Review

Targeting of cancer energy metabolism

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The main purpose of this review is to update and analyze the effect of several antineoplastic drugs (adriamycin, apoptodilin, casiopeinas, cisplatin, clotrimazole, cyclophosphamide, ditercalinium, NSAIDs, tamoxifen, taxol, 6-mercaptopurine, and α-tocopheryl succinate) and energy metabolism inhibitors (2-DOG, gossypol, delocalized lipophilic cations, and uncouplers) on tumor development and progression. The possibility that these antineoplastic drugs currently used in *in vitro* cancer models, in chemo-therapy, or under study in phase I to III clinical trials induce tumor cellular death by altering also metabolite concentration (*i.e.*, ATP), enzyme activities, and/or energy metabolism fluxes is assessed. It is proposed that the use of energy metabolic therapy, as an alternative or complementary strategy, might be a promising novel approach in the treatment of cancer.

Keywords: Anticancer drugs / Glycolysis / Mitochondria / Oxidative phosphorylation

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1 Introduction

The field of cancer energy metabolism seemed resolved by 1956 when Warburg [1] proposed that the prime cause of cancer was an energy deficiency caused by an irreversible damage to the mitochondrial function that induced an increased glycolysis. Since then, several researchers have thought that the Warburg hypothesis applies to all or most cancer cell types [2 – 14], because one of the most notorious and well-known alterations in tumor cells is certainly an increased glycolytic capacity, even in the presence of high O_2 concentration [3, 4, 15 – 18].

The observation that tumors have a higher glycolytic capacity than normal cells has found application in the use of positron emission tomography (PET) for diagnosis, monitoring, and treatment of cancer [19, 20]. With ^{18}fluoro-deoxyglucose (FDG) as tracer, PET has established that the vast majority of metastatic tumors (>90%) are highly glycolytic; and has also allowed for the accurate detection (>90%) of solitary pulmonary nodules, mediastinal and axillary lymph nodes, colorectal cancers, lymphomas, melanomas, breast cancers, and head and neck cancers [19].

2 Glycolysis in tumor cells

2.1 General

Fast-growth cancers from human (leukemia, HeLa) and rodent (AS-30D, Morris 7800, Dunings LC18, Novikoff hepatomas) show an increased glycolytic rate (2- to 17-times) in comparison with nontumorigenic cells [21 – 23]. This metabolic feature is the consequence of glycolytic enzymes over-expression induced by (i) oncogene *c-myc* (glucose transporter 1 (GLUT1), hexosephosphate isomerase (HPI), phosphofructo kinase type 1 (PFK1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), and enolase (ENO)) [5] and (ii) HIF-1α (hypoxia inducible factor 1α), GLUT1, glucose trans-

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Abbreviations: ALDO, aldolase; **AMF,** autocrine motility factor; **Cas IIgly,** casiopeina II gly; **CCCP,** carbonyl cyanide-3-chlorophenylhydrazone; **CML,** chronic myelogenous leukemia; **CP,** cyclophosphamide; **DLC,** delocalized lipophilic cation; **2-DOG,** 2-deoxyglucose; **ENO,** enolase; **F1,6BP,** fructose 1,6 bisphosphate; **F2,6BP,** fructose 2,6 bisphosphate; **FDG,** ^{18}fluoro-deoxyglucose; **GAPDH,** glyceraldehyde 3-phosphate dehydrogenase; **GLUT1,** glucose transporter 1; **GLUT3,** glucose transporter 3; **G6P,** glucose 6-phosphate; **HKI,** hexokinase type I; **HKII,** hexokinase type II; **HPI,** hexosephosphate isomerase; **LDH,** lactate dehydrogenase; **6MP,** 6-mercaptopurine; **3MPA,** 3-mercaptopicolinic acid; **MPT,** mitochondrial permeability transition; **NSAID,** nonsteroidal anti-inflammatory drug; **PET,** positron emission tomography; **PFK1,** phosphofructo kinase type 1; **PFK2,** phosphofructo kinase type 2; **PGK,** phosphoglycerate kinase; **P-gly,** glycoprotein type P; **α-TOS,** α-tocopheryl succinate

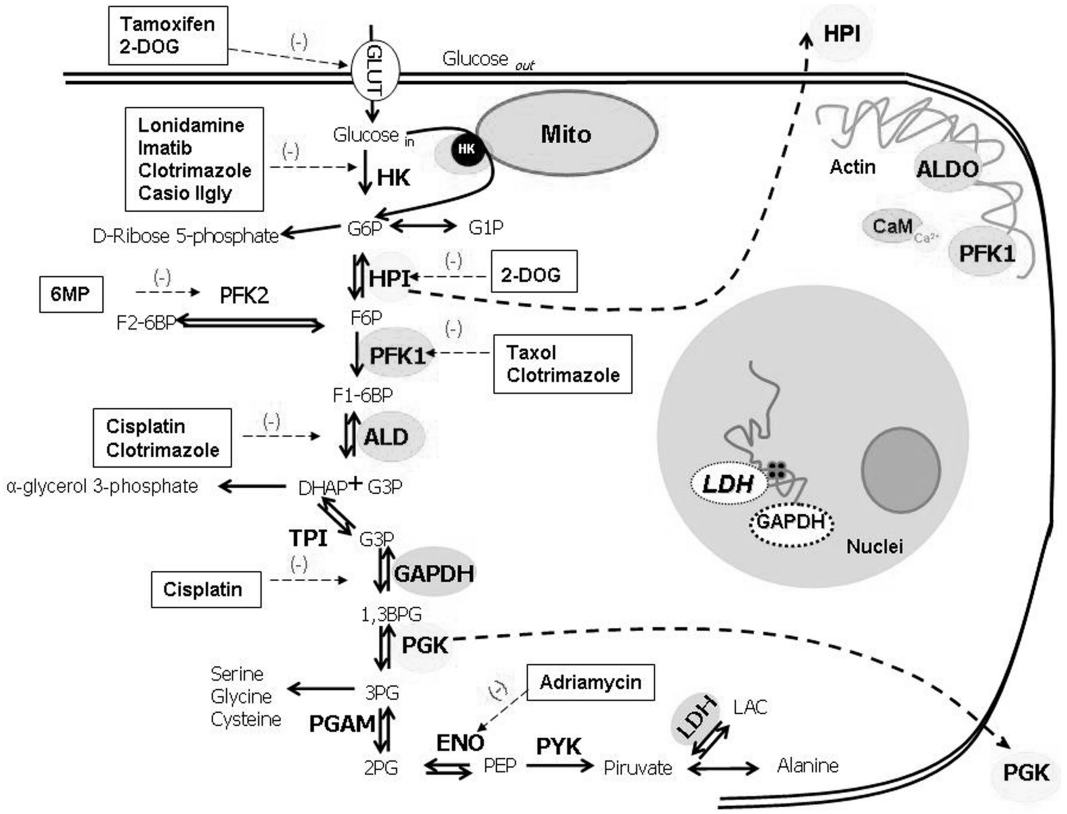

Figure 1. Glycolytic enzyme targeting by anticancer drugs. Phosphoglycerate mutase (PGAM), phosphoglycerate mutase; F6P, fructose 6-phosphate; 1,3BG, 1,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 6MP; and LAC, lactate.

porter 1 (GLUT3), hexokinase type I (HKI), hexokinase type II (HKII), PFK1, phosphofructo kinase type 2 (PFK2), ALD-A, ALD-C, GAPDH, PGK1, ENO1, PYK-M, and lactate dehydrogenase (LDH)-A) [24].

2.2 Additional functions of glycolytic enzymes

Although, the main role of the glycolytic pathway is the generation of ATP under anaerobic conditions, in cells with high proliferation rates (*i.e.*, tumor cells), glycolytic intermediaries are demanded by other secondary pathways. For example, pyruvate and 3-phosphoglycerate (3PG) feed alanine, serine, cysteine, and glycine pools used for protein synthesis; glucose 6-phosphate (G6P) generates intermediaries for nucleic acids and glycogen synthesis (ribose 5-phosphate, glucose 1-phosphate (G1P)); and dihydroxyacetone phosphate (DHAP) is a precursor of α-glycerol 3-phosphate used for triacylglycerides and phospholipids synthesis (Fig. 1). Furthermore, it has been recently documented that some glycolytic enzymes have other cellular functions: (i) HKI and HKII may regulate the release of cytochrome *c* by interacting with the mitochondrial voltage-dependent anion channel [25]. Besides, HK antagonizes the proapoptotic effect of Bid by activating Bax and Bak in the apoptotic cascade [26]. (ii) GAPDH and LDH may bind to a single-strand DNA through a region close to the coenzyme-binding site. In fact, NADH addition diminishes the formation of GAPDH- or LDH-DNA complexes indicating that the NADH/NAD^+ ratio may regulate the glycolytic DNA-binding enzymes [27]. GAPDH and LDH constitute the transcriptional factor complex OCA-S, which increases histone transcription (H2Bgene) to maintain the replication process and function of eukaryotic chromosomes [27]. GAPDH participates also in transcriptional regulation as a nuclear tRNA export protein, and in replication and repair of DNA. It can also mediate endocytosis by its interaction with tubulin and it can be a requirement for programed neuronal cell death [28]. (iii) The autocrine motility factor (AMF) enhances motility and metastatic ability of tumor cells. AMF is identical to both neuroleukin, a neurotrophic growth factor that supports the survival of spinal and sensory neurons, and the maturation factor, which mediates the differentiation of human myeloid leukemic HL-60 cells to terminal monocytic cells. The identity of the AMF factor is the glycolytic enzyme HPI [29]. The AMF/HPI activity appears elevated in the serum or urine of patients (Fig. 1) with disseminated malignant tumors, such as gastrointestinal, kidney, breast, colorectal, and lung carcinomas, thereby being useful as a tumor-dissemination marker. (iv) A transcriptional suppressor of Myc, known as MBP-I, has 95% of identity with ENO [27].Diminution in

