hepatomas than in normal hepatocytes [3,11]. It has been proposed that this increase in the glycolytic flux is a metabolic strategy of tumor cells to ensure survival and growth in environments with low O_2 concentrations [10]. Several mechanisms for the enhanced glycolysis in tumor cells have been advanced and documented (Table 1). It has to be emphasized

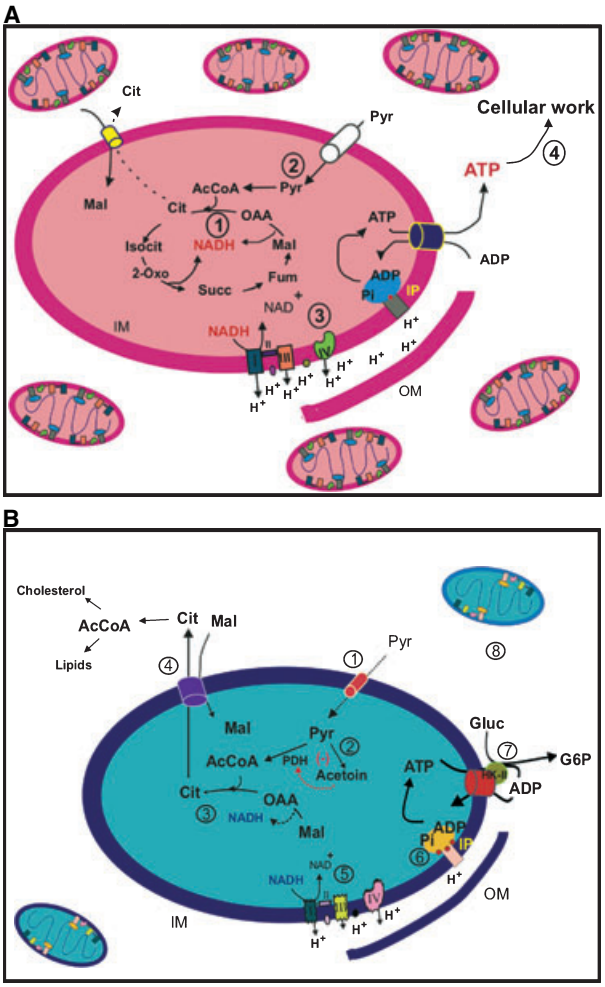

Fig. 2. (A) Metabolic pathways in normal mitochondria. (1) The Krebs cycle generates high NADH levels; (2) the concerted action of the pyruvate (Pyr) transporter and pyruvate dehydrogenase complex (PDH) generate adequate levels of acetyl-CoA; (3) NADH from the Krebs cycle is a substrate for the respiratory chain, which generates a high H^+ electrochemical gradient that drives ATP synthesis by ATP synthase; and (4) ATP is exported by adenine nucleotide translocase (ANT) to the cytosol to be used for cellular work. (B) Principal metabolic perturbations, described or proposed for some tumor mitochondria, which lead to a damaged oxidative phosphorylation. (1) Cytosolic pyruvate is transported into mitochondria through a deficient Pyr transporter; (2) mitochondrial Pyr is decarboxylated to acetoin, which inhibits the tumor PDH; (3) truncated Krebs cycle with low aconitase and isocitrate dehydrogenase activities; (4) a high citrate efflux for cholesterol and fatty acids synthesis is developed; (5) low activity and expression of several respiratory chain complexes promotes a low H^+ electrochemical gradient; (6) an increase in the inhibitory protein (IP; red circles) decreases the ATP synthase hydrolytic activity; (7) the close vicinity of hexokinase-II (HK-II) and ANT favors the direct transfer of mitochondrial ATP to HK-II for glucose phosphorylation; and (8) tumor cells have a lower number of mitochondria which, in turn, have a lower number of respiratory chain copies. IM, inner mitochondrial membrane; OM, outer mitochondrial membrane.

that there is no reason to apply the mechanisms, described below, to all cancer cells automatically; each particular tumor cell line has its own combination of mechanisms and degree of expression for increasing glycolysis.

Glycolytic enzymes and transporters in tumor cells

Transcriptional regulation of the glycolytic genes

A great body of evidence suggests that the main mechanism by which glycolysis is substantially higher in tumor cells than in nontumorigenic cells is the enhanced transcription of genes of several or all pathway enzymes and transporters, which is accompanied by an enhanced protein synthesis [12–15]; activity has, however, rarely been determined.

For instance, in comparison to normal rat hepatocytes (Fig. 1), all glycolytic enzymes are over-expressed by two- to fourfold in rat AS-30D hepatoma [hexose-6-phosphate isomerase, aldolase (ALD), triose-phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase, phosphoglycerate mutase, enolase and lactate dehydrogenase (LDH)], pyruvate kinase is over-expressed by eight- to 10-fold, and hexokinase (HK) and phosphofructokinase type 1 (PFK-1) are over-expressed by up to 17- to 300-fold (Fig. 1) [11,16]. For human cervix HeLa cells, all enzymes, including HK and PFK-1, are over-expressed by two- to sevenfold, with the exception of phosphoglycerate mutase and LDH, which are expressed at a level two- to seven-fold lower than in rat hepatocytes [11]. However, for this last case a more rigorous comparison should be made with normal uterine cervix epithelial cells (i.e. the original source) when data become available. In Morris hepatomas, the activity of HK, PFK and pyruvate kinase is 5- to 500-fold higher than in liver [17], whereas the activity of HK, ALD, pyruvate kinase and LDH is 3.7- to 7-times higher in human breast cancer than in normal tissue [18].

Perhaps the prime driving mechanism for the enhanced glycolysis is activation, via the hypoxia inducible factor 1 (HIF-1), of the transcription and translation of glycolytic genes in tumor cells. HIF-1 is a transcription factor constituted by two subunits, HIF-1α and HIF-1β. Factor stability mostly depends on HIF-1α. Under aerobiosis, an active process of HIF-1α degradation is promoted, whereas in anaerobiosis, HIF-1α becomes highly stable [19,20]. In addition to hypoxia, HIF-1α may be induced, under aerobiosis, by cytokines, growth factors, reactive oxygen species and

Table 1. Mechanisms explaining the accelerated glycolytic rate in fast-growing tumor cells. GLUT, glucose transporter; HK, hexokinase; PFK-1, phosphofructokinase type 1; PFK-2, phosphofructokinase type 2.

	Tumor cell type	
	Rodent	Human
1. Increase in the isoform expression of the glycolytic enzymes and glucose transporters		
GLUT	AS-30D, Novikoff, Ehrlich, and Morris 3924A hepatomas; ependymoblastoma; thyroid and Lewis lung carcinomas [34]	HepG2 carcinomas; brain tumors (A-172, H4) [34]; breast cancer (MCF-7 and T47D); leukemias (Jurkat, HL60, U937,U1); pancreatic, lung, renal (HEK-293), cutaneous, gastric and esophageal tumors [35]
HK	AS-30D hepatoma [11,16]; Morris 7800,5123-D, 7288-C, 3924-A; H19 cells [31]; 3683 and Novikoff hepatomas [44]	HeLa carcinoma [11], ependymoma, astrocytoma, glioma [45]
PFK-1	AS-30D hepatoma [11,16]; mouse ascites carcinoma [33]; thyroid carcinoma [51]; Morris (7800,5123-D,7288-C, 3924-A, 3683); Ehrlich Lettré [53]	HL-60, KG-1, K-562 myeloid leukemia,MOLT-4 leukemia, lymphoma [32], HeLa and KB carcinoma [32], glioma [45]
PFK-2	Ehrlich hepatoma [25]	HeLa, HepG2 [55,57], Hek-293, Lewis lung carcinoma, K562 leukemia, MCF-7 breast carcinoma, TD47 cells [15]
All enzymes	AS-30D hepatoma [11]	HeLa [11] and CaSKi carcinoma, U87 glioblastoma, DU145 prostate tumor, renal RCC4 tumor [24]
2. Decreased expression of mitochondrial oxidative enzymes and transporters		
	Ehrlich [59,60], Morris (16, 44, 777, 3924A, 7794A, 7800) [1,61,66,72], Novikoff, Yoshida, Reuber H-35, and BW7756 hepatomas [1,69,75]; L1210 leukemia; leukemic B82T tumor; SV40-transformed fibroblast [1]	HeLa carcinoma; mammary tumors (Cf7, C3H) [1]; meningioma; ependymoma; pituitary adenoma [74]; human kidney carcinoma [77]
3. Lowering in the amount of mitochondria per cell		
	C-57, HC-252 carcinomas [1], mammary adenocarcinoma [73]	
4. Inhibition of oxidative phosphorylation by glycolysis activation (Crabtree effect)		
	Ehrlich-Lettré [80], AS-30D [64] hepatomas; EL-4 thymoma [83]; sarcoma 180 [81]; tumor pancreatic islet cells; insulinoma RINm5F [82]	HeLa [84], HT29 [85]
5. Increased amount in the natural inhibitor protein (IF1) of the mitochondrial ATP synthase		
	Zadjela and Yoshida sarcomas [90], AS-30D hepatoma [91]	
6. Higher sensitivity of mitochondrial DNA to oxidative stress		
		Breast, colon, stomach, liver, kidney, bladder, head/neck and lung tumors; leukemia; lymphoma [93]

nitric oxide; or by the energy-metabolism intermediates pyruvate (Pyr), lactate and oxaloacetate [20–22]. The von Hippel–Lindau protein, a tumor suppressor, binds to HIF-1α and induces its degradation by the proteosome; in some aggressive tumors, the von Hippel-Lindau protein is mutated, thus becoming ineffective in promoting HIF-1α degradation. This might be the reason why HIF-1α is only detected in malignant tumors, but not in normal, healthy tissues or benign tumors [20,23]. In turn, HIF-1 enhancement promotes the expression of HK, PFK-1, phosphofructokinase type 2 (PFK-2), ALD, GAPDH, phosphoglycerate kinase, enolase, pyruvate kinase and LDH [24,25], which leads to a stimulation of the glycolytic flux. Notwithstanding the O_2 level, metastatic tumor cell lines (breast MDA, U87 glioblastoma, DU145 prostate, renal RCC4 and CaSKi) show high levels of HIF-1α, over-expression of glycolytic enzymes and high glycolysis, whereas non-metastatic tumor cells (breast MCF-7, HT-29 colon, MiaPaCa pancreatic, A549 lung, BX-PC3 prostate) increase HIF-1α, enzyme over-expression and glycolysis only under hypoxia [23].

