

Canonical and new generation anticancer drugs also target energy metabolism

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Abstract Significant efforts have been made for the development of new anticancer drugs (protein kinase or proteasome inhibitors, monoclonal humanized antibodies) with presumably low or negligible side effects and high specificity. However, an in-depth analysis of the side effects of several currently used canonical (platin-based drugs, taxanes, anthracyclines, etoposides, antimetabolites) and new generation anticancer drugs as the first line of clinical treatment reveals significant perturbation of glycolysis and oxidative phosphorylation. Canonical and new generation drug side effects include decreased (1) intracellular ATP levels, (2) glycolytic/mitochondrial enzyme/transporter activities and/or (3) mitochondrial electrical membrane potentials. Furthermore, the anti-proliferative effects of these drugs are markedly attenuated in tumor rho (0) cells, in which functional mitochondria are absent; in addition, several anticancer drugs directly interact with isolated mitochondria affecting their functions. Therefore, several anticancer

drugs also target the energy metabolism, and hence, the documented inhibitory effect of anticancer drugs on cancer growth should also be linked to the blocking of ATP supply pathways. These often overlooked effects of canonical and new generation anticancer drugs emphasize the role of energy metabolism in maintaining cancer cells viable and its targeting as a complementary and successful strategy for cancer treatment.

Keywords Energy metabolism · Humanized monoclonal antibodies · Metabolic therapy · Protein kinase inhibitors · Tumor cells

Abbreviations

ETC Electron transport chain
OxPhos Oxidative phosphorylation
2-OGDH 2-Oxoglutarate dehydrogenase complex

Introduction

Despite considerable advances in biomedical research and clinical applications in the field, cancer remains a leading cause of mortality worldwide. The severe side effects caused by the frequent use of conventional chemotherapeutics diminish, unfortunately, the patients' quality of life. Therefore, the development of safe and efficacious alternatives with well-known mechanism of action is mandatory. First-line anticancer drugs such as antibody–drug conjugates and protein kinase (PK) small-molecule inhibitors have become widely used in the clinical treatment of cancer presumably because they are designed to be selective for one protein or cellular process (Ott and Adams 2011; Li et al. 2013; Miller et al. 2013). However, as occurs with other canonical and traditional chemotherapy drugs

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(platin-based drugs, taxanes, anthracyclines, etoposides, antimetabolites), these new anticancer drugs also have multiple side effects, disturbing important cellular processes present in normal cells, at the doses used during cancer treatment (Hansel et al. 2010; Mellor et al. 2011; Baldo 2013). Thus, for a more comprehensive understanding of the global inhibitory effects of these drugs on cancer and non-cancer cells, an integral functional analysis of the additive or synergistic drug effect on both the protein or pathway for which it was designed and the multiple non-specific inhibition of a priori untargeted pathways or proteins should be undertaken.

In this regard, it has been demonstrated that several cellular processes such as cellular proliferation and survival, apoptosis, endothelial permeability, vascular remodeling and ion channel homeostasis are affected by low doses of monoclonal antibodies, antibody–drug conjugates and PK small-molecule inhibitors (Mellor et al. 2011; Ott and Adams 2011; Baldo 2013). Certainly monoclonal antibodies (mAbs) and PK inhibitors interact directly with their specific receptor- or kinase target. However, structural analysis, performed for instance with the tyrosine kinase (TK) inhibitor imatinib, shows that the drug also interacts directly with other non-kinase proteins in non-tumor cells such as the yeast vacuolar-type H^+ -ATPase and the human NAD(P)H dehydrogenase quinone oxidoreductase 2 (NQO2) (reviewed in Lee and Wang 2009). Although mAbs bind to their specific extracellular receptors, it has been demonstrated that some mAbs may be transported across the plasma membrane and affect intracellular targets (Dao et al. 2013). In addition, TK inhibitors and mAbs may block non-specifically other transduction pathways such as those mediated by AMPK, platelet-derived growth factor receptor (PDGFR), PI3K-mTOR and MAPK (Mellor et al. 2011; Ott and Adams 2011).

In particular, energy metabolism appears to be highly sensitive to clinical doses of several antineoplastic drugs (reviewed in Rodríguez-Enríquez et al. 2009). This observation becomes relevant by taking into consideration several cellular processes occurring in tumor cells (proliferation, metastasis, macromolecules synthesis, stress-defense, ATPases activation) and non-tumor cells that depend on the intracellular ATP concentration which in turn depend on the ATP production rate by both glycolysis and oxidative phosphorylation (OxPhos) (Müller et al. 1986; Schmidt et al. 1991; Rolfe and Brown 1997). Thus, despite the well-documented genetic and biochemical heterogeneity of cancer cells, it has to be recalled that they all depend on glycolysis and OxPhos for the ATP supply. The downside of targeting ATP supply in cancer cells is that this same function is also essential for non-tumor cells, and hence, this approach becomes extremely toxic. However, this strategy may still be particularly relevant for cancer cells

as they demand higher ATP provisions for their accelerated cell proliferation and associated processes (Schmidt et al. 1991), i.e., cancer cells are more susceptible to variations in their ATP supply than non-tumor cells. This central and essential role of the ATP supply has no parallel in other metabolic and signal transduction pathways. In those, the perturbations trigger compensatory mechanisms by turning on and off other interlinked pathways. In contrast, for cellular ATP supply, there are no alternative compensatory ATP-producing pathways. The use of multi-action drugs (acting for instance on their specific targets and on energy metabolism) or a combination of drugs might become an asset in cancer chemotherapy once their modes of action are understood.