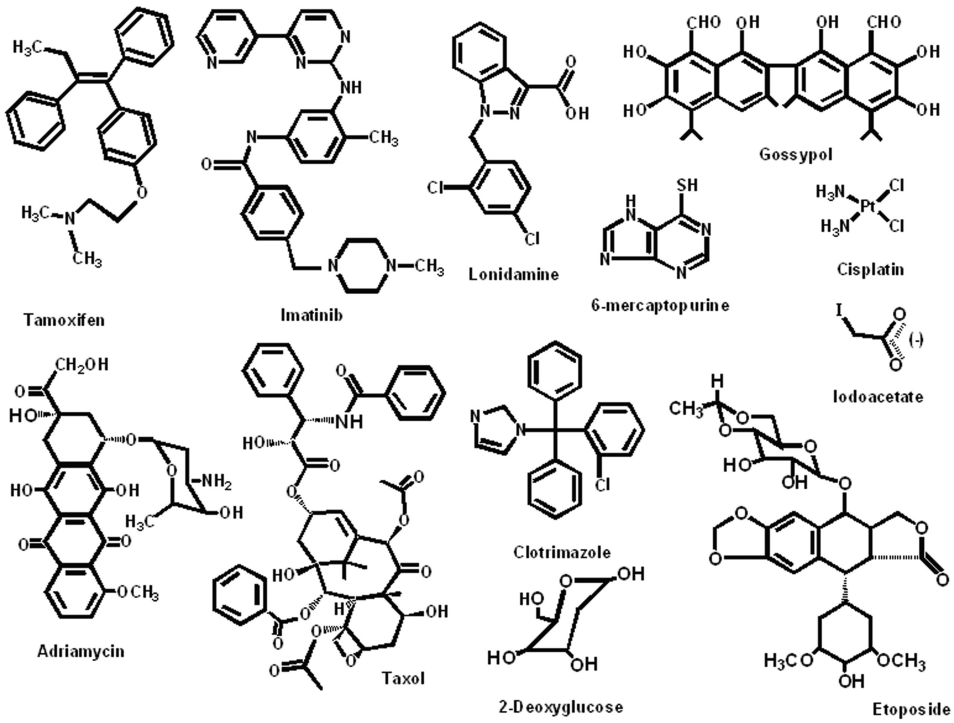

Figure 2. Antineoplastic glycolytic inhibitors.

ENO expression has been associated also with the low survival in patients with lung cancer. (v) The glycolytic enzyme PGK is secreted by tumor cells (Fig. 1) and acts as disulfide reductase that facilitates the cleavage of disulfide bonds in plasmin, and triggers proteolytic release of the angiogenesis inhibitor, angiostatin [30].

Some authors have proposed that glycolytic activity correlates with the degree of tumor malignancy, so that glycolysis is faster in highly de-differentiated and fast-growing tumors than in slow-growing tumors or normal cells [2, 21]. In fact, a high level of lactate has been proposed as predictor of malignancy [31]. Moreover, high glycolysis in tumor cells has been related with resistance to chemo- and radiotherapy treatment [11, 32 – 34]. Therefore, these observations suggest that blocking of glycolysis might diminish tumor progression and enhance efficacy of chemo- and radio-therapy; on the other hand, inhibition of glycolytic enzymes is expected to have secondary effects on the cell physiology, due to the additional functions of these proteins.

Indeed, studies *in vivo* (human osteosarcoma, MV522 lung carcinoma [32], ovarian cancer [35]) and *in vitro* (glioma BMG-1 and U-87, squamous carcinomas 4451 and 4197 [36], human leukemia HL-60, and lymphoma Raji cells [11], breast cancer MCF-7 cells [37], human LoVo colon carcinoma cells [33]) have revealed that inhibition of tumor glycolysis with 2-deoxyglucose (2-DOG), 3-bromopyruvate or lonidamide increases the sensitivity toward anticancer drugs commonly used in chemo-therapy, such as alkylating agents (cisplatin, 4-hydroperoxycyclophosphamide, melphalan, and carmustine), antimetabolites (1-β-D-arabinofuranosylcytocine), antimicrotubule agents (vincristine and taxol), intercalating DNA agents (adriamycin), and topoisomerase inhibitors (etoposide, camptothecin) [11, 32, 33, 35 – 37].

3 Antineoplastic-drugs targeting glycolysis

3.1 Metal-based drugs

The anticancer drug cisplatin (*cis*-diammine-dichloroplatinum (II)) (Fig. 2) is commonly used in the clinical treatment of testicular, ovarian, cervical, head and neck, nonsmall cell lung cancers, and relapsed lymphoma [38]. This drug induces cytotoxicity by (i) forming mono-adducts with the N7 atom of guanosine and adenosine of nucleic acids, (ii) interacting with single DNA strands, and (iii) forming an inter strand-crosslink with the duplex DNA. In consequence, DNA transcription and replication is abolished promoting apoptosis [38]. Thus, cisplatin induces growth inhibition in cancers from different origins [39 – 41] (Table 1).

Cisplatin inhibits glycolytic enzymes in normal cells [42, 43] (Table 2) at the same doses assayed to arrest tumor progression. For example, in normal rabbit muscle, cisplatin (100 μM) diminishes both aldolase (ALDO) and GAPDH activities by 45 and 87%, respectively, through the formation of protein-S-cisplatin complexes [42]. Besides, in the FL512 BcL-X_L lymphocytic cell line, 16 μM cisplatin

Table 1. Potency of anticancer drugs commonly used in chemo-therapy and experimental models

Drugs	Cellular type	IC_{50}	Reference
Adria	Human osteosarcoma 14b and ρ^0 osteosarcoma; human LoVo colon carcinoma	1.8 and 50 nM	Fanciulli, *et al.* 2000 [33]; Liu *et al.* 2001 [61]
	Human melanoma	12 μM	Gupta and Krishan 1982 [41]
Carboplatin	Human ovarian cancer (CAOV-3, OVCAR-3, SKOV-3, ES-2, OV-90, TOV-112D, TOV-21G)	0.7–1.8 nM	Smith *et al.* 2005 [40]
Carmustine	Human melanoma	37 μM	Gupta and Krishan 1982 [41]
Cas IIgly	Human cervix cancer (HeLa, SiHa, CaSki and C33-A); rat C6 glioma; AS-30D hepatoma-bearing rat	0.4–30 μM	Gracia-Mora *et al.* 2001 [47]; Rodríguez-Enríquez *et al.* 2006 [50]
	Human lymphocytes; rat fibroblast	20–34 μM	Trejo-Solís *et al.* 2005 [49]; Rodríguez-Enríquez *et al.* 2006 [50]
Cisplatin	Human cervix cancer (HeLa, SiHa, CaSki and C33-A); human ovarian cancer (CAOV-3, OVCAR-3, SKOV-3, ES-2, OV-90, TOV-112D, TOV-21G); human melanoma (LOX); human bladder carcinoma (EJ); human colon carcinoma (CX-1); human breast cancer (MCF-7); human pancreas carcinoma (CRL1420).	0.8–600 μM	Gupta and Krishan 1982 [41]; Koya *et al.* 1996 [39]; Gracia-Mora *et al.* 2001 [47]; Smit *et al.* 2005 [40]
	Monkey normal kidney epithelial (CV-1)	4.2	Koya *et al.* 1996 [39]
Clotrimazole	Human breast cancer (MCF-7)	89 μM	Meira *et al.* 2005 [70]
Imatinib	Human leukemia BCR-ABL-positive K562 and CML-T1	0.47 and 0.69 μM	Gottschalk *et al.* 2004 [76]
Lonidamine	Human and rodent fibrosarcomas	155–467 μM	Ning and Hahn 1990 [78]
Mercaptopurine	Human acute lymphoblastic leukemia (CCRF-CEM)	1.5 μM	Dervieux *et al.* 2001 [55]; Kano *et al.* 2006 [56]
	Human promyelocytic leukemia (HL60), CML (KU-812), human lymphoblastic leukemia (MOLT-3), Burkitt's lymphoma	1.7–10 μM[a)]	
Tamoxifen	Human breast cancer (MCF-7)	4.4 μM	Dalenc, F *et al.*, 2005 [181]; Treeck O *et al.*, 2006 [182]
	Human ovarian (OVCAR-3, SK-OV-3) and endometrial cancers (HEC-1A)	>1 μM	
Taxol	Human ovarian cancer (CAOV-3, OVCAR-3, SKOV-3, ES-2, OV-90, TOV-112D, TOV-21G); human osteosarcoma 14b and ρ^0	0.8–59 nM	Liu *et al.* 2001 [61]; Smith *et al.* 2005 [40]
	B16 F10 mouse melanoma	10 μM	Glass-Marmor *et al.* 1999 [62]
Vinblastine	Human osteosarcoma 14b and ρ^0	0.9 nM	Liu *et al.* 2001 [61]
	Human melanoma	0.4 μM	Gupta and Krishan 1982 [41]
2-DOG	Osteosarcoma cell line 14b with glucose 5 and 25 mM ρ^0 Osteosarcoma cell line 14b with glucose 5 and 25 mM	0.6 and 6 mM 0.03 and 0.1 mM	Liu *et al.* 2001 [61]
Gossypol	Endometrial tumor cell lines (BP1295, KF681, and VG5800)	25–50 nM	Tuszynski *et al.* 1984 [88]; Jaroszewski *et al.* 1990 [87]; Rodríguez-Enríquez *et al.* 2006 [50]; Badawy *et al.* 2007 [86]
	Human breast cancer (MCF-7, MDA-MB-468); human cervix cancer (HeLa); human cervical carcinoma (KB-3-1, KB-A1, and KB-V1); human colon carcinoma (SW407, SW1084, and SW1116); human melanoma (FEMX, FEMX 4A, P 550, WM9, WM56, and WM164); human ovarian carcinoma (A2780 and A2780/CP70); Chinese hamster lung cancinoma (DC-3F and DC-3F/AD X)	3.4–8.2 μM	
	Human erythroleukemia (K562) and human mammary adenocarcinoma (HT23)	27.5 and 34 μM	AS-30D hepatoma cells 198 μM
	Dog kidney tubular cells (MDCK and MDR-MDCK); diploid myo-epithelial breast cells (Hs578Bst) and human embryonic lung fibroblasts (WI38)	4.5, 19.8, and 26.5 μM	
Iodoacetate	Human cervix cancer (HeLa) and AS-30D hepatoma cells	24 and 99 μM	Rodríguez-Enríquez *et al.* 2006 [50]
Oxamate	Human ρ^0 osteosarcoma 14b and osteosarcoma 14b	9.8 and 47 μM	Liu *et al.* 2001 [61]