HIF-1α also favors the glycolytic flux by increasing the expression of pyruvate dehydrogenase complex (PDH) kinase 1, which inhibits, by phosphorylation, the PDH complex activity, thus decreasing Pyr oxidation in the Krebs cycle and increasing the generation of lactate from Pyr [26]. Further association of HIF-1α with the expression of other mitochondrial proteins has yet to be found.

The oncogene, *c-myc*, encodes the transcription factor, c-Myc, which in transformed cells may also activate glycolytic genes, such as those for glucose transporter 1 (GLUT1), hexose-6-phosphate isomerase, PFK-1, GAPDH, phosphoglycerate kinase, enolase and LDH, thus increasing glycolysis under aerobiosis [13,14].

Isoform expression and activity

HK and PFK-1 are among the main controlling steps of the glycolytic flux in erythrocytes, hepatocytes, and cardiac and skeletal muscle cells [27–30]. Changes in the isoform pattern of HK and PFK-1 expression occur in several tumor cells in comparison to normal cells (Fig. 1) [1,2,31–33]. As described below, it seems that such modifications in these and other glycolytic steps are also part of the mechanisms involved in the increased glycolytic flux of tumor cells.

Glucose transporter

It is well documented that GLUT levels of mRNA and protein are higher in tumor cells than in normal, healthy tissues [34–36]. This increase in the protein levels of GLUT might be part of the mechanisms promoting the increased glycolysis in tumor cells as long as the GLUT activity also increases and significant control of the pathway resides in this step (discussed in more detail in the section entitled 'Metabolic control analysis of glycolysis and oxidative phosphorylation in intact tumor cells').

There are several isoforms of GLUT expressed in mammalian cells. The GLUT1 isoform is present in all tissues; GLUT2 is abundant in liver, pancreas, intestine and kidney; GLUT3 prevails in brain; GLUT4 is present in skeletal muscle, heart, brain and adipose tissue; GLUT5 is present in small intestine, testis, skeletal muscle, adipose tissue and kidney; GLUT6 is present in spleen, leukocytes and brain; GLUT7 is the less-well known member of the family and the sites of expression are unknown; GLUT8 is present in testis and brain; GLUT9 is present in liver and kidney; GLUT10 is present in liver and pancreas; GLUT11 is present in heart and skeletal muscle; and GLUT12 is present in heart, small intestine and prostate [34].

In several tumor cells, the predominant over-expressed isoform is GLUT1 (Table 1) [34,35]. However, other isoforms, which are not usually found in the tissue of origin, may also be over-expressed. For instance, in some human leukemias (U937, HL60 y U1), GLUT5, which is an isoform not found in normal leukocytes, is over-expressed [34]. GLUT3 is detected in lung, ovarian and gastric cancers, but not in the corresponding normal tissues [35].

In most studies on GLUT expression in tumor cells, an enhanced mRNA or protein content has certainly been detected, but unfortunately these results have not been accompanied by an effort to determine whether indeed an increased GLUT activity is also achieved, perhaps because it is not an easy assay. Nevertheless, some kinetic parameters of GLUT in tumor cells have been reported [37,38]. However, these last experiments were not carried out with glucose, but with glucose analogues (some of which are indeed nonmetabolizable, although 2-deoxyglucose can be phosphorylated by HK and dehydrogenated by glucose-6-phosphate dehydrogenase) and under noninitial rate conditions (incubation at 37 °C for long time periods). By taking into account the last criticisms, our group has improved the assay of the glucose transport in AS-30D hepatoma and HeLa cells. Our data indicate that the tumor GLUT activity is 10–12-fold higher than that found in nontumorigenic cells [39,40], and that it is also highly sensitive to cytochalasin B and phloretin, two common GLUT inhibitors (S. Rodríguez-Enríquez, F. Flores-Rodríguez, A. Marín-Hernández, L. Ruiz-Azuara & R Moreno-Sánchez, unpublished results).

Hexokinase

In mammalian cells there are four different isoforms of HK (HK-I, -II, -III, and -IV, or glucokinase), which differ in their kinetic properties as well as in their tissue-specific expression and subcellular localization [41,42]. The predominant isoform in brain, mammary gland, kidney and retina is HK-I [42]. HK-II predominates in skeletal muscle and adipose cells, although its activity is relatively low [43]. Because they contain a specific hydrophobic N-terminal segment, HK-I and HK-II may be either bound to the outer mitochondrial membrane or free in the cytosol [43].

In fast-growth tumor cells, HK-II seems to be the predominant isoform, except for brain tumors in which HK-I is the over-expressed isoform [16,42,43]. In hepatomas of Novikoff, H19 and AS-30D, the HK-II activity is 20–306 times higher than the HK activity in liver cells [11,16,31,44]; however, in Hela cells the HK activity was only seven times higher than in hepatocytes [11] (Table 1). There is some discrepancy in the reported HK activity in human brain tumors. Lowry *et al.* [4] described that the HK activity in gliomas, meduloblastomas and schwannomas, obtained from terminal patients, was 78% lower than the HK activity in nontumorigenic brain tissue. In contrast, others have reported that in rat ependymoma, and in human

astrocitoma and gliomas, the HK activity was similar to or higher than that in control tissue [45]. It is recalled that for a rigorous comparison of an enzyme activity from different biological sources, experimental determination should proceed under V_{max} conditions (i.e. with a saturating substrate concentration at least 10 times higher than the K_m value) and in the absence of products.

The apparent specific site of HK-II binding to the outer mitochondrial membrane is the voltage-dependent anion channel or porin [46]; such interaction protects HK-II from proteases and provides direct access to the newly synthesized ATP by the ATP synthase (Figs 1 and 2B). It is hypothesized that the pro-apoptotic protein, Bax, forms (with the voltage-dependent anion channel) a channel for the release of cytochrome *c* under stress conditions [47]. Hence, the enhanced binding of HK-II found in fast-growth tumor cells, and in normal brain cells, may have the additional role of protecting cells from Bax action, thus blocking the initiation of apoptosis [48,49].

The accumulation of products may decrease the forward reaction. In this regard, it is well established that glucose-6-phosphate (G6P) is a potent inhibitor of HK-I, HK-II and HK-III [42]. In consequence, the enhanced HK activity in tumor cells might be counterbalanced by product inhibition. HK binding to mitochondria was proposed as a mechanism to circumvent the G6P blockade [16,31]. However, when assayed under near-physiological conditions of pH (7.0), temperature (37 °C) and concentrations of glucose and G6P (> 1 mM), the mitochondrial HK exhibited a sensitivity to G6P similar to that of the cytosolic HK in AS-30D tumor cells [11]. The presence of this G6P regulatory mechanism of tumor HK supports an essential role for this enzyme in the control of tumor glycolysis, despite its elevated overexpression [11].

PFK-1

There are three types of PFK-1 subunits in mammalian cells. In liver and kidney, the L subunit is the most abundant; in skeletal muscle the M subunit predominates; platelets only have C subunits; whereas in brain, the C, L and M subunits are all present [33,50]. In different malignant human and rat tumor types and established tumor cell lines (Table 1) subunits C, L, or both, prevail over the M subunit [32,33,51]. On the other hand, the expression of both L and M isoforms increases in human gliomas [52], whereas in human T-cell leukemias and cervix carcinomas (HeLa, KB) the C subunit predominates [32].

Each PFK-1 subunit shows different kinetic properties. For example, the C subunit has a lower sensitivity to phospho*enol*pyruvate, one of the physiological allosteric inhibitors of PFK-1, which may contribute to the increased glycolytic flux [53]. Then, the kinetic and regulatory properties of the heterotetrameric PFK-1 depends on the type and proportion of the different subunits [50]. It is known that tumor PFK-1 (rat thyroid cells, rat anaplastic medullary thyroid carcinomas and human gliomas) is less sensitive to inhibition by ATP and citrate than normal PFK-1 [51,52,54]. K_i values of PFK-1 for citrate of 0.1 mM (human normal brain) and 0.75 mM (human glioma) have been determined [52]. The human glioma, PFK-1, is also more sensitive to activation by fructose-2,6-bisphosphate (F2,6BP), with a K_a of 1 μM, with respect to the K_a of 5 μM in the normal brain enzyme [52]. AMP activation of tumor PFK-1 also appears to be enhanced, but this has not been evaluated further, probably because AMP is ineffective at relieving the citrate and ATP inhibition [11,54].

In several malignant rodent and human cells and established human tumor cell lines (Table 1), the PFK-1 activity is one- to 56 times higher than in nontumorigenic cells [11,16,32]. In contrast, in human KB, some gliomas, meningiomas, schwannomas (cranial-nerve VIII), meduloblastoma and rat thyroid tumor cells, the PFK-1 activity is similar to, or even 1.3–2.5 times lower than that in nontumorigenic cells [4,32,52,54]. As the glycolytic flux is also enhanced in all above-mentioned human tumor cells, a null increase in PFK-1 activity (i.e. in content of active enzyme) suggests negligible control exerted by this step (6% in AS-30D glycolysis) [11].