Energy metabolism alterations in solid tumors

Oxidative phosphorylation flux and metabolite levels

Oxidative phosphorylation (OxPhos) is the principal ATP producer (>90 %) for several malignant tumor cell types under normoxic conditions (i.e., 21 % atmospheric O_2) (Zu and Guppy 2004; Rodríguez-Enríquez et al. 2010). Therefore, drug therapy targeting OxPhos has emerged as an important alternative for growth arrest of oxidative type tumors (reviewed in Moreno-Sánchez et al. 2007; Rodríguez-Enríquez et al. 2011). However, in solid tumors (Fig. 1), the development of unorganized, fragile and leaky vessels promotes intermittent, rather than constant, blood flow and the onset of episodes of hypoxia (from 31 to 86 μM dissolved O_2 or 24–66 mmHg under normoxia to 3–13 μM dissolved O_2 or 0.1–1 % atmospheric O_2 under hypoxia; Vaupel et al. 1989; Höckel and Vaupel 2001) and low-carbon source supply (for instance, extracellular

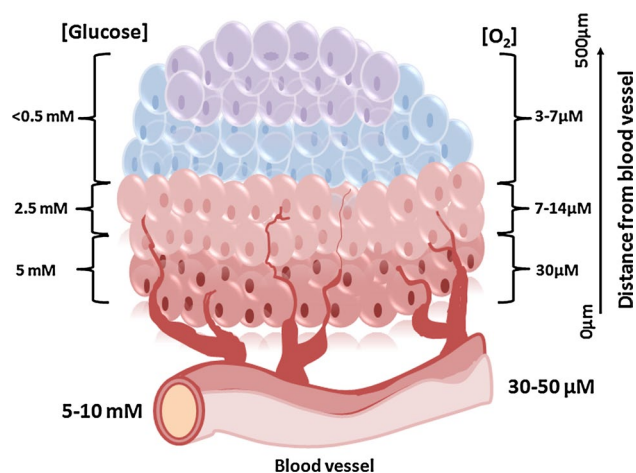


Fig. 1 Gradients of glucose and O_2 in solid tumors

glucose decreases from 5–10 to 0.5–2.5 mM; Tamulevicius and Streffer 1995; Hirayama et al. 2009) in regions distant from the blood vessels. The transient or permanent shortage in O₂ and carbon sources may, directly and indirectly, affect OxPhos machinery and induce an energy metabolic shift toward a greater dependence on glycolysis (Sonveaux et al. 2008; Hirschhaeuser et al. 2011; Mathews et al. 2011).

Long-term (0.1–2 % atmospheric O₂, 18–24 h) hypoxia affects OxPhos in HeLa (cervix), MCF-7 (breast), HCT116 and RKO (colon) and RCC4 (renal) cancer cells at different levels of regulation inducing diminution of (1) the contents and activities of the 2OGDH and GA (Rodríguez-Enríquez et al. 2010) and (2) the assembly of the respiratory chain complexes I and IV which in turn influences their enzymatic activities (Chen et al. 2010; Tello et al. 2011). Although OxPhos flux (Rodríguez-Enríquez et al. 2011; reviewed in Moreno-Sánchez et al. 2014) and total O₂ consumption (Cairns et al. 2007; Chen et al. 2010; Tello et al. 2011) severely diminish (80–90 %) in cancer cells under long-term hypoxia, cellular viability remains high at 70–80 %. This phenomenon occurs perhaps because rescue pathways are activated (i.e., increased glycolysis, decreased ATP-dependent processes) and cellular proliferation decreases, thus maintaining the intracellular ATP pool constant and high. It has been documented that hypoxia (0.05 % atmospheric O₂) and low glucose (1 mM) up-regulate the expression of the mitochondrial proline oxidase (POX) for glutamate and 2OG production. However, it has not been determined whether under hypoxia and low glucose, proline oxidation may support OxPhos flux and in turn help to preserve the high cell viability observed under this stress condition (Liu and Phang 2012; Liu et al. 2012).

HIF-1 α stabilization is reached under normoxia in paragangliomas, pheochromocytomas and renal cell cancer in a process mediated by mutations in the Krebs cycle fumarate hydratase or succinate dehydrogenase. The mutant enzymes become inactive promoting the accumulation of succinate and fumarate (Isaacs et al. 2005; Pollard et al. 2005; Selak et al. 2005). These two metabolites competitively inhibit prolyl-hydroxylase (PHD) activity preventing HIF-1 α hydroxylation and its proteasomal degradation (Isaacs et al. 2005; Selak et al. 2005).