a) IC_{80} values.

Table 2. Effect of several anticancer drugs on glycolytic enzymes activities

Drugs	Enzyme target	Doses (μM)	Cellular type	Effect	Reference
Adria	ENO	20 mg/kg	Mice C57BL/6 heart	Enzyme inhibition by 25%	Chen *et al.* 2006 [67]
Cas IIgly	HK	10–100	AS-30D hepatoma cells	Enzyme inhibition by 50%	Marín-Hernández *et al.* (unpublished data)
Cisplatin	GAPDH, ALDO	100	Rabbit muscle	Enzyme inhibition by 45–85%	Aull *et al.* 1979 [42]
	GLUT1, GLUT3, HKI, HKII, PFK1, ENO, PYK, LDH	16	Human lymphocytic cell line (FL5.12 BcL-x1)	Decrease in gene expression by 60–80%	Zhou *et al* 2002 [43]
Clotrimazole	Mitochondrial-bound HK, PFK, ALDO	50	Mouse melanoma (B16F10); Lewis lung cancer; human colon carcinoma (CT-26).	Detachment of mitochondria 80% and cytoskeleton 50%	Penso and Beitner 2002 [69, 71]; Penso and Beitner 1998 [72]; Meira *et al.* 2005 [70]
Etoposide	GLUT1, GLUT3, HKI, HKII, PFK1	16	Human lymphocytic cell line (FL5.12 BcL-x1)	Decrease in gene expression by 60–80%.	Zhou *et al.* 2002 [43]
Imatinib	HK	0.68–6.8	Human leukemia (K562)	Enzyme Inhibition by 25%	Boren *et al.* 2001 [77]
Lonidamine	Cytosolic HK Mitochondrial-bound HK	75 5	Ehrlich ascites tumor cells	Enzyme Inhibition by 17–65%	Floridi *et al.* 1981 [79]
Mercaptopurine	PFK2, HK	1000	Rat liver, rat lymphocytes, bovine heart, HTC rat hepatoma cells, chick embryo fibroblast, HeLa and IM-9 cells, regenerating rat liver	Inhibition of activity by 25–90%	Lea *et al.* 1970 [57]; Mojeda *et al.* 1992 [58]
Tamoxifen	GLUT1	0.3–6.8	Human erythrocytes	Inhibition of GLUT by 50%	Martin *et al.* 2003 [73]; Afzal *et al.* 2002 [74]
Taxol	PFK1	25	B16F10 mouse melanoma cells	Decrease cytoskeleton bound enzyme 50%	Glass-Marmor *et al.* 1999 [62]

diminishes protein expression (60–80%) of GLUT1, GLUT3, HKI, HKII, PFK1, ENO, PYK, and LDH with the concomitant diminution in the glycolytic rate (40%) [43] (Table 2). A direct cisplatin effect on tumor glycolytic enzymes has not been assessed yet.

Etoposides show a similar inhibition pattern to that observed with cisplatin on glycolysis (Table 2, Fig. 2) [43]. Expression of GLUT1, GLUT3, HKI, HKII, PFK1, ENO, PYK, and LDH decreases, inducing an 80% diminution in the glycolysis rate, after etoposide treatment. Changes induced by etoposide in glycolysis gene-expression lead to mitochondrial dysfunction and severe DNA damages, triggering apoptotic events [43].

At doses used to kill tumor cells, cisplatin also affects other metabolic pathways in normal tissues (Table 1). At low doses (10–75 μM), the drug promotes apoptosis in canine kidney cells [44] and mouse fibroblasts [45]. At higher doses (>100 μM), lipoperoxidation, inhibition of pyruvate-stimulated gluconeogenesis, and massive accumulation of *p*-aminohippurate (a characteristic signal of organic transport system inhibition) are observed in the kidney [46]. Perhaps more importantly, cisplatin induces significant side effects (at nephro- and neurological levels) in the host at therapeutic doses [38]. Therefore, other new platinum compounds with apparently less toxic effect have been developed (*i.e.*, carboplatin, oxaliplatin, and satraplatin), although the effect of these cisplatin-derivatives on glycolytic rate has not been fully evaluated yet.

Casiopeinas are antitumor drugs with a copper center (Fig. 3). Casiopeina II gly (Cas IIgly) has shown a potent inhibitory effect on growth of human (HeLa, SiHa, CaSki, C33-A, CH1 ovarian and rodent AS-30D, L1210 leukemia, glioma C6) tumors [47–50] (Table 1). Interestingly, normal cells (lymphocytes, fibroblasts) are less sensitive to Cas IIgly than tumor cells (HeLa and glioma C6) suggesting that Cas IIgly may be a promising alternative drug to reduce tumor proliferation without affecting healthy tissues [49, 50].

Cas IIgly (0.4 nM–30 μM) induces cellular cytotoxicity through DNA-adduct formation and release of cytochrome *c* as apoptotic signal [51]. However, at low doses (<10 μM), Cas IIgly also inhibits 40% OxPhos and 40–50% glycolysis in HeLa cells and AS-30D hepatoma [50, 51]. Enzymes in the OxPhos pathway inhibited by Cas IIgly were the 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase complexes [51]. In the glycolytic pathway, HK was probably the main target of Cas IIgly because it is one of the main controlling steps [18] and, due to its high K_m value for ATP (0.9 mM, [18]), a diminution in the ATP level may limit HK activity. To this regard, we have observed that a high dose of

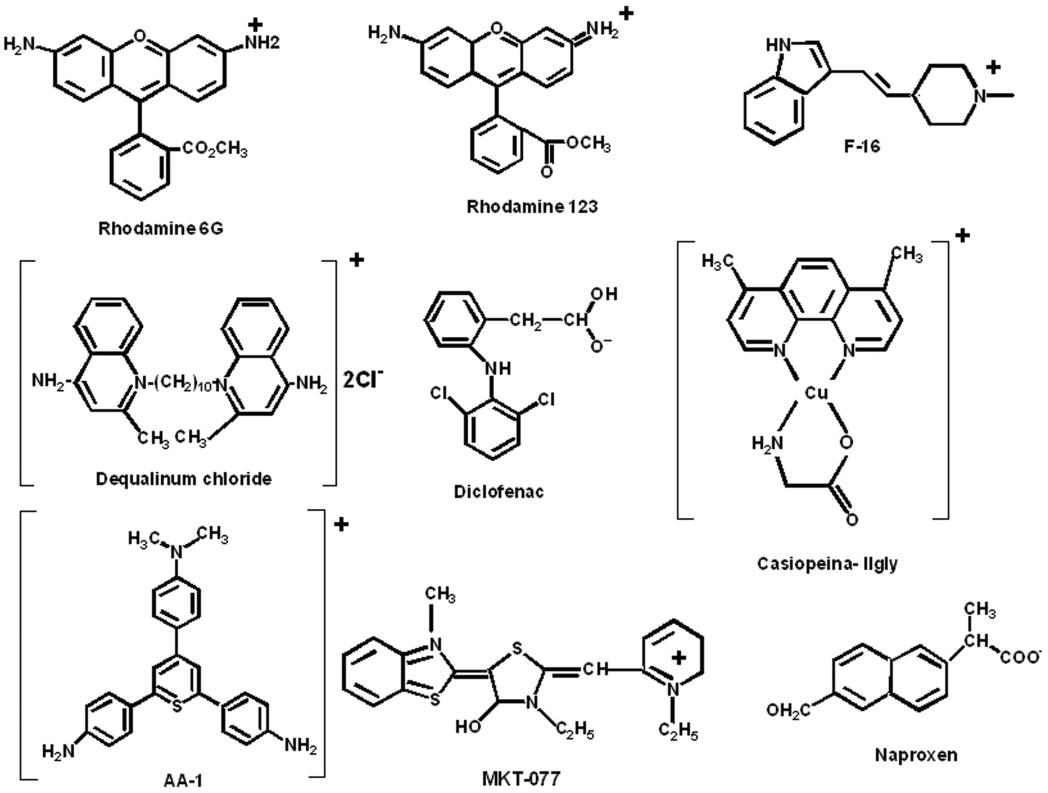

Figure 3. Lipophilic cationic antineoplastic drugs with delocalized charge.