PFK-2

In mammalian cells there are several isoforms of PFK-2, which are encoded by four genes. The expression of these genes is tissue- and development-dependent [55,56]. PFK-2 is a bifunctional enzyme with activities of kinase and phosphatase that modulate the cellular level of F2,6BP, the most potent activator of PFK-1 in normal and tumor cells. The *Pfkfb3* gene encodes both ubiquitous PFK-2 (the isoform with the highest kinase/bisphosphatase ratio) and inducible PFK-2 (which is produced through alternative splicing) [55]. The over-expression of PFK-2 PB3 (HIF-1α inducible) brings about an increase of F2,6BP in several tumor cells (Table 1) [15,25,57]. This mechanism may very probably contribute to the increased glycolytic flux in tumor cells, because F2,6BP activation of PFK-1 may readily overcome the citrate and ATP inhibition [11].

Mitochondrial oxidative metabolism in tumor cells

Warburg [58] originally proposed that the driving force of the enhanced glycolysis in tumor cells was the energy deficiency caused by an irreversible damage of the mitochondrial function. There indeed seems to be a diminished oxidative metabolism in many tumor cell types [1]. Several explanations have been advocated (Fig. 2), although, in some, not-so-solid arguments or plainly flawed assumptions have been considered. These are discussed below.

A lower Pyr oxidation, owing to inhibition of the PDH complex by acetoin [59,60] and a diminished Pyr transporter activity [60,61]

It has been described, for AS-30D and Ehrlich hepatomas, that a significant fraction of mitochondrial Pyr is decarboxylated to an active acetaldehyde through a 'nonoxidative' reaction (assuming that tumor mitochondria operate in a low-oxygen environment) catalyzed by the E1-PDH, via bound β-hydroxyethylthiamine pyrophosphate [59]. The active acetaldehyde formed is condensed with a second acetaldehyde to generate acetoin, which competitively inhibits PDH ($K_i = 41$ μM) [59]. Tumor cells may maintain high levels of acetaldehyde as a result of the presence of an atypical aldehyde dehydrogenase isoform (IV) with low affinity for this substrate [62]. Tumor PDH is activated by 0.5–1 mM AMP, which does not occur in normal PDH [63]. The intracellular AMP concentration is 0.6–3.3 mM in AS-30D cells [11,64], but no data on the intramitochondrial AMP level are available. However, an enhanced Pyr decarboxylation by an AMP-activated PDH may lead to an increased acetoin formation, which would affect the enzyme in a product-inhibition manner and would establish a fine regulatory mechanism of tumor PDH (Fig. 2B).

Indeed, acetoin inhibits the CO_2 generation in Pyr-stimulated mitochondria [59]. However, it remains to be demonstrated whether PDH inhibition, by acetoin, affects oxidative phosphorylation in tumor cells, as glutamine oxidation, which is highly active in tumor cells, may not be sensitive to acetoin inhibition [59].

On the other hand, in mitochondria isolated from Morris 44 and 3924A hepatomas, the Pyr transporter is slightly slower ($V_{max} = 5$–12 nmol·min^{-1}·mg of protein^{-1}) and has a lower Pyr affinity ($K_m = 0.74$–1.1 mM) than that of liver mitochondria ($V_{max} = 20$ nmol·min^{-1}·mg of protein^{-1} and $K_m = 0.64$ mM) [61]. In Ehrlich hepatoma, the Pyr uptake is similar to that found in liver slices and isolated mitochondria [65]. Such a small difference in transporter activity casts doubt on the role of this site in decreasing oxidative phosphorylation in tumor cells (Fig. 2B).

Truncated Krebs cycle and lower reducing equivalents transfer

Parlo & Coleman [66] proposed that the high glycolytic activity in some tumor cells is caused by mitochondrial dysfunction at the level of the Krebs cycle, which leads to a lower availability of reducing equivalents for the respiratory chain and hence a lower oxidative phosphorylation. The same authors detected that in Morris 3924A hepatoma, Pyr-derived citrate was preferentially expelled from tumor mitochondria (four times faster than in liver mitochondria) owing to a defect in the transformation of citrate into 2-oxoglutarate (i.e. failure in both aconitase and isocitrate dehydrogenase activities), which induces citrate accumulation in the mitochondrial matrix and hence citrate efflux (Fig. 2B). In the cytosol, citrate stimulates the synthesis of cholesterol, triacylglycerides and phospholipids (Fig. 2B), but does not inhibit glycolysis because tumor cells over-express the citrate-insensitive PFK-1 isoform (Fig. 1) [6,51,52,54] (see the section entitled 'Glycolytic enzymes and transporters in tumor cells').

However, other authors [67,68] challenged the truncated Krebs cycle hypothesis. These authors determined the rates of Pyr, malate, citrate, acetoacetate and acetate decarboxylation in AS-30D cells and mitochondria, and found that they were similar to those of nontumorigenic cells and mitochondria, thus indicating that the citrate flux through the Krebs cycle is not truncated, at least in AS-30D hepatoma (Fig. 3). In fact, the activities of all Krebs cycle enzymes are 1–30 times higher in AS-30D mitochondria than in normal liver mitochondria [67] (Fig. 3).

Likewise, the activities of the aspartate/malate and α-glycerophosphate shuttles seem to be diminished in some tumor cell types (Table 1), which would impede the efficient transfer of reducing equivalents to mitochondria from the cytosol [1,69]; in consequence, the higher availability of cytosolic NADH may accelerate the LDH activity. However, the rate of reducing equivalent transfer from the cytosol to the mitochondrial matrix in Ehrlich hepatoma was similar to that observed in hepatocytes (rate of transfer = 2.78 and 2.61 μmol·min^{-1}·g of wet weight^{-1}, respectively) [70].

Therefore, the results regarding a deficiency in transfer equivalents from cytosol to mitochondria, as well as those on acetoin inhibition and the truncated Krebs cycle, are not sufficiently strong to establish a lower

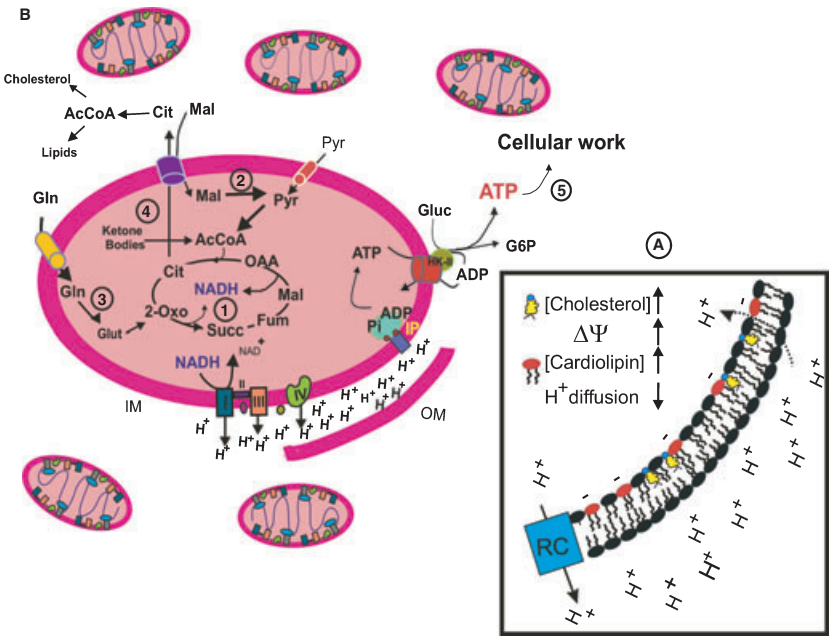

Fig. 3. Tumor mitochondria may have a normal or even an over-expressed enzyme set and have a highly active oxidative phosphorylation. (A) Changes in the lipid composition of the inner mitochondrial membrane brings about a lower passive H^+ permeability and a higher H^+ gradient across the inner mitochondrial membrane; (B) (1) a complete and fully functional Krebs cycle; (2) malate is transformed to pyruvate (Pyr) by an increased $NADP^+$-dependent intramitochondrial malic enzyme; (3) glutamine is actively taken up by a specific and over-expressed glutamine transporter (an enhanced phosphate-dependent glutaminase transforms glutamine into glutamate, which enters the Krebs cycle as 2-oxoglutarate); (4) acetoacetate and β-hydroxibutyrate are actively oxidized to acetyl CoA by means of an increased succinyl-CoA acetoacetyl transferase; and (5) a fraction of mitochondrial ATP is exported to the cytosol to be used for cellular work. Tumor cells may have a normal number of mitochondria which, in turn, may have a normal number of respiratory chain copies.

mitochondrial function in tumor cells. Furthermore, significant and rather high differences should be found in the mechanism proposed for decreasing oxidative phosphorylation in several tumor cell lines, not only in a selected one or two tumor cell lines. Again, owing to the genetic heterogeneity among the different tumor cell types, it should not be expected to find a similar degree of modification in the mechanism proposed, but at least it should be observed to be occurring in several tumor cell lines.

Lower content of mitochondria per cell and defective respiratory chain

In 1978, Pedersen [1] proposed that the respiratory activity of isolated tumor mitochondria was as efficient as that of normal mitochondria, but that the diminished oxidative phosphorylation observed in tumor cells was the result of a lower content of mitochondria (20–50% lower mitochondrial content) (Fig. 2; Table 1). This conclusion extended the original 1956 argument by Warburg [58], that the high glycolytic rate in tumor cells was the result of a damaged respiratory chain (Fig. 2B). A lower number of mitochondria per cell implies that in tumor cells there are more active degradation mechanisms of mitochondria (i.e. mitophagy [71]) and/or a diminished rate of organelle proliferation, which has yet to be explored. However, the mitochondrial content of Morris 16 and 7800 hepatomas was similar to that of liver cells (reviewed in [1]).