OxPhos also plays an important role in 3D physiological models such as multi-cellular tumor spheroids (MCTSs, Kunz-Schughart 1999; Rodríguez-Enríquez et al. 2008) and tumor xenotransplants (Menon et al. 2003; Yaromina et al. 2012). The MCTSs resemble the physiologically heterogeneous architecture (hypoxic and normoxic micro-areas) found in the initial avascular stages of solid tumors (Sutherland 1988; Kunz-Schughart et al. 2004). These micro-areas generate O₂ (1–120 mmHg) and glucose (3 to >21 μ mol/g of cellular protein) gradients between the inner and outer

cell layers, which cannot occur in the extensively used monolayer or bi-dimensional culture model (Hirschhaeuser et al. 2010). Thus, the 3D tumor spheroid architecture induces transcriptional and biochemical changes in the energy metabolism and in consequence changes in the sensitivity to metabolic inhibitors (Rodríguez-Enríquez et al. 2008; Mandujano-Tinoco et al. 2013), metastasis onset (Gallardo-Pérez et al. 2014) and the development of anti-apoptotic pathways (Barbone et al. 2012).

It has been reported that HeLa, Hek293 and MCF-7 MCTSs strongly depend (>80 %) on mitochondrial ATP for their growth. Therefore, proliferation is highly sensitive to OxPhos inhibitors (Rodríguez-Enríquez et al. 2008; Mandujano-Tinoco et al. 2013). In contrast, mature MCTSs are destabilized and disaggregated by OxPhos *plus* glycolytic inhibitors, indicating that both ATP sources are essential for tumor maintenance and survival (Rodríguez-Enríquez et al. 2008). Ductal infiltrating breast (DIB) cancer human biopsies, hormone receptor-positive (HR+) subtype show similar values for mitochondrial and glycolytic protein contents to those of human breast MCF-7 MCTSs (which are also HR+), in which 2OGDH and respiratory complex IV are over-expressed compared to normal tissue (Kim et al. 2014; Pacheco-Velázquez et al. manuscript in preparation). In triple-negative DIB samples, 2OGDH was particularly (2–3 times) over-expressed versus other cancer subtypes, although the glycolytic GLUT-1, HKI and LDH protein contents were similar (Pacheco-Velázquez et al. manuscript in preparation). These data further support a significant role for mitochondria in cancer cell physiology. Consequently, anti-mitochondrial therapy could be an alternative against triple-negative breast cancer subtype. In renal biopsies of oncocytoma and stage-3 urothelial cancer, the mRNA contents of the adenine nucleotide translocator ANT2 and ATP synthase subunit B are up-regulated (Faure-Vigny et al. 1996), which may also indicate an active OxPhos in these cancers.

Glycolytic flux and metabolite levels

Increased glycolysis in tumor cells is associated with the activation of the transcriptional factors HIF-1 α , c-MYC and probably H-RAS, all of which promote increased transcription of the genes and increased contents of all glycolytic enzymes/transporters (Gillies et al. 2008; Dang et al. 2009; Marín-Hernández et al. 2009; Chesney and Telang 2013). In fact, HIF-1 α induces the over-expression of glycolytic isoforms with high affinity to their substrates and/or activators and low affinity to their physiological inhibitors (Marín-Hernández et al. 2009). This ensures the supply of cytosolic ATP, NADH and glycolytic intermediaries for protein synthesis (alanine, serine, cysteine and glycine), nucleic acid and glycogen synthesis (ribose-5-phosphate,

glucose-1-phosphate) and lipid synthesis (glycerol-3-phosphate) even under stress conditions.

Conversely, it has been shown that several glycolytic enzymes have other roles in addition to their canonic metabolic function acting as (1) apoptotic inhibitors (HKI and HKII); (2) transcriptional factors (GAPDH/LDH) regulating DNA replication and repair processes; (3) metastatic and epithelial–mesenchymal transition inducers (HPI); and (4) potential biomarkers for aggressive carcinomas such as lung cancer (ENO) (Marín-Hernández et al. 2009; Funasaka et al. 2009; reviewed in Rodríguez-Enríquez et al. 2009; Gallardo-Pérez et al. 2014). Therefore, the use of anticancer drugs that affect the expression and activity of glycolytic or OxPhos proteins, in addition to their effects on the targeted proteins for which they were designed, may have clinical relevance against glycolytic- or oxidative-dependent tumors, respectively.