100 μM Cas IIgly and a prolonged incubation of 60 min induce a 50% inhibition of tumor HK (Marín-Hernández, A., Gallardo-Pérez, J. C., Moreno-Sánchez, R., Rodríguez-Enríquez, S., unpublished data). Accordingly, Cas II gly also induces diminution in cardiac work (IC_{50} = 4 μM) and in organ oxygen consumption when glucose is the only oxidizable substrate [52]. In contrast, with a more physiological oxidizable substrate, such as a fatty acid (octanoate), the heart function sensitivity toward Cas IIgly decreases 50% [52]. These data suggest that glycolysis is more susceptible than OxPhos to Cas IIgly inhibition.

3.2 Nonmetal-based drugs

Alkylating drugs, such as cyclophosphamide (CP), carmustine and dacarbazine, insert into guanosine or adenosine residues to generate mono-adducts that interfere with DNA transcription and replication. CP has been employed against non-Hodgkin lymphoma, multiple myeloma, and small-cell lung carcinoma [53]. A single but high dose of CP (300 mg/kg) reduced 60% the size and glycolytic activity of fibrosarcoma RIF-1 in tumor-bearing mice [53]. Low but chronic doses (6.8 mg/kg *per* day for 9 wk) of CP did not affect ALDO and LDH activities [54], suggesting that other glycolytic enzymes may be the target of CP (*i.e.*, HK or GLUT [18]). Effects of carmustine and dacarbazine on glycolysis have not been reported yet for any cell type.

3.3 Antimetabolites

Antimetabolites are structural analogs of cellular metabolites, such as folic acid (methotrexate), pyrimidines (fluorouracil and cytarabine), and purines (6-mercaptopurine (6MP)) (Fig. 2), that block the synthesis of nucleotides inducing diminution in DNA and RNA synthesis. These drugs have been used in the treatment of several malignant cancers, such as uterus choriocarcinoma, acute leukemia, and skin cancer, but their toxicity on energy pathway enzymes has not been systematically evaluated. In several leukemia types (Table 1) and Burkitt's lymphoma, 6MP inhibits tumor progression with an IC_{50} of 1.5 μM and and IC_{80} of 1.7 – 10 μM, respectively (Table 1) [55, 56]. Because 6MP has high structural analogy with purine nucleotides, cells may incorporate it to the newly synthesized DNA and arrest cellular proliferation. Only extremely high doses of 6MP (0.1 – 0.2 and 1 mM) can reduce 50 and 90% the PFK2 activity in several normal and cancer-type tissues (Table 2). In tumor bearing-rats, 40 mg/kg of 6MP diminished 3.2-fold the volume of a hepatoma 777, whereas, at the same dose, the drug reduced HK activity by 25% in regenerating

rat liver (Table 2) [57]. These data indicate that 6MP antineoplastic activity is not mediated by altering energy metabolism.

Mojeda *et al.* [58], proposed that the inhibition of PFK2 by 6MP may involve the direct interaction between 6MP sulfhydryl groups (Fig. 2) and the essential thiol-groups of the enzyme. This interaction was prevented by thiol-reducing agents (DTT, β-mercaptoethanol) reverting 6MP's inhibitory effect. However, the same 6MP dose also inhibited GAPDH, suggesting that other thiol-dependent enzymes may also be targeted by this drug [58].

The 6MP (micromolar range) reduced significantly the fructose 2,6 bisphosphate (F2,6BP) concentration (20–55%) and lactate release (20–60%) in rat spleen and in IM-9 human lymphocytes. In HeLa cells, 6MP induced a drastic diminution in the F2,6BP content (80%) without affecting lactate formation [58]. In contrast, in rat hepatocytes and glucose-perfused heart, addition of 6MP at millimolar concentrations did not modify the F2,6BP levels, although enzyme activity was not assayed in the presence of the drug [58]. The different cellular response to 6MP may be explained by the intrinsic glutathione level; in hepatocytes, glutathione concentration is higher than in lymphocytes (5 *vs.* 0.2 mM, respectively), which might prevent the interaction between 6MP and the thiol-enzyme [59, 60].

3.4 Drugs affecting microtubule assembly

Other agents involved in the arresting of tumor progression are the microtubule-destabilizing drugs. Vinblastine, vincristine, and taxol are used in clinical treatment of lymphomas, ovarian, breast, small-cell lung, and malignant brain cancers. In ovarian carcinomas and 14b osteosarcoma, taxol (IC_{50} of 0.7–59 nM) and vinblastine (IC_{50} of 0.9 nM) inhibit tumor growth at extremely low doses [40, 61]. In contrast, in mouse and human melanomas, the taxol and vinblastine concentrations required to diminish tumor growth are 500–2000 times higher [62] (Table 1). At micromolar concentrations, taxol also promoted cytoskeleton-detachment of PFK1, diminution of G1,6P, and fructose 1,6 bisphosphate (F1,6BP) levels, as well as diminution in the ATP content in mouse melanoma [62]. Therefore, at the high doses used for melanoma treatment, taxol, and vinblastine may have toxic effects on nontumorigenic cells through glycolysis inhibition, because the drug concentration required to perturb glycolytic enzymes is in the micromolar range (Table 2).

3.5 Intercalating DNA drugs

Intercalating DNA agents show high affinity toward DNA and RNA [63]. The anthracyclines, adriamycin (adria), bleomycin, mitomycin, and daunomycin, intercalate in the B-form of the DNA helix with guanine–cytosine specific regions, promoting a distorted DNA helix, and preventing DNA-helicase, DNA-topoisomerase, and DNA-polymerases association. Adria stimulates free radical production through one-electron addition to quinone to generate semiquinone and reduces molecular oxygen to superoxide anion and hydrogen peroxide. In consequence, lipoperoxidation and protein oxidation events are attained and finally cellular death. In spite of its relevant antitumor activity in human tumor lines (Table 1), adria induces several severe side-effects in human and rodent models, such as cardiotoxicity and palmar-plantar erythrodysesthesia [64–66]. In heart tissue of adria-treated C57BL/6 mice, at doses equivalent to those employed in patients (20 mg/kg body weight), the drug induces diminution in ENO activity (25%), whereas triose phosphate isomerase (TPI) remains unaltered (Table 2) [67].

Several intercalating-drugs are ineffective against brain tumors. Clotrimazole, an antifungal-drug (Fig. 2), has been successfully used to diminish the size and development of intracranial gliomas (C6 and 9L), prolonging survival in rodents [68]. One of its mechanisms of action involves inhibition of Ca^{2+}-dependent calmodulin, and diminution of cellular proliferation and viability in mouse LL/2 Lewis lung cancer, CT-26 colon carcinoma, and human breast cancer MCF-7 (Table 1) [69, 70]. The Ca^{2+}-calmodulin complex promotes HK-binding to the outer mitochondrial membrane to ensure direct access to mitochondrial-derived ATP for glycolytic stimulation, and attachment of PFK1 and ALDO to the cytoskeleton (Fig. 1). It is noted that the activity of soluble enzymes is two to three times lower than that of enzymes bound to the cytoskeleton [71]. Thus, in breast cancer, the first action of clotrimazole (50 μM) could be the destabilization of HK, PFK-1, and ALDO, lowering the intracellular levels of G6P, F1,6BP, ATP, and the glycolytic flux; consequently, decreasing cellular proliferation (IC_{50} = 89 μM). In other cancers, the clotrimazole effect on glycolysis might be the result of cellular death [69–72] (Table 2; Fig. 1).

3.6 Other drugs affecting glucose metabolism

Antihormones are androgen (flutamide) and estrogen (tamoxifen) receptor agonist-analogs frequently used to stop growth in prostate and breast cancers. Tamoxifen is an estrogen receptor agonist used in the treatment of ovarian, endometrial, and breast cancers (Table 1) that also affects glucose metabolism through strong inhibition (K_i = 0.3–6.7 μM) of human erythrocyte GLUT1 (Table 2; Fig. 1). This drug interacts with four sequences of the cytosolic GLUT1 region that shows high homology with the binding sequences of the estrogen β-receptor [73, 74]. Effects of tamoxifen on glycolytic enzymes or fluxes in normal or tumor cells have not been evaluated yet.