Marked deficiencies have been identified in some respiratory chain components (iron sulfur centers, NADH cytochrome *c* reductase, succinate dehydrogenase and cytochrome *c* oxidase) of mitochondria from several tumors (Table 1) [72–74]. However, an increase (two- to five-fold) in the activity of NADH cytochrome *c* reductase has also been determined in the same brain tumors [74]. In mitochondria isolated from some hepatomas (Table 1), the adenine nucleotide translocase (ANT) activity was lower (5.4-fold) than in normal liver mitochondria [75]. In contrast, an increment in the ANT1 and ANT2 mRNA levels (eight-fold) in SV40-transformed cells has been detected [76]; however, the ANT kinetic parameters in the transformed fibroblasts were not elucidated. In mitochondria (synthetic activity) and submitochondrial particles (hydrolytic activity) from human hepatocellular carcinoma

(a fast-growing tumor), the synthetic and hydrolytic activity (V_{max}) and affinity ($K_{max\ ATP}$) of the ATP synthase is reduced by 50–70% by comparison with human liver [77]. In contrast, no differences in oxidative enzyme activities with normal cells have been detected for Morris hepatomas 3924A, 9618A and 7800, and Novikoff hepatomas [78,79] (Fig. 3).

It is pertinent to emphasize that diminution of one enzyme or transporter does not automatically lead to a diminution in the pathway flux or metabolite concentration; the altered steps have to exert significant metabolic control, otherwise the alteration would be of no relevance. Unfortunately, determination of the enzyme activities in tumor cells has not always been accompanied by measurements of flux rate and steady-state metabolite concentrations. Likewise, detection of protein levels by western blot, or of gene transcription by northern blot, provides information with little functional meaning unless these measurements are accompanied by determination of activity and pathway flux.

Crabtree effect (inhibition of oxidative phosphorylation by glycolysis)

Partial inhibition of oxidative phosphorylation by the addition of glucose and other hexoses in fast-growth tumor cells (Table 1) [64,80–85] and normal, proliferative cells (hamster and neural rat embryos and rat thymocyte proliferating cells) is well documented [86,87]. After glucose addition in AS-30D cells the glycolytic flux elevates, but the ATP and P_i contents decrease and the cytosolic pH lowers from 7.2 to 6.8; in addition, the concentrations of phosphorylated hexoses (G6P, fructose-6-phosphate, fructose-1,6-bisphosphate) show a substantial increase [64]. The variation in ATP, P_i and hexose phosphate indicates a glycolysis activation that surpasses the mitochondrial capacity to regenerate ATP; P_i might have become limiting for mitochondria. Also, the acidic pH induced by lactate generation may affect highly pH-sensitive oxidative enzymes, such as the 2-oxoglutarate dehydrogenase complex [88] and the cytochrome bc_1 complex [89].

Increased content of the inhibitory peptide of ATP synthase

An increased content of the ATP synthase inhibitory subunit has been described for some tumor cells (Table 1) [90,91]. This has been interpreted as causing the diminution of the ATP-generating capability of tumor cells (Fig. 2B). However, there seems to be a misconception on the role of the inhibitory subunit, because it inhibits the hydrolytic (reverse) reaction under conditions of low inner membrane electrical potential, but it does not affect the synthetic reaction (which occurs at high electrical membrane potential) [92]. Therefore, this alteration in tumor cells should not affect the mitochondrial capacity for supplying ATP (Fig. 3), thus discarding the involvement of the ATP synthase inhibitor protein in decreasing oxidative phosphorylation in tumor cells.

Increased sensitivity of mtDNA to oxidative stress

mtDNA lacks histones, which makes it more susceptible to interaction with free radicals [93]. As several subunits of respiratory chain site I (seven subunits: ND1–ND6 and ND4L), site II (apocytochrome *b* subunit) and site III [three subunits: (COX)I, COXII and COXIII], and ATP synthase (two subunits: ATPase6 and ATPase8), are encoded by mtDNA, it is thought that the enhanced oxidative stress in tumor cells induces a decrease in the transcription and translation of mitochondrial genes [93]. Likewise, the higher frequency of mtDNA mutations found in breast and other human cancers might also presumably contribute to mitochondrial dysfunction, as only a few are known to have pathological significance [94,95]. In addition, there seems to be an attenuated capacity for DNA repair in normal mitochondria in comparison with the nuclear DNA [93]. The mtDNA repair capacity has not been examined in tumor cells.

Mutations in the mtDNA (ND1 and a nonconservative substitution in cytochrome *b*) of oncocytic thyroid carcinomas correlate with low viability, low respiratory rate, decreased complex I and III activities, reduced ATP content and a high reactive oxygen species production [96]. On the other hand, in human renal carcinomas, a low level of mtDNA mutations is observed, indicating that the decreased aerobic energy capacity in this tumor is rather mediated by a nuclear regulated mechanism [95].

In conclusion, there are examples of tumor cell lines which certainly exhibit a decreased mitochondrial function, mediated by any of the above-described mechanisms, but that observation does not seem to apply to all tumor cell types. Therefore, owing to the genetic heterogeneity of tumor cells, the oxidative phosphorylation capacity should be experimentally evaluated for each particular tumor cell line to assess whether the enhanced glycolysis is indeed accompanied by a significantly depressed mitochondrial function. This last statement, widely spread in the field [1,2,6,9,14,15,17,23,57,58,93,97–100], has been taken as an established fact for tumor cell metabolism for many

years, but because of the absence of hard experimental data, it has rather become the metabolic central dogma of tumor cells.

Re-evaluation of oxidative phosphorylation in tumor cells

Oxygen concentration

The increased glycolysis and the diminished mitochondrial activity found in the pioneering studies with solid and ascites tumor cells led Warburg in 1956 [58], and other authors subsequently [1,2,6,9,14,15,17,23,57,60,97–100], to propose, as a universal mechanism, that all tumor cell types were energetically dependent mainly or only on glycolysis. In particular, glycolysis seems to be the main energy pathway in slow-growing solid tumors (human melanomas, mammary adenocarcinoma [101], rat rhabdomyosarcomas [102]) as oxidative phosphorylation is apparently limited by the low O_2 availability inside the tumor [103]. In many human solid, hypoxic, tumors, the concentration of O_2 is lower than 20 μM [104].

It is worth noting that the glycolytic rate is usually determined by measuring the lactate production in cells incubated with added glucose, but other nonglycolytic reactions, catalyzed by alanine transaminase and malic enzyme, may also contribute to the formation of L-lactate. To correct for any overestimation of the glycolytic rate, lactate formation should also be determined in the absence of added glucose and in the presence of a glycolytic (GAPDH) inhibitor (i.e. arsenite, iodoacetate).

It should also be considered that, in addition to lower O_2 availability in solid tumors, especially in the initial and avascular developmental stages under which a poor vascularization occurs, glucose supply can be similarly affected [105], thus inducing a severe decrease in the generation of glycolytic ATP. Moreover, in the center of glioma and carcinoma multicellular spheroids (a model that simulates the avascular stages of solid tumors) and in the hypoxic regions of human tumors, the O_2 concentration was determined to be 8–57 μM [103,106–108], which resembles the range of values usually found in several normal tissues with normal blood irrigation (femoral muscle, mammary gland tissue) [109]. The ascites fluid may have a high (50 μM) [7], or a low (< 7 μM) [110], O_2 concentration.

Oxidative phosphorylation may be compromised at O_2 concentration values lower than 1 μM because the $K_{m\ O2}$ of cytochrome *c* oxidase is 0.1–0.15 μM in submitochondrial particles and pure enzyme [111], 0.4–0.8 μM in human umbilical vein endothelial cells [112] and 0.39 μM in intact skin fibroblasts [113]. In turn, a saturating O_2 concentration for cytochrome *c* oxidase and for oxidative phosphorylation would be > 4–8 μM (i.e. a substrate concentration of 10 times its K_m value). Therefore, tumor mitochondrial metabolism would not be affected by the hypoxia level found in tumors, unless prolonged exposure (weeks or months) to the hypoxic microenvironment somehow alters the expression of mitochondrial enzymes, perhaps through a p53-mediated mechanism [98]. Furthermore, the O_2 concentration in the tumor microenvironment could not always reach such low values, unless an O_2 gradient develops so that the O_2 concentration surrounding mitochondria falls below the critical level of 1 μM.

By assuming, but not experimentally determined, that oxidative phosphorylation is negligible under hypoxic conditions, the enhanced glycolysis of tumor cells is usually considered as a sufficiently good reason for proposing that the ATP supply only or mainly depends on glycolysis [1,2,6,9,14,15,17,23,57,93,98,99]; in turn, tumor glycolysis may be either marginally affected (0–5%) or be further increased by 50–60% under hypoxia [101,114]. However, the quantitive contribution of each energy pathway to ATP supply has rarely been determined.

It also remains to be analyzed whether the accelerated glycolysis under hypoxia indeed serves only for ATP supply or, alternatively, whether its role is the supply of intermediates for biosynthesis of polysaccharides, and precursors for nucleic acids, lipids and amino acids. Moreover, the active angiogenesis in solid tumors suggests a dependence on oxidative metabolism, at least in the regions close to the blood vessels [103].

Substrate utilization

It is postulated that both glucose and glutamine (an exclusive mitochondrial-oxidizable substrate) are the substrates preferentially consumed by fast-growth tumor cells [1,7,115,116]. However, it is not clearly established which of these two (or other) oxidizable substrates supports the accelerated cell proliferation; in glycolytic tumors an increased oxidation of glutamine is also observed [6]. Some tumors, such as HeLa cells, may adapt their metabolism towards the available external carbon source: in the absence of external glucose, HeLa cells activate the *de novo* synthesis of mtDNA, which prompts the synthesis of the respiratory complexes and citrate synthase [99]. For HeLa cells, the ATP demand is supported by the aerobic oxidation of both glucose and glutamine [116,117], which indicates that glycolysis and oxidative phosphorylation

Table 2. Predominant energy metabolism (glycolysis or oxidative phosphorylation) in different types of tumor cells. Gly, glycolysis; OxPhos, oxidative phosphorylation.