Energy metabolism and metastasis

Metastatic tumor cells constitute a subpopulation originated in the primary tumor that displays significant reprogramming in their intermediary and energy metabolism and signaling pathways. These alterations endow the cells with the ability to survive after detachment from the primary tumor, migrate through the circulatory and/or lymphatic systems and eventually colonize distant tissues. Anchorage independence implies the avoidance of anoikis, a form of programmed cell death due to the loss of contact with neighboring cells or the extracellular matrix. Anchorage independence (i.e., anoikis resistance), migration and colonization may be considered separate functional events as they probably result from the subversion of distinct regulatory pathways. Notwithstanding, all of them are subordinated to the energy supply that encompasses the progression from the benign to malignant states. Analysis of energy metabolism in HeLa cells, a metastatic cervix cancer cell line, has shown that OxPhos provides most (>70 %) of the ATP required for cellular work (Rodríguez-Enríquez et al. 2006) such as proliferation and metastasis onset.

As metastasis is a major challenge to cancer therapy, it becomes relevant to dissect those biochemical mechanisms associated with the malignant trait aiming not only at understanding the underlying biochemical mechanisms of metastasis, but also at identifying susceptible targets for drug intervention. Dereglulation of many PKs has been shown to affect cell transformation, tumor progression and metastasis. Because PKs constitute regulatory nodes in many signaling pathways, frequently they also affect the reactions that involve directly or indirectly in the energy metabolism. Thus, oncogenic kinases as well

as transcription factors have become potential targets for pharmacological intervention.

Src family kinases (SFKs) are non-receptor tyrosine kinases (TKs) that respond to several stimuli all of them bearing on cell transformation, tumor progression and metastasis. Interestingly, SFKs can be activated by reactive oxygen species (ROS), linking up oncogenesis mainly to mitochondrial activity. The p21-activated kinases (PAKs) are serine and threonine PKs also involve in cell transformation, metastasis and angiogenesis (Radu et al. 2014). PAKs modulate the cell cycle through many nuclear signaling pathways and prevent apoptosis by the dual inhibition of caspases and mitochondrial BCL-2.

The transcription factor TP63, a member of the p53 family involved in epithelial morphogenesis and differentiation and a regulator of microRNAs (miRNAs), is generally recognized as a metastasis suppressor factor in epithelial tumors. For instance, knockdown of p63 abrogates the invasion led by specialized breast cancer cells (Cheung et al. 2013) and induces GA 2 expression (Giacobbe et al. 2013). p63 knockout mice exhibit obesity, glucose intolerance and insulin resistance and also display mitochondrial fatty acid oxidation dysfunction (Candi et al. 2014). These data suggest a connection between p63 and the oxidative metabolism, in which p63 may depend on functional mitochondria for promoting metastasis.

Pyruvate kinase isoform M2 (PYKM2) is a glycolytic enzyme over-expressed in highly proliferating cells and most types of cancer cells. It has been shown that the interaction of PYKM2 with phosphotyrosine-containing proteins inhibits its activity and increases upstream glycolytic metabolite levels to support cell proliferation (Mazurek 2011). Exposure to non-physiological PYKM2 activators such as TEPP-46 (a thieno [3,2-*b*] pyrrole [3,2-*d*] pyridazinone family member) or DASA-58 (a *N,N'*-diarylsulfonamide family member) induces inhibition of tumor growth under mild hypoxic conditions (1 % atmospheric O₂) and decrease in proliferation-linked metabolites such as ribose-5-phosphate and serine. Binding of these activators to PYKM2 promotes tetramer formation that in turn renders it resistant to inhibition by tyrosine-phosphorylated proteins and suppresses tumor growth (Anastasiou et al. 2012).

However, PYKM2 seems to transcend its metabolic role in tumor glycolysis (Marín-Hernández et al. 2009). (1) Recent results have shown that PYKM2 acquires PK activity, using PEP instead of ATP, upon succinyl-5-aminoimidazole-4-carboxamide-1-ribose-5-phosphate (SAICAR) binding (Keller et al. 2014), an abundant metabolite in proliferating cells. PYKM2 PK activity phosphorylates many proteins such as histones and kinases, particularly Erk1/2, promoting cancer cell proliferation (Keller et al. 2014). (2) MicroRNA-122 (miR-122) is increased in normal liver

tissue and decreased in hepatocellular carcinomas. Overexpression of miRNA-122 in hepatocellular cancer cells displays diminished PYKM2 expression (Liu et al. 2014) as well as decreased proliferation and metastasis, and increased sensitivity to anticancer drugs (Bai et al. 2009). Thus, PYKM2 activity in glycolysis (by increasing it) and as PK (by decreasing it) may be attractive novel targets for counteracting tumorigenesis and metastasis.

Another novel anticancer chemotherapeutic approach targets the fine redox balance dealing with oxidative stress. A consensus is emerging in which low ROS levels seem to promote tumor growth and invasiveness, whereas high ROS levels induce tumor cell death. Therefore, pro-oxidant pharmacological interventions based on either increasing ROS production or decreasing ROS scavenging are proving to be effective (Pathania et al. 2014). In this respect, recent results with pancreatic cancer cells have shown that quinazoline derivatives act as redox modulators and substantially increase rates of O₂ consumption and ROS production, which in turn induce oxidative stress, apoptosis (Pathania et al. 2014) and tumor vascularity impairment without affecting cellular proliferation. These results suggest that quinazoline derivatives may target specifically those aspects pertaining to anoikis resistance during metastasis (Bilbro et al. 2013), and which seem intimately linked to energy metabolism.