Other anticancer drugs used in chemotherapy with effects on glucose metabolism are imatinib (gleevec) and lonidamine. Imatinib (Fig. 2) is used in the treatment of

chronic myelogenous leukemia (CML), and competitively inhibits the tyrosine kinase-fusion protein (BCR-ALB) involved in the phosphorylation of diverse signaling proteins (ras-GTPase, Raf, JNK, and c-Jun) required for tumor development [75]. This drug inhibits proliferation of human leukemia BCR-ABL-positive cell lines, CML-T1 (IC_{50} = 0.69 μM) and K562 (IC_{50} = 0.47 μM) (Table 1), but does not affect growth of BCR-ABL-negative cells [76, 77], and tumor cell lines that do not express BCR-ABL oncogene (MIA pancreatic adenocarcinoma cells: HC-1 leukemia cells). Submicromolar imatinib doses (0.1–1 μM) inhibit glucose consumption (25–77%) in K562 human leukemia and CML-T1 cells. In addition, in K562 leukemia, the drug diminishes HK activity (Table 2, Fig. 1), and glycolytic flux (25 and 65%, respectively) [76, 77].

Lonidamine is a derivate of indazole-3-carboxilic acid (Fig. 2) that inhibits growth of human and rodent fibrosarcomas at 0.1–0.4 mM [78] (Table 1). At lower doses (5, 75 μM), both mitochondrial membrane-bound (65%) and cytosolic HK (17%) activities diminish and, in consequence, glycolytic flux (Table 2; Fig. 1). At 90 μM, the drug reduces 50% cellular oxygen consumption [79]. In Ehrlich ascites tumor, lonidamine diminishes the activities of NADH, succinate, acylCoA, and α-glycerophosphate dehydrogenases, suggesting that its inhibitory effect on oxygen consumption is related with inactivation of several pathways, such as the Krebs cycle, respiratory chain, and β-oxidation [80]. Lonidamine enhances the cytotoxic effect of cisplatin, 4-hydroperoxycyclo phosphamide, melphalan, carmustine, doxorubicin, placlitaxel, epirubicin, and CP in human breast cancer MCF-7, human Lo Vo colon carcinoma, and in clinical trial phases I and III against ovarian cancer, early breast cancer, and glioblastoma multiforme [35, 81, 82], suggesting that parallel administration of energy metabolism inhibitors may improve efficacy of currently used antineoplastic drugs.

3.7 Selective glycolytic inhibitors with antitumoral activity

The 2-DOG is not a human anticancer-drug commonly used in clinical trials. Its structural analogy with glucose allows it to be recognized by glucose transporters and HK (and G6P dehydrogenase), but it inhibits HPI, thus diminishing glycolytic flux. In consequence, 2-DOG may block growth in presumably glycolysis-dependent tumors (Figs. 1 and 2; Table 1). The use of 2-DOG could be advantageous *versus* other glycolysis-targeted drugs because there seem to be no side effects at therapeutic doses [83], but this has not been clinically evaluated. However, because of its competitive nature, the 2-DOG concentrations required to diminish tumor growth are high (IC_{50} = 0.6–6 mM), due to the elevated exogenous glucose concentration usually present (5–25 mM) [61]. Interestingly, toxic effects of 2-DOG are accentuated in tumor cells with defects in mitochondrial metabolism and in cells exposed to hypoxia. For example, in mtDNA-lacking osteosarcoma (osteosarcoma ρ^0), 2-DOG results more toxic than in parental osteosarcoma (IC_{50} = 32 and 100 μM, respectively). In osteosarcoma nude mouse xenografts and nonsmall cell lung cancer, 2-DOG treatment (500 mg/kg weight) is ineffective to diminish tumor development [32], suggesting that these tumors depend rather on mitochondrial ATP.

In addition, 2-DOG increases two to four times the efficacy of therapeutic drugs, such as etoposide, camptothecin, or Hoechst-33342 in cerebral glioma BMG-1, squamous carcinomas 4451 and 4197, and malignant glioma U-87 cells [36]. The combination of 2-DOG with other antineoplastic drugs, such as adria or placlitaxel, diminishes the size and proliferation of human osteosarcoma, and mice-transplanted MV522 lung carcinoma in comparison with tumors treated with 2-DOG but without placlitaxel or adria [32]. This response has been observed also in etoposide-treated Ehrlich hepatoma-bearing mice, in which 2-DOG increases 11–22% the etoposide efficiency [84]. This increased sensitivity toward anticancer drugs induced by 2-DOG is attributed to the high glycolysis-dependence of the tumor for ATP supply. Recently, other glucose halogenated analogs derived from 2-DOG, such as 2-fluoro-2-deoxy-D-glucose, 2-chloro-2-deoxy-D-glucose, and 2-bromo-2-deoxy-D-glucose, have been administered successfully against some hypoxic tumor lines (osteosarcoma 143 B and ρ^0 wild type). Similarly to 2-DOG, the doses assayed of the analogs were in the millimolar range; toxicity of halogenated analogs on normal cells has not been assessed [85].

Gossypol is a polyphenolic component of cotton seeds used as male anticonceptive (Fig. 2). This drug inhibits NAD^+(P)-dependent enzymes, such as GAPDH and LDH, but may affect also some mitochondrial dehydrogenases. Gossypol affects other cellular functions associated with cellular proliferation [50], including Ca^{2+}-ATPase activity, glucose uptake, and induces changes in membrane fluidity [86–88]. Jaroszewski *et al.* [87] showed that gossypol (3.4 μM) inhibits 50% MCF-7 breast cancer growth; however, at 10 μM, the drug induces a higher glucose uptake and lactate production, suggesting glycolysis activation rather than inhibition induced by gossypol. At low doses (30 mg/kg), the drug reduces 65% the tumor size and induces tumor mortality (8%) in nude-mice SW-13 adrenocortical carcinoma [89]. In phase I trials, gossypol decreased glial and adrenal tumor size by 10–50% [90] (Table 1).

Glycolysis can be inhibited by other compounds, such as arsenite, iodoacetate, oxalate, and oxamate (Table 1). It is noted that the low efficacy of glycolytic inhibitors to stop tumor growth shown in several studies is also usually accompanied by severe side effects in the host [91–93]. Furthermore, the assumption that cancer cells have increased glucose utilization does not apply to all tumors [21, 22, 94–96].

4 Antineoplastic drugs targeting mitochondria

The Warburg hypothesis that cancer cells have an enhanced glycolysis and damaged mitochondria [1] have led to the general belief that ATP is mainly or only provided by glycolysis [2 – 14], which; however, has not usually been accompanied by experimental evaluation. Increased glycolysis in cancer cells might certainly be the result of impaired mitochondrial respiration induced by mtDNA mutations (prostate cancer, breast cancer, gastric cancer, and leukemia) or by the presence of damaged respiratory complexes (renal cell carcinomas) [61, 97 – 100].

On the other hand, it is frequently encountered that tumors with low glycolytic activity, such as adenomas, bronchioloalveolar carcinomas, mucin-induced low cellular density of metastatic tumors, carcinoid tumors, low grade lymphomas, small sized tumors, and thyroid carcinoma, yield false negative results on the FDG/PET scan [101, 102]. Both aerobic and anaerobic tumor cells may metabolize FDG. However, accumulation of FDG in malignant tumors is related mainly to regional hypoxia because of the lower capacity (50%) of aerobic cells to consume FDG as compared with hypoxic cells [103]. However, because other factors affecting FDG uptake might be more predominant in chronic hypoxia, a poor correlation between hypoxia and FDG uptake can appear. Therefore, FDG/PET cannot reliably differentiate hypoxic (*i.e.*, glycolytic) from normoxic tumors in frequently hypoxic (head and neck cancer and glioblastoma multiforme) and less frequently hypoxic tumors (breast cancers) [104].

Mitochondria are the main organelles supplying cellular ATP in highly aerobic tissues, such as heart and brain [105]. In turn, it has been documented that, in tumor cells, ATP production may derive exclusively from glycolysis (rat glioma C6, human medulloblastoma, CT-26, and LoVo colon tumors), OxPhos (human sarcoma, HeLa carcinoma, AS-30D, and Reuber H-35 hepatomas), or both (human glioblastoma, astrocytoma C6, and breast MCF-7 carcinoma) energy pathways [21]. In some tumor cells (MCF-7 carcinoma, Walker 256), the sole application of glycolytic drugs, such as 2-DOG or 3-mercaptopicolinic acid (3MPA), does not significantly decrease tumor progression [106, 107]. However, when glycolytic inhibitors (2-DOG, 0.3 mM or 0.5 g/kg; 3MPA, 40 mg/kg weight) are combined with antimitochondrial drugs (rhodamine 123, 1.3 μM or 15 mg/kg; rhodamine 6G, 0.8 mg/kg), the proliferation rate is drastically decreased (human MCF-7 carcinoma) or tumor size is significantly reduced (Ehrlich- and Walker 256-bearing rodents) [83, 106, 107]. These observations indicate that mitochondria may contribute to sustain tumor development and, therefore, they acquire clinical importance as a target for antineoplastic therapy.