Tissue of origin	Tumor cell type	Predominant energy metabolism	References
Brain	Glioma C6, oligodendroglioma, meningioma, medulloblastoma	Gly	[4,5,190]
	Glioblastoma multiforme, astrocytoma C6	Gly and OxPhos	[4]
	Transformed hamster brain	OxPhos	[191]
Bone	Sarcoma	OxPhos	[3]
Colon	CT-26, LoVo colon adenocarcinoma	Gly	[192]
	Novikoff	Gly	[3,193]
Liver	Ehrlich Lettré, Ehrlich, Walker-256, Morris 3683 and Dunings LC18 hepatomas; ascites mouse cancer; sarcoma 27; MCF-7 carcinoma	Gly and OxPhos	[3,193]
	Reuber H-35, Morris (7793, 7795, 7800, 5123) and AS-30D hepatomas	OxPhos	[3,7,193]
Lung	Lung carcinoma	OxPhos	[123,191]
Mammary gland	Breast cancer	OxPhos	[123]
	MCF7	Gly and OxPhos	[141]
Skin	Melanoma	OxPhos	[3]
Uterine cervix	HeLa, ovarian and uterus carcinomas	OxPhos	[3,117,123]

are both essential for ATP supply in this human tumor cell line.

The ascitic medium developed in rodents during AS-30D hepatoma growth contains high glutamine (> 4 mM), whereas glucose is scarce (0.026 mM) [7]; a low glucose concentration (< 0.11 mM) was also described for the ascites fluid produced by MM1 hepatoma cells in rats [110]. Other oxidizable substrates, also present in the ascites fluid, are ketone bodies (0.9 mM acetoacetate, 0.04 mM β-hydroxybutyrate), glutamate (0.15 mM), Pyr (0.16 mM) and lactate (3.3 mM) [7]. In contrast, the blood plasma, and most of the commercial culture media for mammalian tumor and normal cells, contain high glucose (5–25 mM) and glutamine (4–5 mM) [118]; thus, culture media are used without having determined the prevalent type of energy metabolism (glycolytic versus oxidative) in each tumor cell type (Table 2).

Contribution of glycolysis and oxidative phosphorylation to ATP supply

It is intriguing that despite the accelerated glycolysis in many fast-growth tumor cells (Table 2), its total contribution to the cellular ATP supply only reaches 10% (reviewed in ref. 3). In marked contrast, in other tumor cell lines also considered of fast growth (Table 2), glycolysis indeed supports 50–70% of the ATP demand, a contribution value also estimated by Warburg [58]. Moreover, the contribution to the ATP demand from glycolysis and oxidative phosphorylation for protein and nucleic acids synthesis, and for ion transport (Na^+/K^+, Ca^{2+}), during the proliferative phase was similar in tumor cells with a deficient oxidative system (Table 2) [119]. The ATP content in Ehrlich hepatoma cells during the cell cycle transition from G_0 to S phase diminishes by 50%, which correlates with a similar diminution in oxidative phosphorylation, whereas glycolysis remains constant [120]. This suggests that when Ehrlich tumor cells undergo a transition from resting to an active, highly proliferative state, only mitochondrial ATP supports the enhanced energy demand.

Some human and rodent gliomas exhibit high or moderate susceptibility to respiratory inhibitors, indicating the presence of fully functional mitochondria and dependency on oxidative phosphorylation; furthermore, gliomas with a glycolytic phenotype actively oxidize Pyr and glutamine under conditions of low glucose [9].

An active Pyr oxidation, and a complete and functional Krebs cycle, able to supply NADH for oxidative phosphorylation, operate in AS-30D hepatoma [67] (Fig. 3). Moreover, other oxidative pathways, such as those for glutamate and ketone bodies, are also highly active in these fast-growth tumor cells [7,68]; the succinyl-CoA acetoacetyl transferase enzyme, which initiates oxidation of ketone bodies, is 40-fold more active in AS-30D cells than in hepatocytes [68] (Fig. 3). On the contrary, many brain tumors have lower succinyl-CoA acetoacetyl transferase activity than normal neurons and glia and are unable to metabolize ketone

bodies. Then, as fatty acids do not pass the blood–brain barrier, brain tumors seem to depend only on glucose and glycolysis for ATP supply [100].

As originally claimed by Weinhouse [121], a significant number of tumor cell lines exhibit elevated rates of respiration (recently reviewed in ref. 3); whether this activity is fully associated with oxidative phosphorylation remains to be determined. For instance, in AS-30D and HeLa cells, the rate of respiration was 85–90% sensitive to oligomycin, a specific ATP synthase inhibitor, indicating that the remaining 10–15% of the cellular O_2 consumption was not associated with oxidative phosphorylation. Tumor cell lines with high rates of respiration are Ehrlich Letreé hyperdipliod chain [80], human colon cancer HT29 [85], Lewis lung carcinoma [122], human breast MCF-7 carcinoma, HeLa cells [117], *in vivo* human tumor xenographs [123], mouse fibrosarcoma 1929 [124] and *Neu* mammary epithelial mice tumor and LDH-A-deficient clones [125], although unfortunately, in these studies, oligomycin sensitivity was not evaluated.

Other observations supporting the existence of highly active mitochondria in some tumors are the presence of mitochondrial proteins ($NADP^+$-malic enzyme, glutaminase and glutamine transporter) with high activities and affinities toward their substrates. Mitochondrial tumor malic enzyme is 10–20 times more active in tumor cells than in its original tissue counterpart [126] (Fig. 3). The role of malic enzyme in tumor cells has not yet been defined, although the enzyme might remove excess malate to generate Pyr for oxidative phosphorylation [127]. Glutamine oxidation is another pathway that functions at high rates during the logarithmic and stationary growth phases of AS-30D hepatoma and HeLa cells [117]. Cytosolic glutamine is transported faster (4–10 times) into tumor mitochondria and further oxidized to glutamate by a P_i-dependent glutaminase with also higher activity (10–20 times versus liver mitochondria) [128,129] (Fig. 3).

The glycolytic pathway has other functions, in addition to providing cytosolic ATP and NADH. In human and other mammalian normal cells, glycolysis also contributes to the generation of metabolites for anabolic pathways (G6P for glycogen and ribose-5-phosphate synthesis; dihydroxyacetone phosphate (DHAP) for triacylglyceride and phospholipid synthesis; 3 phosphoglycerate for serine, cysteine, and glycine synthesis; and Pyr for oxidative phosphorylation, and for alanine and malate synthesis) and the maintenance of the pyridine nucleotide redox state in the cytosol (Fig. 1). These other functions in normal cells may change in tumor cells, but unfortunately they have not been studied in detail.

The contribution of oxidative phosphorylation has been mostly determined in the presence of glucose, which favors the Crabtree effect. High glucose may or may not be present in the tumor microenvironment. The availability of glucose versus glutamine (and other mitochondrial substrates such as ketone bodies, glutamate and proline, and the Krebs cycle intermediaries 2-oxoglutarate and malate) for different tumor cells has not been examined, and neither has the magnitude of the Crabtree effect.

Therefore, the generalized statement that glycolysis predominates over oxidative phosphorylation for ATP supply in tumor cells should be re-evaluated and experimentally determined for each particular type of tumor cells. An energy deficiency caused by a deteriorated oxidative phosphorylation might indeed be the driving force behind the enhanced glycolysis in hypoxic tumors, in a process mediated by HIF-1α. However, in nonhypoxic oxidative tumors, oxidative phosphorylation-independent mechanisms do clearly operate to enhance glycolysis, under which HIF-1α may also be involved. Thus, the main thermodynamic reason for increasing glycolysis in tumor cells (associated with either a damaged or an unaltered oxidative phosphorylation) might rather be an energy deficiency induced by highly ATP-demanding processes, such as an accelerated cellular proliferation and/or a stimulated nucleic acid, protein and cholesterol synthesis.

Metabolic control analysis of glycolysis and oxidative phosphorylation in intact tumor cells

The main controlling steps of the glycolytic flux in mammalian, normal cells (erythrocytes, skeletal and cardiac muscle, hepatocytes) are GLUT, HK and PFK-1, with the other steps having a minor contribution [27–30]. As previously discussed, in fast-growth tumor cells these three proteins are several-fold over-expressed and therefore their activities are enhanced (see the section entitled 'Glycolytic enzymes and transporters in tumor cells'); the rest of the glycolytic enzymes are also over-expressed in tumor cells, although to a lesser extent (Fig. 1). Thus, it seems difficult to extrapolate the elucidated control mechanisms of glycolysis in normal cells towards tumor cells. To solve this problem, the flux control distribution, and the regulatory mechanisms involved, should be explicitly determined in tumor cells.

Metabolic control analysis establishes how to determine, quantitatively, the degree of control that each pathway enzyme (E_i) exerts on flux (J) and metabolite (M) concentration (termed flux control coefficient C^J_{Ei} and metabolite concentration control coefficient C^M_{Ei})

[130]. For oxidative phosphorylation, C^J_{Ei} values can be determined by titrating flux with specific, classical inhibitors (rotenone, antimycin, cyanide, carboxyatractyloside, oligomycin) [131,132]. However, there are no specific, permeable inhibitors for each glycolytic step. An alternative approach, called elasticity analysis [130], consists of the experimental determination of the sensitivity [or elasticity coefficient (ε^{Ei}_M)] of enzyme blocks towards a common intermediate, M. Variations in the steady-state activity of enzyme blocks can be attained by adding different concentrations of the initial substrate or inhibitors of either block, which do not have to be specific for one step but they do have to inhibit only one block. The block of enzymes that generates the common intermediate M is named the producer block (E1), whereas the block consuming that intermediate is named the consumer block (E2). By applying the summation ($\Sigma C_{Ei} = 1$) and connectivity ($C^J_{E1}/C^J_{E2} = -\varepsilon^{E2}_M/\varepsilon^{E1}_M$) theorems of metabolic control analysis, the C^J_{Ei} values can then be calculated [130].