Small-molecule drugs used in oncology that also affect glycolytic and mitochondrial enzymes/transporters

Several commonly used chemotherapeutic drugs such as imatinib, sorafenib, temsirolimus, lapatinib, decitabine, azacytidine, bortezomib, bevacizumab, trastuzumab, cetuximab, rituximab, metformin, phemformin and letrozole (Fig. 2) have a direct effect on several glycolytic and mitochondrial proteins at the transcriptional (PK inhibitors), posttranslational (PK inhibitors, monoclonal humanized antibodies) or kinetic (PK inhibitors, monoclonal humanized antibodies, proteasome inhibitors) levels (Figs. 3, 4).

Tyrosine kinase (TK) inhibitors

Imatinib, sunitinib, dasatinib, erlotinib, axitinib, lapatinib, sorafenib, nilotinib, busotinib and temsirolimus (Fig. 2) are currently the most frequently used TK inhibitors in the treatment of cancer (Mellor et al. 2011; Ott and Adams 2011). All of them are signal transduction inhibitors that presumably only affect specific kinases in the tumor cells, but which, unfortunately, also affect other protein functions unrelated to the respective kinase network. For example, imatinib directly interacts with the stem cell factor receptor (c-KIT) and the platelet-derived growth factor receptor (PDGF-R) blocking their respective signal pathways.

Also, TK inhibitors directly interact with different oncogenes such as BCR/ABL (imatinib) and B-RAF (sorafenib) affecting their oncogenic function (Arora and Scholar 2005; Fabian et al. 2005; Rix et al. 2007; Kessler et al. 2010; Waller 2010). These observations cast doubt on the cellular specificity of the TK inhibitors.

The intended action of the TK small-molecule inhibitors is the direct interaction with the receptor/enzyme ATP-binding site at relatively low, therapeutically viable, concentrations. For *imatinib*, computational models with TK crystals revealed a submicromolar inhibition constant ($K_i = 0.4 \mu\text{M}$, Lin et al. 2013). However, decitabine ($\text{IC}_{50} = 9 \text{ nM}$) and nilotinib ($\text{IC}_{50} = 45 \text{ nM}$) showed more potency for abolishing erythroleukemia K562 growth than imatinib ($\text{IC}_{50} = 400 \text{ nM}$) (Rix et al. 2007). As there is a multitude of ATP-interacting enzymes in cancer metabolism, it can be expected that the TK inhibitors may also interact with the ATP-binding pocket of a variety of nontargeted enzymes such as the glycolytic enzymes HK, PFK-1, PGK and PYK.

Imatinib and lapatinib at therapeutic doses also inhibit several glycolytic steps (Fig. 2; Table 1) including the tumor glycolytic flux-controlling GLUT (Marín-Hernández et al. 2011). Interestingly, in leukemia BCR–ABL-positive (K562) cell line, but not in BCR–ABL-negative (HC-1) cells, imatinib (2.5 $\mu\text{M}/96 \text{ h}$) induced a significant decrease in lactate production. The glycolytic flux is 1.3–1.5 times higher in BCR–ABL-positive cells than in their negative counterparts. Moreover, despite significant TK inhibition, imatinib does not affect glycolysis of other BCR–ABL-positive cells (CML-T1) whose glycolysis is significantly lower (1.6 times) than that of BCR–ABL-negative HC-1 cells (Gottschalk et al. 2004). Thus, these data suggest that the imatinib metabolic effect is associated with high glycolytic rates in tumor cells. In other studies, imatinib-positive and imatinib-resistant BCR–ABL cells DA1-3b/M2 cells increase their glycolytic flux correlating with a significant increment in PGK-1 protein and HKII, HPI, PFK1 and ALDO mRNAs contents through a mechanism presumably associated with HIF-1 α stabilization (Zhao et al. 2010; Kluza et al. 2011). In other diseases, such as pulmonary arterial hypertension, imatinib and sunitinib also affect 18-fluorodeoxyglucose (FDG) uptake (Zhao et al. 2013) indicating that the imatinib effect on glycolysis is not a typical response of cancer cells.