On the other hand, mitochondria are also involved in apoptosis, a common process required for the normal maturation of individual organs, embryogenesis, and metamorphosis [108]. Mitochondria are the link between proapoptotic mediators (*i.e.*, cytochrome *c*, Smac/Diablo, TRAIL, Bcl-2, AIF) and apoptotic-effectors (*i.e.*, executer caspases). Therefore, mitochondrial alterations in cancer cells are also associated with the development of apoptosis-resistance mechanisms [108 – 110].

5 Mitochondrial inhibitors and uncouplers as antineoplastic drugs

5.1 Delocalized lipophilic cations (DLCs)

Mitochondria are unique organelles in the sense that they have an alkaline ($pH \geq 7.5$) and highly negative charged (up to 180 mV) internal matrix [111], conditions that favor the accumulation of lipophilic cations (Fig. 3). Lipophilic cations carrying a delocalized positive charge can readily permeate plasma and inner mitochondrial membranes. Once inside, they concentrate within the lipid boundary in response to the membrane potential (negative inside). This mechanism may be amplified by the unspecific binding of the DLC to membrane phospholipids and cellular proteins [112, 113].

Moreover, mitochondria from human colon CX-1 tumor [114] cells and mitochondria from MCF-7 breast adenocarcinoma [115], as well as *neu*-, *v-Ha-ras*-, β-*catenin*, and *c-myc*-initiated mouse tumor cell lines [116], develop a mitochondrial membrane potential of higher magnitude than that of normal cells (epithelial cells from spleen, breast, and kidney). According with the Nernst equation, an increase in 60 mV in membrane potential promotes a tenfold increase in accumulation of membrane-permeable cations, whereas a slightly (10 mV) higher membrane potential promotes a 68% higher cation accumulation [117]. The biochemical bases of the difference in the higher mitochondrial membrane potential in tumor cells are unknown, but higher contents of cardiolipin (increasing the density of membrane negative charges) and cholesterol (decreasing the passive diffusion of protons across the membranes) in the tumor plasma and inner mitochondrial membranes could facilitate the DLC uptake and accumulation [2, 117]. In osteosarcoma cells lacking mtDNA (ρ^0) and, hence, lacking respiratory chain and the ability to generate a membrane potential, 50 times more rhodamine 123 is required to inhibit proliferation [118]. Therefore, DLC preferentially accumulate and remain for longer periods in tumor cells than in normal tissues [115], promoting inhibition of mitochondrial function. Difference in DLC retention between normal and tumor cells might originate from a higher activity of the multidrug resistance transport protein, glycoprotein type P (P-gly), in normal cells. However, tumor cells (prostate carcinoma, ovarian carcinoma) [119, 120] express higher activity of P-gly in comparison with nontumorigenic cells (brain cells, liver), suggesting that the rate of DLC uptake and their

Table 3. Effects of DLCs on cellular metabolism

Drug	Cellular type	Cellular effect	Energy-metabolism effect	Doses assayed	Reference
AA-1	Human colon carcinoma (CX-1)	Diminiution on proliferation rate Increment of median mice-survival 41–55 days	Not assayed	IC_{50} = 0.075 μg/mL 3–5 mg/kg	Sun *et al.* 1994 [133]
	MB49-bladder bearing mice, human melanoma (LOX,OVCAR-III) Rat liver mitochondria		Inhibition of coupled-respiration ATP hydrolysis	IC_{50} = 25, 20 μM	
F16	*neu*-initiated mouse tumor; *V-Ha-ras* initiated tumor cell line, human breast cancer (MDA-MB, MCF-7)	Cell cycle arrest Nuclear DNA fragmentation	Not assayed	3 μM for 36 h	Fantin *et al.* 2002 [116]
	Epithelium A6 cells	Mitochondrial abnormalities mitochondrial swelling	ATP depletion	3 μM for 15–24 h	
FJ5002	Human leukemia (U937)	Telomerase inhibitor	Not assayed	50–200 nM for 90 days	Naasani *et al.* 1999 [124]
MKT-077	Human colon cancer (CX1), human breast cancer (MCF-7), human pancreatic cancer (CRL1420), human bladder cancer EJ, human melanoma (LOX), human renal carcinoma, A-498 bearing nude mice	Inhibition of colonies formation Diminution of mean tumor weight	Not assayed	2.3 μM for 24 h 7.5 mg/kg i.v. each 48 h for 1 wk	Koya *et al.* 1996 [39]
	Cx-1 colon mitochondria	Inhibition of ADP-stimulated respiration	–	10 μg/mg protein	Weisberg *et al.* 1996 [126]
	Rat liver mitochondria	mtDNA fragmentation	Inhibition of ADP-stimulated respiration, OxPhos uncoupling	15 mg/kg for 5 + 3 days recovery	Weisberg *et al.* 1996 [126]
	Rat liver mitochondria	mtDNA diminution	Cytochrome *c* oxidase and succinate-cytochrome *c* reductase inhibitors	Drug-photoactivation with 7.5 J/cm^2 plus 11–34 μM	Modica-Napolitano *et al* 1998 [127]
Rhodac	HeLa; HT29 colon; A431 skin	ROS formation	Not assayed	7–10 μM	Delaey *et al.* 2001 [123]
Rhod 6G	Zajdela hepatoma cells	Inhibition of cytochrome c1 and b–c1 complex precursor	Not assayed	1.3 μg/mg protein	Kolarov *et al.* 1984 [183]
	Rat liver mitochondria		Inhibition of ANT and ATP-supported calcium accumulation	3 μM	Gear 1974 [129]
Rhod 6G + 3-MPA	Walker 256-bearing rats	Reduction by 50% tumor weight	Not assayed	24 h -starved rats, rhod 6G 0.8 mg/kg + 3MPA 40 mg/kg	Fearon *et al.* 1987 [107]
Rhod 123	CRL 1420 pancreatic MCF-7 breast EJ bladder CCL 77 Ascites	Induction of 50% cell death	Not assayed	131 μM for 48 h	Lampidis *et al.* 1983 [106]
	Rat liver mitochondria		Inhibition of F_0–F_1-ATPase, adenine nucleotide translocase	13–53 μM	Modica-Napolitano *et al.* 1984 [136]
Rhod 123 + 2-DOG	Ehrlich ascites and MB49 bladder carcinoma-bearing mice	Increase by 250–420% mice survival	Not assayed	Rhod123 15 mg/kg + 2-DOG: 0.5 g/kg, both three times at week	Bernal *et al.* 1983 [83]
	MCF-7 breast	Total Inhibition of clonogenic survival	Not assayed	Rhod 123, 1.3 μM + 2-DOG, 300 μM[a)]	Lampidis *et al.* 1983 [106]
CCCP	Human breast cancer (EMT-6) Human bladder cancer (MGH-U1)	Cell survival diminution by, increasing intracellular acidification (pH 6.1)	Inhibition of glycolysis and glucose uptake ATP levels reduction	5–15 μM for 6 h	Newell and Tannock 1989 [141]

Rhod, rhodamine; human carcinomas: Cx1; HT29; MB49; EMT-6, MGH-U1;MDA-MB; MCF-7; CRL1420; EJ; A-498. Rodent carcinomas: Walker-256; CCL 77.

a) Assays were performed in D-MEM medium which contains at least 25 mM glucose.