There are few reports where metabolic control analysis of the tumor energy metabolism has been carried out. The elasticity analysis of glycolysis in AS-30D tumor cells revealed that the main flux control (71%) resided in the upstream part of the pathway (i.e. GLUT and HK) [11]. The rest of the control (29%) was localized in the ALD-LDH segment, with a negligible contribution by PFK-1 (< 6%). It was shown that, despite the extensive over-expression, tumor HK was strongly inhibited by its product G6P. On the other hand, PFK-1 was moderately over-expressed, but the tumor isoform was highly activated by F2,6BP, which surpassed the citrate and ATP inhibition. These findings provided a mechanistic explanation for the respective high and low flux control exerted by HK and PFK-1 (see Fig. 1). This study also showed that a massive over-expression of glycolytic enzymes does not lead to uncontrolled flux, but rather strict regulatory mechanisms are preserved by tumor cells.

By applying elasticity analysis to oxidative phosphorylation, it was found that the respiratory chain site 1 and the ATP-consuming enzyme block (protein and nucleic acid synthesis; ion ATPases) were the main controlling sites in AS-30D tumor cells [7]. For that experiment (Fig. 4), oxidative phosphorylation flux was measured as the rate of cellular respiration that was sensitive to an excess of oligomycin. Flux was titrated with low concentrations of oligomycin (to calculate the elasticity of the consumer block) and streptomycin (for the producer elasticity); the steady-state concentration of ATP was measured under each condition. In a normalized plot, the slopes of each

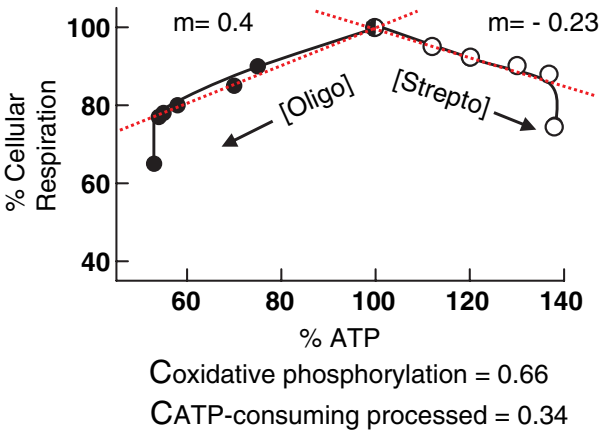

Fig. 4. Determination of flux control coefficients (C_E) in AS-30D hepatoma cells. Cellular respiration and ATP concentration were titrated with oligomycin (100–700 pmol per 10^7 cells) and streptomycin (0.1–0.7 mg per 10^7 cells). In the absence of inhibitors, the rate of cellular respiration, sensitive to an excess of oligomycin, was 60 ng atoms of oxygen·min^{-1} per 10^7 cells, and the ATP concentration was 1.88 nmol per 10^7 cells, or 0.75 mM.

curve at the noninhibited point (Fig. 4) yield the elasticity coefficients. The flux control coefficient, derived from the elasticity coefficient, was 0.66 for the ATP-producing branch (i.e. oxidative phosphorylation) and 0.34 for the ATP-consuming processes [7].

Control analysis of the tumor energy metabolism, either by establishing the main sites of control in glycolysis and oxidative phosphorylation or by assessing the predominant energy pathway, may provide a more rational and quantitive approach for the identification and design of more specific therapeutic strategies. Therefore, it would be highly recommended for this type of analysis to be carried out in many other different types of cancer.

Energy metabolism in tumor cells as therapeutic target

A proportional relationship between the rate of cellular proliferation and the rate of ATP supply has been established for fast-growth tumor cells [120]. However, there is some discrepancy regarding the correlation between the degree of malignancy and the rate of ATP synthesis from glycolysis or oxidative phosphorylation. Some authors have proposed that the glycolytic activity correlates with the degree of tumor malignancy, so that glycolysis is faster and oxidative phosphorylation is slower in highly de-differentiated and fast-growing tumors than in slow-growing tumors or normal cells [1,133]. In fact, a high level of lactate (and choline phospholipid metabolites) has been proposed as a

predictor of malignancy [134]. There is a direct correlation between tumor progression and the HK [11,43] and PFK-1 [11,53,54] activities, which are increased several-fold in fast-growth tumor cells (see above in the section entitled 'Glycolytic enzymes and transporters in tumor cells'). Accordingly, it has been postulated that tumor cells which exhibit deficiencies in their oxidative capacity are more malignant than those that have an active oxidative phosphorylation [135].

Muller *et al.* [119] originally proposed that a biochemical strategy to suppress the accelerated tumor proliferation efficiently was the simultaneous blockade of both ATP-generating pathways. It appears difficult to target the energy metabolism of tumors as the host cells also depend on the same essential pathways for ATP supply. However, by identifying the most significant differences in the energy metabolism between tumor cells and healthy host cells, it might be possible to achieve some suitable potential antineoplastic targets.

Such an unorthodox approach has already been applied to some tumor cells (Table 3). For example, in

Table 3. Compounds assayed as antineoplastic drugs targeting energy metabolism in fast-growing tumor cells. 2-DOG, 2-deoxyglucose; 3-MPA, 3-mercaptopicolinic acid; ANT, adenine nucleotide translocase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HK, hexokinase; HPI, hexose-6-phosphate isomerase; LDH, lactate dehydrogenase; OxPhos, oxidative phosphorylation; PEPCK, phosphoenolpyruvate carboxykinase; 2-OGDH, 2-oxoglutrate dehydrogenase.

			Effects	
Metabolic drug	Tumor cell type	Drug concentration	Growth (% of growth diminution or size reduction	Energy pathway
Casiopeina II-gly	AS-30D hepatoma	10 μM	> 95%	2-OGDH inhibitor Succinate
	HeLa cells [117]	1 μM		DH inhibitor
3-bromopyruvate	Rabbit VX2 tumor [147,148]	500 μM	80%	HK-II inhibitor Krebs cycle enzymes inhibitor
3-bromopyruvate	Human HL-60 leukemia; human lymphoma Raji, C6F leukemia; Raji/C8, HL-60/AR [97]	50–100 μM	90%	HK-II inhibitor Krebs cycle enzymes inhibitor
2-DOG	Breast MCF-7 [140]	3.5 mM[a]	50%	HK-II inhibitor HPI inhibitor
Clofazimine	Chemoresistant bronchial carcinoma WIL [144]	10 μM	40–50%	Mitochondrial uncoupler
F16	Mammary tumor and human breast cancer [145]	3 μM	> 90%	H^+-ATPase inhibitor ANT inhibitor[b]
Lonidamide	Glioblastoma multiforme [152]	200 μM	50%	OxPhos uncoupler Mitochondria-bound HK
MKT-077	Breast MCF-7 carcinoma CRL1420 Carcinoma CX-1 Melanoma LOX [146]	0.5–2.3 μM	> 90%	Mitochondrial uncoupler Damage to mitochondrial DNA
Rhodamine 6G +	Rat implanted Walker-256 carcinosarcoma [137]	0.8–4 mg kg^{-1}	50%	OxPhos uncoupler and ATP/ADP translocase inhibitor
3-MPA		40 mg kg^{-1}		Host hypoglycemia PEPCK inhibitor
Rhodamine 123 +	Human MCF-7 breast, human cervical carcinoma KB-3–1 [140]	3.4–3.8 μM	50–60%	OxPhos uncoupler
Gossypol		0.8–4.3 μM		GAPDH and LDH inhibitor
Rhodamine 123 +	Human MCF-7 breast [142]	1.3 μM	100%	OxPhos uncoupler
2-DOG		300 μM		HK-II inhibitor HPI inhibitor
Rhodamine 123 +	Human osteosarcoma [141]	5 μM	65–80%	
2-DOG		500 μM		
Rhodamine 123 +	Mice-implanted Ehrlich hepatoma MB49 carcinoma [143]	15 mg kg^{-1}	80% (mice survival)	
2-DOG		0.5 g kg^{-1}		

[a] In the presence of 4 mM glucose. [b] Assayed only in liver mitochondria.

Walker-256 tumor-bearing rats, 3-mercaptopicolinate (a phospho*enol*pyruvate carboxykinase inhibitor) was added to block gluconeogenesis in the host by inducing severe hypoglycemia and hence a diminution in tumor glycolysis, together with the hydrophobic cation, rhodamine 6G (Table 3), which acts as an uncoupler (H^+ ionophore) and an ANT inhibitor [136,137], to block oxidative phosphorylation. When added simultaneously, tumor growth decreased by 50%, whereas separately, the drugs did not affect growth [137].

Gossypol is another drug used to block glycolysis in diverse fast-growth tumor cells (Table 3). This drug inhibits NAD^+-dependent enzymes (GAPDH, LDH) [138,139]. The simultaneous inhibition of glycolysis with gossypol and oxidative phosphorylation with rhodamine 123 (Table 3) decreased tumor cell proliferation by 60% [140].

Similarly, treatment of several human and rodent tumors with 2-deoxyglucose and rhodamine 123 induced almost full blockade of growth (> 90%) [141–143]. Clofazimine (Table 3), an antileprotic agent, induced a 40% size reduction in WIL, a human bronchial carcinosarcoma that is resistant to regular chemotherapy, by acting as a mitochondrial uncoupler [144]; clofazimine, in combination with a glycolytic drug, was, however, not assayed. Other lipophilic cationic drugs, such as MKT-077 and F16 [145,146] (Table 3), have proved to be effective against several human and mouse tumors (> 90% growth inhibition by 0.5 μM MKT-077 after 24 h or 3 μM F16 after 7 days). F16 was ineffective against mouse breast *c-myc*-initiated and fibrosarcoma *ras*-initiated tumor cells [145]. Also, F16 inhibited the respiratory rate ($IC_{50} = 25$ μM) and H^+-ATPase activity ($IC_{50} = 6$ μM) of rat liver mitochondria [145].