As a consequence of the glycolysis suppression, imatinib-positive leukemia cells (0.1 $\mu\text{M}/\text{week}$ for 5 weeks or 0.1–2.5 $\mu\text{M}/96 \text{ h}$) as well as imatinib-negative leukemia cells (same treatment doses) switch their energy metabolism to an oxidative type (Table 2) leading to increased mitochondrial metabolite levels such as succinate, fumarate, malate and glutamate (Gottschalk et al. 2004; Klawitter et al. 2009; Kluza et al. 2011). In these cells, a significant increment in NADH/NAD⁺, ATP/ADP, UTP/ADP,

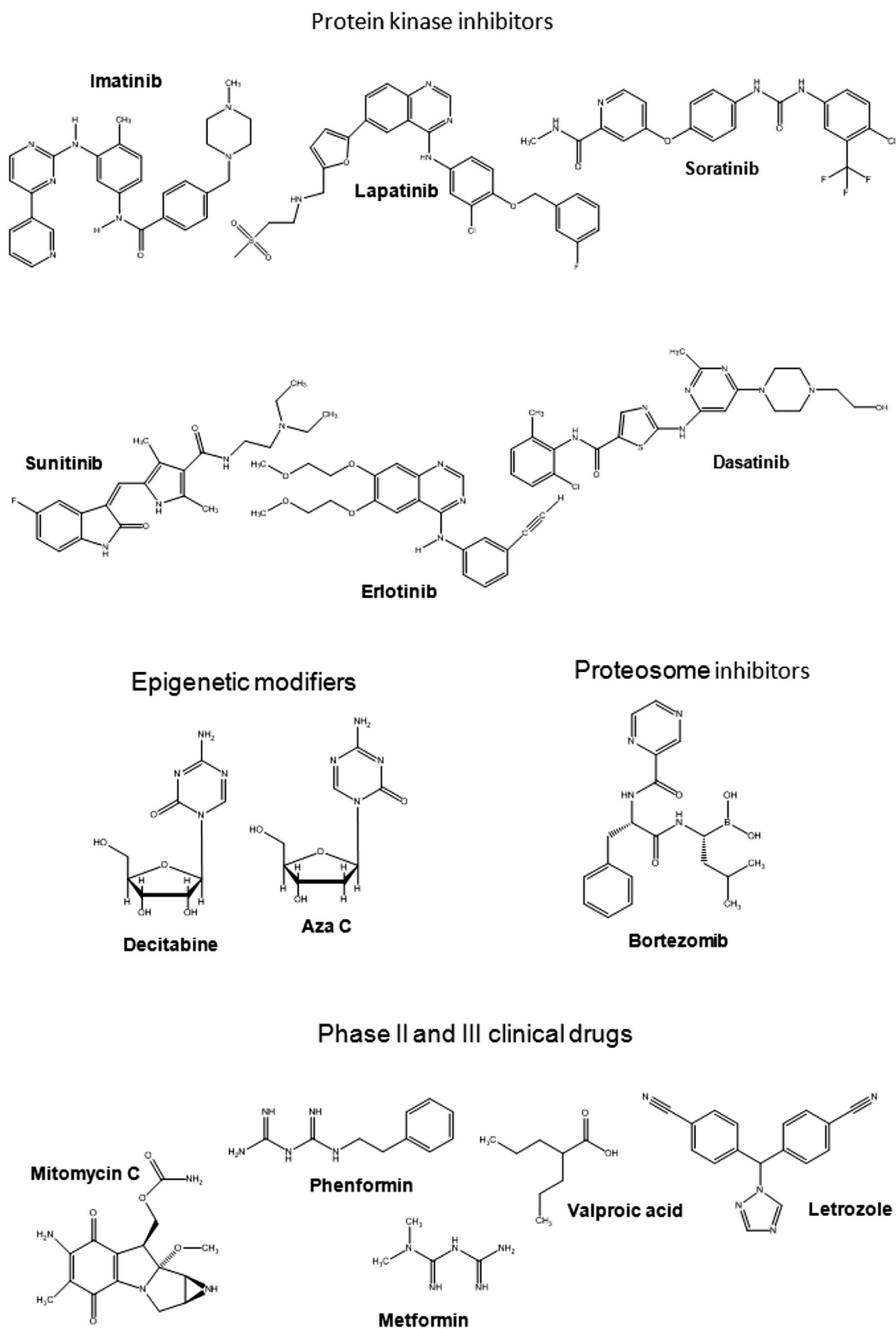
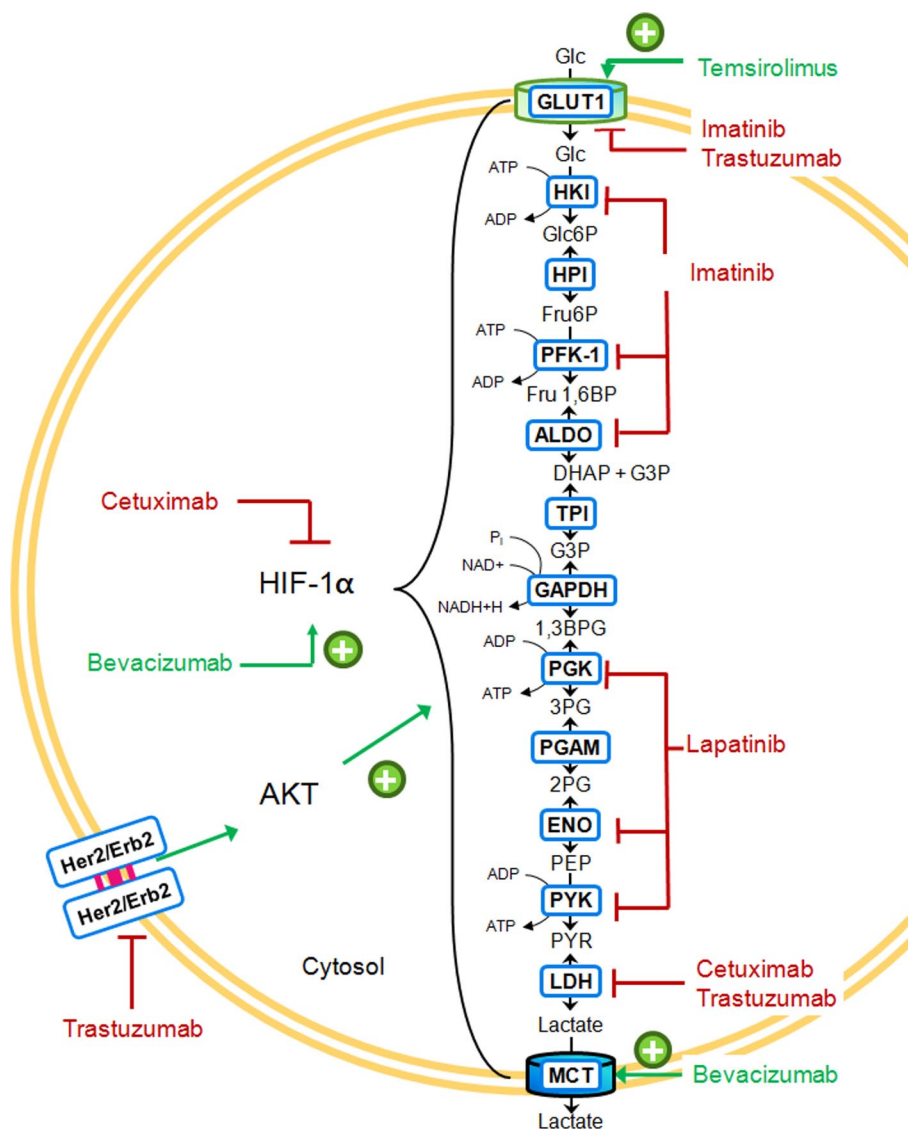