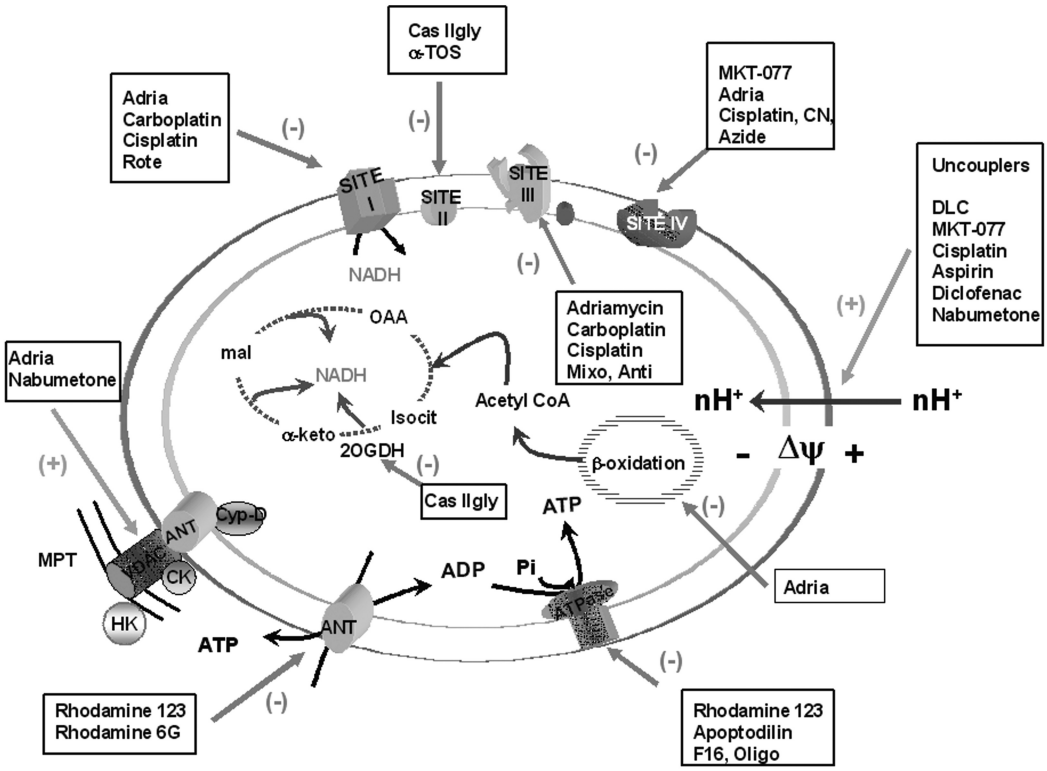

Figure 4. OxPhos enzyme targeting by anticancer drugs. Isocit, isocitrate; mal, malate; OAA, oxaloacetate, α-TOS; ANT, adenine nucleotide translocator; CK, creatine kinase; Cyp-D cyclophilin D; VDAC, voltage-dependent anion channel. Adria and nabumetone induce mitochondrial permeability transition (MPT), which is represented with positive sign and green arrow. Typical and highly membrane-permeable inhibitors of OxPhosp are also illustrated: rotenone (Rote), antimycin, myxothiazol (Myxo), cyanide (CN), azide and oligomycin (Oligo).

binding to anionic sites inside tumor cells and mitochondria are actually faster than the P-gly activity [121, 122].

Table 3 shows different DLCs used as anticancer drugs in several tumor types and their effect on energy metabolism. Rhodacyanines are a DLC family that includes MKT-077 (1-ethyl-2-([3-ethylbenzothiazolin-2-yliden]-4-oxothiazolidin-2-ylidenemethyl) pyridium) [39], the photosensitizer rhodac [123], and the FJ5002 dye [124] (Table 3). MKT-077 (Fig. 3) has been used in phase I clinic trials with encouraging results in advanced solid malignancies [125]. After 5 days of treatment, MKT-077 works as a respiratory inhibitor inducing severe inhibition (60%) of mitochondrial state 3 (ADP-stimulated) respiration. However, after a long recovery period (3 days), this drug functions as uncoupler stimulating mitochondrial respiration by 40% [126]. High-intensity light (photo-activation) increases the MKT-077 toxic effect on mitochondria. For example, low drug doses (11 or 34 μM), in the presence of intense light, inhibit four and six-fold the cytochrome *c* oxidase and succinate-cytochrome *c* reductase activities, respectively (Fig. 4) [127].

Other DLC with high capacity to concentrate in cytosol and mitochondrial matrix are the rhodamine derivatives (123, 123-platinum complex, and 6G) [115, 128 – 130], F16 [116], dequalinium chloride [131], *N*′-bis(2-ethyl-1,3-dioxolane)-krytocyanine [132], AA1 (2,6-bis(4-aminophenyl)-4-[4-(dimethylamino)phenyl] thiopyrylium chloride) [133], and the photosensitizer chalcogenapyrylium dye 8b (Fig. 2) [134]. Moreover, rhodamine dyes are used as specific optical probes to monitor changes in mitochondrial membrane potential. Rhodamine 123 seems highly selective for tumor cells due to its high accumulation and retention efficiency (2 – 5 days) in adenocarcinomas, epidermoid, and squamous cancers as compared with normal cells (16 – 24 h) [135]. Both rhodamines, 123 and 6G, affect mitochondrial functions [50] and, hence, cellular ATP levels, *via* strong inhibition of key OxPhos enzymes (Table 3; Fig. 4) [131, 135, 136]. Rhodamine 123, in combination with 2-DOG, induces a greater diminution of tumor growth *in vitro*, thus increasing the survival of tumor bearing-animals, than when using each drug separately (Table 3) [83, 107].

Rhodamine 6G (Fig. 3) has greater inhibitory potency on tumor cell growth *in vitro* than rhodamine 123 [107] and, therefore, it has been assayed in *in vivo* models in combination with 3MPA, an inhibitor of host gluconeogenesis (Table 3). In Walker 256 tumor-bearing rats treated with low doses of rhodamine 6G plus 3MPA, the size, and weight

of Walker tumor decreases 50%. Hypoglycemia or rhodamine 6G alone also causes significant inhibition of tumor growth, suggesting dependence on both ATP-generating pathways [107]. F16 inhibits growth of *neu*-overexpressing cells and *v-Ha-ras* -initiated tumor cells at low doses (3 μM) (Fig. 3; Table 3), and induces nuclear DNA fragmentation in breast tumor cells [116]. These F16 effects might be related to their preferential accumulation in tumor mitochondria [137].

Other DLC tested, by virtue of their uncoupling effect (*i.e.*, collapsing mitochondrial membrane potential), are 1,4-anthracenediones in MDR-HL60 cells [138], dequalinium chloride in carcinoma and sarcoma cells [139], AA1 in CX1- and CV-1 carcinoma cells [133], and tetrahydrocannabinol in pulmonary transformed A549 cell line [140] (Figs. 3 and 4; Table 3).

5.2 Effect of typical OxPhos uncouplers on tumor development

The proton ionophore carbonyl cyanide-3-chlorophenylhydrazone (CCCP), a classical uncoupler of OxPhos, is a potent inhibitor of EMT-6 and M6H-U1 tumor growth. The ionophore mechanism of action consists in the acidification of the mitochondrial matrix and collapse of the mitochondrial electrochemical membrane potential, resulting in a significant decrease in cellular ATP levels (Fig. 4; Table 3) [141]. Similarly to tetrahydrocannabinol, the uncoupler carbonyl cyanide-*p*-(trifluoromethoxy)-phenyl hydrazone (FCCP) also induces apoptosis and cellular cycle arrest in G2-M in human epithelial cells [142]. In prostate cancer (PC3), CCCP (3 μM) in combination with the ovulation-inducer agent, clomiphene, significantly decreases cell viability [143]. Unfortunately, these typical uncouplers seem to be not specific for tumor cells, as they also affect mitochondrial functions in normal cells at similar doses.

6 Antineoplastic-drugs acting as inhibitors of oxphos enzymes

In perfused rat heart, adria accumulates mainly in mitochondria and nuclei. In mitochondria, adria binds to cardiolipin with high affinity (80-times more than to phosphatidic acid) forming local nonbilayer arrangements between the two mitochondrial membranes [65]. These unusual arrangements affect membrane fluidity and functionality of mitochondrial membrane enzymes, such as NADH oxidoreductase and cytochrome *c* oxidase (Fig. 4, Table 4) [64, 65]. Other well-documented toxic effects of adria on mitochondria include (i) disturbance of mitochondrial Ca^{2+} homeostasis [144], (ii) complex formation with heavy metal ions, such as Fe (III) [145], (iii) free radicals production (Table 4) [65].

Ditercalinium or 7H-pyridocarbazole dimer (bisintercalator), belongs to a new class of antineoplastic intercalating agents and is currently being applied in phase I clinic trials [146]. Low doses of ditercalinium (0.25 – 0.5 μM for 24 h) arrest the cell cycle of mice lymphocytic leukemia L1210 cells [147]. Unfortunately, healthy cells are also susceptible to drug toxicity at the same doses [148]. Recently, it has been described that this compound alters mitochondrial morphology causing selective degradation of mtDNA in hamster lung fibroblast and HeLa tumor cells (Table 4) [148, 149].

Some metal-based drugs, such as cisplatin, oxaliplatin, and casiopeinas, are DNA-intercalating agents that also inhibit mitochondrial functions. Oxidative damage has been proposed as a mechanism of cisplatin-induced renal cell death [150]. Cisplatin and its second generation derivative, oxaliplatinum, decrease mitochondrial membrane potential and respiratory complex I to IV activities (Table 4) at doses assayed to arrest tumor proliferation (Table 1), suggesting that mitochondria are the main targets to induce cellular death. Inhibition of mitochondrial activity by cisplatin promotes a severe diminution of the cellular ATP content (>50%), increasing ROS, lowering mitochondrial Ca^{2+} uptake [150, 151], and, in consequence, decreasing cellular viability [152]; glycolytic enzymes were not affected by these drugs [152].

At low concentration (3 nmol/mg protein), casiopeinas (Figs. 3 and 4), particularly Cas-IIgly, diminish mitochondrial function by inhibiting succinate, 2-oxoglutarate, and pyruvate dehydrogenases in tumor and liver mitochondria [51], and tumor cells [50] (Table 4). Besides, Cas-IIgly induces apoptosis by increasing the release of cytochrome *c* [51]. Cas-IIgly at high doses (≥15 nmol/mg protein) induces uncoupling (perhaps by acting as DLC) followed by potent respiratory inhibition. It promotes also mitochondrial swelling through stimulation of the ATP-dependent K^+ channel and, consequently, cytochrome *c* release and apoptosis.