Geschwind *et al.* [147,148] carried out an exhaustive screening of a multitude of drugs, searching for more specific and potent inhibitors of both glycolysis and oxidative phosphorylation. It was found that 3-bromopyruvate (Table 3), an alkylating agent, was a potent inhibitor of both energy pathways and able to eliminate, almost completely, tumors implanted in rabbits. The specific sites of action were not elucidated, although the authors have claimed that 3-bromopyruvate inhibits bound HK-II and the Krebs cycle [148]. These authors histologically analyzed the host tissues, finding no apparent damage; however, the occurrence of subcellular morphological damage cannot be discarded. Tumor cell lines with high respiratory activity (human HL-60 leukemia; human lymphoma Raji), that are mitochondria-deficient (ρ^-) (C6F leukemia; Raji/C8) or that express a multidrug-resistant phenotype (HL-60/AR), were killed effectively with 3-bromopyruvate under normoxia or anoxia, although at somewhat disappointingly high doses (50–100 μM for 24 h) [97] (Table 3).

In the search for drugs that are more specific for tumor cells than for normal cells, some authors have used the typical mitochondrial inhibitors, such as rotenone and oligomycin, for blocking tumor cell proliferation. For instance, oligomycin at low doses (0.06–0.7 μM), which do not affect normal cells, stopped the cell cycle progression from G_1 to S phase in human promyelocytic leukemia cells (HL-60) and in Jurkat T cells owing to a severe diminution of mitochondrial ATP production [149]. At 3–6 μM oligomycin, over 50% of HL-60 cells arrested in the G2-M phase; however, this drug concentration may also affect normal cells. The respiratory chain site 1 inhibitor, rotenone (0.1–1 μM), arrests the cell cycle at G2/M, promoting a strong inhibition (50–90%) of cell proliferation in human lymphoma WP and 134 B osteosarcoma [150]. Such an effect is related to a severe diminution of the H^+ electrochemical gradient across the inner mitochondrial membrane and hence to inhibition of oxidative phosphorylation, but also to an increase in the membrane fluidity and to the activation of apoptosis [151]. Certainly, rotenone does inhibit the respiratory chain site 1 in normal cells, but this drug might still be advantageously used whether site 1 exerts a significantly higher flux control of oxidative phosphorylation in tumor cells (see the section entitled 'Metabolic control analysis of glycolysis and oxidative phosphorylation in intact tumor cells').

Other glycolytic drugs, such as lonidamide, also diminish the growth of human breast cancer cells [152], but severe side-effects are observed in the host [153]. Tyrosine kinase inhibitors, such as imatinib and genistein, are used in the therapy against hematological malignancies as a result of their effect on tumor energy metabolism [154,155]. In BCR leukemia cells, imatinib decreases glucose uptake and glycolysis by 65–77% and increases the activity of several mitochondrial Krebs cycle enzymes by 40–70% (i.e. imatinib induces a switch in the energy metabolism of leukemia cells). Thus, despite a drastic inhibition of glycolysis by imatinib, the cellular ATP content increases because of oxidative phosphorylation activation [154]; therefore, imatinib seems not to be adequate for targeting tumor energy metabolism.

It is known that several human tumors (colon and esophagus squamous cell carcinomas; skin cancer) over-express cyclooxygenase (COX-2), which seems essential for inhibiting apoptosis and stimulating angiogenesis and invasiveness [156]. COX-2 synthesizes (a) prostaglandin E2, which stimulates bcl-2 and inhibits apoptosis, and (b) interleukin-6, which enhances

haptoglobin synthesis. Prostaglandin E2 is associated with tumor metastases, interleukin-6 is associated with cancer cell invasion, and haptoglobin is associated with implantation and angiogenesis [156].

Aspirin, a nonsteroidal anti-inflammatory drug (NSAID) that inhibits COX activity, may either reduce or delay the appearance of colorectal adenoma, or decrease the progression of colorectal cancer, because a lower incidence of colorectal cancer among regular aspirin users was originally reported by Kune *et al.* [157]. Other NSAIDs, such as indomethacin, may also reduce cancer progression and prolong the survival of patients with established metastatic solid tumors [158]. However, the ability of NSAIDs to attenuate tumor growth does not correlate with the tumor content of COX-2, the NSAIDs target protein, or even of COX-1 [159]. Interestingly, several NSAIDs, such as nimesulide, meloxicam, nabumetone, diclofenac and naproxen, potently inhibit oxidative phosphorylation in intact cells and isolated mitochondria from AS-30D hepatoma [160]. Therefore, tumor mitochondrial function might be directly targeted by some NSAIDs.

Once established that the energy metabolism in rodent AS-30D hepatoma and human HeLa cells was mainly of the oxidative type, drugs that presumably are specific for mitochondria, such as rhodamines and casiopeinas, were utilized to test the hypothesis that oxidative phosphorylation is the principal ATP supplier in these tumor cells [117]. Interestingly, the lipophilic cationic drugs, rhodamines 6G and 123, and casiopeina-IIgly, at low micromolar concentrations drastically abolished oxidative phosphorylation and cell proliferation, whereas glycolytic drugs, such as gossypol, arsenite and iodoacetate, were ineffective in these tumor cells [117].

We have recently determined that the growth of HeLa cells in multicellular spheroids involves changes in their energy metabolism in comparison to their counterpart in monolayer (bidimensional) culture. In the initial formation of spheroid, mitochondria support over 80% of the cellular ATP supply. However, at later spheroid stages, mitochondrial metabolism fails and glycolysis becomes predominant (S. Rodríguez-Enríquez, J.C. Gallardo-Pérez, A. Aviles-Salas, V. Maldonado-Lagunas & R. Moreno-Sánchez, unpublished results). Casiopeina II-gly blocks the initial cell cluster formation and growth of Hela multicellular spheroids by directly inhibiting oxidative phosphorylation (S. Rodríguez-Enríquez, J.C. Gallardo-Pérez, A. Aviles-Salas, V. Maldonado-Lagunas & R. Moreno-Sánchez, unpublished results).

Certainly, drug efficacy, delivery and side-effects are problems that need to be solved in developing new chemotherapies. In solid tumors, delivery to a hypoxic region may be difficult whether the drug does not easily permeate through the different cellular layers. To eliminate these uncertainties, it seems relevant to continue searching and designing new specific drugs (i.e. molecules with inhibition constants in, at least, the submicromolar range and with superior membrane permeability). However, the 'error and trial' strategy, followed to date, by assuming that the application of a given drug for a 'key' or 'rate-limiting' step may yield full inhibition, is a misleading concept. It has now been widely shown that control of glycolysis and oxidative phosphorylation is shared by several steps (see the section entitled 'Metabolic control analysis of glycolysis and oxidative phosphorylation in intact tumor cells'). It appears more rational for drug design to gather information by applying the metabolic control analysis, which allows the quantitative identification of the main controlling steps in a pathway, along with providing understanding of the underlying regulatory mechanisms and faciliting the prediction of the system behavior.

The encouraging results obtained with energy-metabolism drugs indicate that to block the growth of oxidative or partially oxidative tumors successfully (Table 2), specific drugs for glycolysis and oxidative phosphorylation, which may cross the cellular permeability barriers, need to be used simultaneously (Table 3). It may be argued that cancer cells are genetic and phenotypically heterogeneous from line to line. However, all tumor cell lines depend on glycolysis and oxidative phosphorylation for ATP supply. The so-called 'metabolic therapy' searches for physico- and biochemical differences between tumor and normal cells to facilitate the design of strategies that preferentially affect tumor metabolism and growth, without altering drastically the host tissue and organ functionality. This approach may complement the existent chemotherapeutic treatments, so that in combination they may successfully stop tumor growth, invasiveness and drug resistance. Traditional chemotherapy currently offers little long-term benefit for most malignant gliomas and is often associated with adverse side-effects that diminish the length or quality of life. Hence, new approaches are required that can provide long-term management of malignant brain tumors while permitting a decent quality of life [161].

Lipophilic cationic drugs, such as the rhodamines, MKT-077, F16, and perhaps the casiopeinas, are accumulated in the cytosol and mitochondria of tumor and normal cells because of the elevated electrical potential gradient ($\Delta\psi$, negative inside) generated across both the plasma membrane and the inner mitochondrial membrane. However, the causes underlying the observed higher selectivity of tumor mitochondria towards rhodamines have not been elucidated [162]. In this regard, it is documented that mitochondria

from human colon CX-1 tumor [143], cells and mitochondria from MCF-7 breast adenocarcinoma [163], and *neu*-, *v-Va-ras*-, *β-catenin* and *c-myc*-initiated mouse tumor cell lines [145] develop a $\Delta\psi$ of a higher magnitude than that of normal cells (epithelial cells from spleen, breast, kidney) (Fig. 3). The reasons for the higher $\Delta\psi$ might be related to the higher content of cardiolipin (increasing the density of membrane negative charges) and cholesterol (which may decrease the passive diffusion of H^+ and other ions) in the tumor plasma and inner mitochondrial membranes [1,164] (Fig. 3). In osteosarcoma cells lacking mtDNA (ρ°) and hence lacking a respiratory chain and the ability to generate an electrical gradient, 50 times more rhodamine 123 is required to inhibit proliferation [165]. The fluorometric techniques used in detecting a higher $\Delta\psi$ do not permit a quantitative estimation of the absolute values or of the difference in $\Delta\psi$ between normal and tumor cells and mitochondria. However, by taking into account the quantitative determinations of $\Delta\psi$ in cells and mitochondria [92,166–168], it is expected that the absolute values are in the range of 120–150 mV and the difference to be not higher than 20–40 mV, as that is the magnitude of the increase in $\Delta\psi$ when an oxidative phosphorylation inhibitor (oligomycin or carboxyatractyloside) is added to respiring cells and mitochondria [167].

Metabolic changes associated with the tumor resistance to radio- and chemotherapy

It is documented that the lack of effectiveness of clinical treatments, such as radiation or antineoplastic drugs, to diminish tumor progression is related to the development of a hypoxic and acidic microenvironment surrounding the tumor cells [104–108]. In particular, the growth of solid tumors may surpass the O_2 diffusion from the blood vessels, thus developing hypoxic areas. Such an O_2 gradient has been detected in human cervix carcinomas, breast cancers, head and neck cancers, soft tissue sarcomas and glioblastoma multiforme [106,107,169]. Apparently, both radio- and chemotherapy require the presence of O_2 (and hence blood microcirculation) to become effective treatments against tumor cells. Moreover, apoptosis mediated by antineoplastic drugs also requires a highly oxygenated environment and intracellular oxidant agents, which are not always available in solid, glycolytic tumors [170]. In fact, it seems that a functional oxidative phosphorylation is required for apoptosis, as ρ° tumor cells develop an apoptotic-resistant phenotype [171]. Most solid tumors cannot grow without a blood supply (oxidizable substrates, O_2), and metastasis depends on neovascularization of the primary tumor. In multispheroids of human U118MG colon cancer, changing the prevalent glycolytic metabolism to oxidative, by adding oxamate, an LDH inhibitor, provokes an increase in the tumor sensitivity to radiation [107]. These observations suggest that the hypoxic microenvironment in tumors facilltates survival.

Traditional chemotherapy targets dividing, proliferating cells. Unfortunately, all the clinically accepted chemotherapeutic treatments use large drug doses that also induce profound damage to normal, proliferative host cells [172]. Therefore, more selective targeting is required for the treatment of cancer. Another problem associated with chemotherapy is that in many tumor types there is either inherent or acquired resistance to antineoplastic drugs. A significant advance has been elucidation of the metabolic changes developed by the tumor cells for drug resistance. The drug-resistant cells decrease the mitochondrial H^+ electrochemical gradient by over-expressing uncoupler protein 2, which acts as a mitochondrial H^+ channel, thus collapsing the H^+ gradient generated by the respiratory chain; a potent uncoupler protein 2 inhibitor is guanosine 5′-diphosphate (GDP). These drug-resistant cells also increase the utilization of alternative oxidizable substrates (fatty acids) [173]. Furthermore, drug-resistant tumor cells may also over-express the P-glycoprotein, an organic anion ATPase that efficiently expels xenobiotics, after exposure to drugs such as doxorubicin, paclitaxel, vinblastine and epirubicin [174].

Tumor cell metabolism and positron emission tomography (PET)

In recent years, PET, sometimes combined with computed tomography (CT) has been applied for the diagnosis, monitoring and treatment of cancer [175,176]. PET has mainly used glucose derivatives [^{18}fluoro-deoxyglucose (FDG)] as tracers under the assumption that tumors have a higher glycolytic capacity than normal cells [177]. Unfortunately, the FDG/PET results have been contradictory in some cases [178,179].

On the one hand, FDG/PET has established that the vast majority of metastatic tumors ($> 90\%$) are highly glycolytic and allowed the accurate detection ($> 90\%$) of solitary pulmonary nodules, mediastinal and axillary lymph nodes; colorectal cancers; lymphomas; melanomas; breast cancers; and head and neck cancers [175]. On the other hand, false positives of FDG/PET in benign diseases have been reported. Infectious diseases (mycobacterial, fungal, bacterial), inflammatory cells (neutrophils, activated macrophages), fibrotic lesions, sarcoidosis, radiation pneu-

monitis and postoperative surgical conditions have shown an intense FDG signal in PET. Moreover, it is frequently encountered that tumors with low glycolytic activity, such as adenomas and bronchioloalveolar carcinomas, and a low cellular density of metastatic tumors caused by the presence of mucin, carcinoid tumors, low-grade lymphomas and small-size tumors, have revealed false-negative results on FDG/PET [180].

In thyroid carcinoma (an advanced state and highly differentiated human tumor), FDG was not concentrated, despite administration of high and repetitive doses; instead, such treatment induced a secondary effect, consisting of hematic toxicity [181], suggesting that the relevant energy pathway in thyroid carcinoma was not glycolysis. Accumulation of FDG in malignant tumors is related to regional hypoxia, but because other factors affecting FDG uptake may be more predominant in chronic hypoxia, there may be a poor correlation between hypoxia and FDG uptake. Therefore, FDG/PET cannot reliably differentiate hypoxic (i.e. glycolytic) from normoxic tumors in frequently hypoxic (head and neck cancer and glioblastoma multiforme), and in less frequently hypoxic (breast cancers), tumors [182].

Certainly, glycolysis may be activated under hypoxia in tumor cells, but glucose catabolism and O_2 levels may not follow a simple relationship. Glycolysis can be affected by processes not related to hypoxia, such as cell proliferation and maintenance of ion gradients [169]. Moreover, the glycolytic pathway has other functions, in addition to providing cytosolic ATP. These additional, secondary functions in normal cells may change in tumor cells.

Knowing that not all tumors are glycolytic, but that some have a predominant oxidative type of energy metabolism (see Table 2 and the section entitled 'Re-evaluation of oxidative phosphorylation in tumor cells'), it may be justifiable to apply PET-CT with tracers directed to mitochondria. There are already some examples: pyruvate-1-[11]C was used in the analysis of mitochondrial encephalomyopathy and Leigh's disease [183]; copper (II)-pyruvaldehyde-bis (N4-methylthiosemicarbazone) was used for monitoring electron transport chain in normal brain mitochondria and Ehrlich ascites [184]; TC-99M-tetrofosmin was found to accumulate more in tumor mitochondria (breast adenocarcinoma MCF-7 and SK-BR-3 cells, synovial sarcoma SW 982 cells and chondrosarcoma SW 1353 cells) than in normal mitochondria [185]; and in prostate and hepatocellular carcinoma, the use of ^{11}C-acetate/PET has been successfully validated by both clinical and experimental studies [186], probably because the predominant energy pathway in these cancers is not glycolysis, but fatty acid oxidation [187].

A family of novel copper II -mixed chelate compounds, termed casiopeinas®, have been tested with success against several human (HeLa, SiHa, CaSKi, CaLo) and murine (B16 melanoma, AS-30D) tumor cell lines [117,168,188]. Because of their hydrophobic and cationic nature (Table 3), these antineoplastic drugs (^{11}C-casiopeinas) might be useful in PET diagnosis of nonglycolytic tumors. Of course, the use of mitochondrial tracers in PET analysis may also encounter the same difficulties described for FDG, in regard to revealing false negatives in low oxidative tumors, and false positives in normal tissues with high oxidative activity. The question of whether mitochondrial PET tracers may be more specific for tumors than for normal, healthy cells will be answered when more data become available, but it is worth noting that mitochondria of oxidative tumors develop a higher electrical membrane potential than mitochondria of normal tissue (see the section entitled 'Mitochondrial metabolism as therapeutic target'), thus favoring the accumulation of lipophilic, cationic drugs and facilitating the detection of oxidative tumors.

Therefore, it now seems a more rational strategy to first elucidate the energy metabolic properties of each tumor type, to help establish the most appropriate therapeutic strategies.

Conclusions

All tumor cell types show an enhanced glycolytic flux; however, not all have a diminished mitochondrial metabolic capacity. Therefore, the take-home message is that not all tumor cell types depend exclusively on glycolysis for ATP supply; some may equally or predominantly rely on oxidative phosphorylation. In consequence, the driving force for the enhanced glycolysis in tumor cells cannot be an energy deficiency induced only by a damaged oxidative phosphorylation. The accelerated cellular proliferation may also impose an energy deficiency (as well as a higher demand for glycolytic and Krebs cycle biosynthetic intermediaries), which can only be covered by an increased glycolysis together with an unperturbed oxidative phosphorylation.

Certainly there is genetic, biochemical and morphological heterogeneity in cancer cells, but all depend only on glycolysis and oxidative phosphorylation for ATP supply. The enhanced tumor glycolysis results from a generalized over-expression of most or all the enzymes and transporters of the pathway, with HK and PFK-1

being markedly over-expressed in a different isoform (with different kinetic properties from that of the tissue of origin). At the genetic level, the enhanced glycolysis seems to be activated by the transcription factor, HIF-1α, although other factors, such as p53, may also be involved. These two transcription factors might also affect the mitochondrial function.

The metabolic control analysis of glycolysis and oxidative phosphorylation, under conditions close to their natural environment, and the elucidation of their regulatory mechanisms, may allow us to predict the pathway behavior and identify the relevant alterations in tumor cells. This type of analysis has shown that despite the enhanced glycolysis in all tumor cell types, the same pathway regulatory mechanisms observed in normal cells seem to be in place, with the notable exception of those for PFK-1. In other words, GLUT and HK do control glycolytic flux in some fast-growth tumor cells (rodent AS-30D; human HeLa) as well as in normal cells, despite the drastic over-expression of these two proteins and the shift in the over-expressed isoform: for HK, the regulatory mechanism is a strong product inhibition. It would be interesting to establish whether the two controlling steps (GLUT, HK) might also be suitable therapeutic targets for glycolytic, hypoxic tumors. However, it first has to be determined whether these proteins also mainly control glycolytic flux in these other tumors. In turn, the better understanding of the energy metabolism of tumor cells may lead to design strategies for an efficient modulation of the ATP supply and cellular processes that are highly ATP-dependent, such as cellular proliferation.

The relevant role of HIF-1α in mediating angiogenesis, proliferation and invasion, and in regulating the expression of glycolytic enzymes in tumor cells, has led to the proposal of the blockade of the HIF-1α signal as a novel, promising therapeutic target in hypoxic tumors [189]. However, the several unsuccessful efforts in this direction indicate that the multiple functions of this transcription factor have to be fully elucidated before embarking on clinical trials.

In tumor types (such as oxidative tumors) in which glycolysis is not the predominant energy pathway, the application of mitochondria-directed drugs (such as cationic lipophilic molecules), as well as the use of mitochondria-directed tracers (such as ^{11}C-acetate or glutamine or ^{11}C-rhodamines or casiopeinas) in PET-CT analysis may be considered as alternative detection and therapeutic strategies. These observations emphasize the necessity in advancing the understanding of tumor energy metabolism for improvement in diagnosis, drug design and chemotherapy of cancer.

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