Apoptolidin and its analogs selectively sensitize cancer cells to proceed toward apoptosis [153, 154]. These macrocyclic polyketide drugs have been successfully used at micromolar doses (0.8 – 8 μM) on many human cancers, such as leukemia (HL-50, MOLT-4, and CCRF-CEM); lung cancers (A549, HOP-62, and NCl-H226); colon cancers (COLO205, HT29, and SW-620) central nervous system cancers (SNB-19, U251); melanomas; ovarian cancers (OVCAR-3, – 5, – 8); renal cancers (CAKl-1, UO-31, PC-3); prostate cancer (DU-145); and breast cancers (MCF-7; MDA-MB, T-47D).

Apoptolidin at very low doses is a strong inhibitor of F_0–F_1-ATPase in leukemia cells (Fig. 4; Table 4) [153, 154]. Apoptolidin shows effects on tumor cells similar to those of some polyketide inhibitors (ossamycin and oligomycin), which have high structural similitude [153]. Therefore, it has been hypothesized that tumor proliferation arrest is a consequence of F_0–F_1-ATPase inhibition. However, potent ATPase inhibition with the drug does not significantly modify the proliferation of E1A-transformed rat fibroblasts,

Table 4. Effect of anticancer drugs on oxidative phosphorylation

Drug	Source	Mechanism	Effect	Doses assayed	Reference
Adria	Rat liver, rat heart, and bovine heart	Cardiolipin-interaction	Inhibition of complex I, III and IV	IC50 = 175–450 μM	Nicolay and de Kruijff 1987 [64]
	Submitochondrial particles	Formation of semiquinone radicals	Inhibition of complex I	25–50 μM by 15 min-1 h	Marcillat *et al* 1989 [184]
	Rat heart mitochondria	SH groups oxidation	Calcium efflux through activation of membrane permeability transition	Rat treated with 2 mg/kg weight for 1–9 wk	Solem *et al.* 1996 [144]
	Rat heart cells	Formation of Adria-Fe(III) complex	Abnormal cell contractility and Rythmicity. Decrease in palmitate utilization	Rat treated with 4–8 mg/kg weight on day 0 and 7	Link *et al.* 1996 [145]
Apoptolidin	Yeast mitochondria Triton X-100-solubilized ATPase	Inhibition of F_0–F_1 ATPase	Inhibition of OxPhos	Ki = 4–5 μM	Salomon *et al.* 2001 [154]
Casio Ilgly	Liver, kidney, heart, and AS-30D hepatoma mitochondria	2-OGDH activity cytochrome *c* release SDH activity	Inhibition of OxPhos	3–8 nmol/mg prot 25 nmol/mg prot 250 nmol/mg prot	Marín-Hernández *et al.* 2003 [51]
	HeLa and AS-30D hepatoma cells in culture	Inhibition of mitochondrial enzymes	Inhibition of OxPhos	1–10 μM for 24–48 h (AS-30D) and 72–96 h (HeLa)	Rodríguez-Enríquez *et al.* 2006 [50]
Cisplatin	Kidney pig mitochondria	Inhibition of complex I to IV; diminution on Dy	Inhibition of OxPhos; and low viability and ATP content	50 μM for 20 min	Kruidering *et al.* 1997 [152]
Ditercalinium	Lung fibroblast	Mitochondrial DNA degradation	Ultrastructural modifications in mitochondria including cristae loss	1.4 μM for 24–72 h	Segal-Bendirdjian *et al.* 1988 [148]
F16	Rat liver mitochondria	Inhibition of F_0–F_1 ATPase	Inhibition of OxPhos	IC_{50} = 6 μM	Fantin *et al.* 2002 [116]
NSAIDS					
Aspirin, diclofenac	Rat perfused liver; rat liver mitochondria	OxPhos uncoupler	–	1–10 mM 10–100 μM	Petrescu *et al.* 1997 [185]
Diclofenac, Naproxen, Nimesulide, Meloxicam, Nabumetone	AS-30D isolated cells, rat liver mitochondria	–	Inhibition of OxPhos	17–900 nmol/mg protein	Moreno-Sanchez *et al.* 1999 [167]
Diclofenac	Ganglioneuroma, neuroblastoma cells Rat bearing-neuroblastoma xenografts	OxPhos uncoupler	85–90% abolishment tumor development	0.4 mM for 24 h 200–250 mg/L for 6–7 days	Johnsen*et al.* 2004 [164]
Nabumetone	Rat liver mitochondria Liver carcinoma HepG2	MPT inducer; Ca^{2+} efflux inducer; OxPhos uncoupler; MPT inducer,	Inhibition OxPhosp Cell death, OxPhos inhibitor	5–50 μM	Freitas *et al.* 2007 [168]

2-OGDH, 2-oxoglutarate dehydrogenase; SDH, succinate dehydrogenase; and MPT, mitochondrial membrane permeability transition.

suggesting a secondary biological apoptodilin-target or that glycolysis predominates in the ATP supply for E1A- fibroblast growth. Encouragingly, no effect of apoptodilin on normal tissues at tumor toxic levels is attained [154].

Another potent anticancer drug of recent development is α-tocopheryl succinate (α-TOS), a redox-silent vitamin E derivative, which has shown high efficacy against various malignant tumor cells, such as mesothelioma, neuroblastoma, and prostate cancer while being largely nontoxic to normal cells [155–162]. This drug specifically targets mitochondria inhibiting respiratory chain complex II by blocking ubiquinone binding (Fig. 4). Impaired respiratory chain generates ROS, which promote oxidative stress, trigger mitochondrial destabilization, and promote apoptosis [156]; effects of α-TOS on cytochrome bc1 complex, another ubiquinone-using enzyme, or on other respiratory complexes have not yet been examined. α-TOS apparently targets cancer cells because normal cells have stronger antioxidant defense mechanisms [159, 161].

Several nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, indomethacin, sulindac, nimesulide, and diclofenac inhibit cyclooxygenase activity and reduce tumor progression of human colorectal cancer, neuroblastomas, and nonsmall lung cancer through apoptosis induction [163–165] or by increasing sensitivity to radiotherapy [166]. NSAIDs also affects OxPhos in normal and tumor cells, and mitochondria [164, 167–169] (Table 4) acting as OxPhos uncouplers and inhibitors (Fig. 4). Tumor cells and mitochondria show higher NSAIDs retention capacity than normal cells [170]. It is noted that the NSAID chemical structures reveal some similarity with DLC structures. Therefore, NSAIDs use as effective anticancer drugs could

be related with the greater capacity of tumor mitochondria to accumulate preferentially these drugs than mitochondria of normal cells (Fig. 3).

Other drugs, currently under study in phase I, II, or, III clinical trials as adjuvant chemotherapy that affect mitochondrial metabolism are 5-fluorouracil, gemcitabine, paclitaxel, tirapazamine, diphenylamine, and sulfonylurea-based compounds. It has been documented that all of them induce apoptosis and cellular death. Gemcitabine, 5-fluoracil, and paclitaxel generate ROS and, in consequence, a drastic loss of mitochondrial membrane potential [171 – 173]. On the other hand, tirapazamine, diphenylamine, and sulfonylurea-based compounds act as OxPhos uncouplers [174, 175].

7 Concluding remarks

Each particular type of cancer shows different DNA mutations and perturbations in its intermediary metabolism (cancer cells are genetically and phenotypically heterogeneous from line to line). However, the ATP supply continues to depend solely on glycolysis and OxPhos in all cancer types. Therefore, selective inhibition of prevalent tumor energy metabolism (metabolic therapy) may have significant impact on cancer treatment. It can be argued that such a treatment may also severely affect host energy metabolism. In fact, some of the secondary side-effects of currently used antineoplastic drugs may derive from inhibition of energy metabolism in healthy cells, as analyzed in the present work. However, the increased glycolysis in cancer cells together with redistribution of flux control for both glycolysis [18] and OxPhos [15] in fast-growth tumor cells are biochemical characteristics that may favor the selective application of energy metabolism-affecting drugs.

Current chemotherapy 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 to be able to provide long-term management of malignant brain tumors while permitting a better quality of life. Metabolic therapy searches for physical and biochemical differences between tumor and nontumor intermediate metabolism to facilitate the design of drugs that preferentially affect tumor metabolism and growth, without altering drastically the host tissue and organ functionality. Metabolic therapy could complement the existent chemotherapeutic treatments, so that, in combination, they might successfully stop tumor growth, invasiveness, and drug resistance.

Certainly, drug efficacy, delivery, and side-effects are problems to be solved in developing new chemotherapies. In solid tumors and in multicellular tumor spheroids, delivery to a hypoxic region may be difficult because the drug does not easily permeate through the different cellular layers. Indeed, diffusion of hydrophobic and hydrophilic molecules (drugs, antibodies, dyes, *etc*.) from the periphery to the center of a tumor is really low [176]. Therefore, drug inaccessibility accounts mainly for the relative inactivity of these drugs on the inner cellular layers. Besides, P-gly activity is higher in tumor spheroids as compared with monolayer-cultured tumor cells, which may promote a rapid expulsion of chemotherapy drugs, thus contributing to the drug-resistance observed in solid tumors [119, 120]. 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 up to now, 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 OxPhos is shared by several steps [15, 18, 177, 178]. It appears more rational for drug design to gather information by applying the metabolic control analysis [179, 180], which allows the quantitative identification of the main controlling steps in a pathway, along with providing an understanding of the underlying regulatory mechanisms and facilitating the prediction of the system behavior.

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