Fig. 2 Chemical structures of some first-line anticancer drugs that block energy metabolism

Fig. 3 Glycolytic targets of PK inhibitors and mAbs. *GLUT* glucose transporter, *HK* hexokinase, *HPI* hexosephosphate isomerase, *PFK1* phosphofructokinase type 1, *ALDO* aldolase, *TPI* triosephosphate isomerase, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *PGK* phosphoglycerate kinase, *PGAM* phosphoglycerate mutase, *ENO* enolase, *PYK* pyruvate kinase, *LDH* lactate dehydrogenase, *MCT* monocarboxylate transporter, *Glc* glucose, *Glc6P* glucose 6-phosphate, *Fru6P* fructose 6-phosphate, *Fru1,6BP* fructose 1,6-bisphosphate, *DHAP* dihydroxyacetone phosphate, *G3P* glyceraldehyde-3-phosphate, *1,3BPG* 1,3-bisphosphoglycerate, *3PG* 3-phosphoglycerate, *2PG* 2-phosphoglycerate, *PEP* phosphoenolpyruvate, *PYR* pyruvate. Red lines indicate drug inhibition; green arrows indicate drug activation

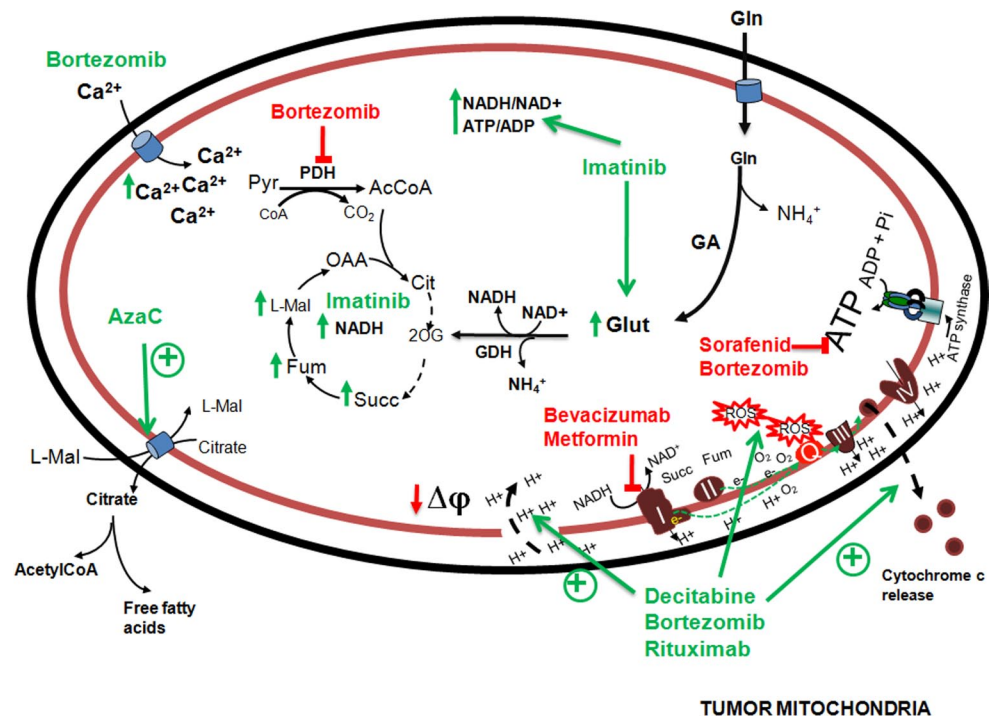


CTP/CDP and GTP/GDP ratios, energy charge and mRNA contents of several enzymes involved in glutamine oxidation is observed (Table 2); also, perhaps as a consequence of mitochondrial metabolism activation, decreased phosphocreatine and acetate contents are detected (Gottschalk et al. 2004; Klawitter et al. 2009; Kluza et al. 2011) (Table 2). The OxPhos activation induced by imatinib in K562-positive leukemia and HC-1-negative leukemia cells was not observed in imatinib-positive and imatinib-resistant leukemia cells such as DA1-3b/M2 cells, in which imatinib actually induced a significant diminution in the total O_2 consumption (Kluza et al. 2011), suggesting decreased mitochondrial energy metabolism. The OxPhos inhibitory effect of imatinib on DA1-3b/M2 cells is supported by the observation that under physiological conditions, the piperazinyl group of imatinib (Fig. 2) is predominantly protonated, thus carrying a net positive charge (Lin et al. 2013). The relevance of the last observation can be fully

understood by considering that tumor mitochondria accumulate positive charge lipophilic molecules, to a higher extent than non-tumor mitochondria, due to their greater magnitude of the electrical potential difference across the inner membrane or $\Delta\psi$ (reviewed in Rodríguez-Enríquez et al. 2009). Thus, it can be predicted that imatinib may preferentially accumulate in tumor mitochondria versus normal mitochondria. The molecular mechanisms involved in the apparent imatinib activation or inhibition, at the translational, transcriptional or kinetic levels, of mitochondrial enzymes and metabolism clearly require further investigation.

The OxPhos activation induced by imatinib in K562 and HC-1 leukemia cells has not been observed for other TK small-molecule inhibitors. On the contrary, *sorafenib* induces a decrease in the ATP intracellular content in hepatoma cells at clinical doses (Table 2; Fiume et al. 2011). Furthermore, in non-tumor cells, imatinib, suratinib,

Fig. 4 Mitochondrial targets of first-line or experimental drugs in cancer treatment. *Cit* citrate, *Gln* glutamine, *GA* glutaminase, *GDH* glutamate dehydrogenase, *Mal* malate, *OAA* oxaloacetate, *2-OG* 2-oxoglutarate, *Succ* succinate, *Fum* fumarate



dasatinib, lapatinib and sorafenib cause (1) cardiac failure (Ksienki 2011; Mellor et al. 2011); (2) heart mitochondrial swelling (Chu et al. 2007); (3) inhibition of several heart mitochondrial enzyme activities (COX IC_{50} = 124 μ M; ATP synthase IC_{50} = 5–96 μ M; CI-NADH dehydrogenase IC_{50} = 31–220 μ M; and CII-SDH + CIII IC_{50} = 3 μ M; Will et al. 2008); and (4) lowering in the PYK-M2 protein content (Hegde et al. 2007). Thus, it seems that imatinib may promote differential effects on mitochondrial energy metabolism of cancer cells versus normal cells, activating in the former and depressing in the latter.

Temsirolimus is presumably a selective inhibitor of the mTOR complex, a serine–threonine PK involved in several tumor-promoting signaling pathways and controlling many cellular functions such as proliferation, survival, cell cycle regulation (PI kinase, Akt/PK B) and stabilization of transcription factors such as HIF-1 α and VEGF (Koh et al. 2010). In clinical practice, this drug is used in patients with advanced renal cell carcinoma (Ott and Adams 2011; Polivka and Janku 2014). In murine xenograft tumor models, therapeutic doses of temsirolimus increase the content of GLUT1 by 50 % (Ma et al. 2009); other glycolytic proteins were not evaluated.

Epigenetic modifiers

It has been proposed that DNA hypermethylation is associated with tumorigenesis onset (Esteller 2007). As guanine and cytosine are the predominant targets for methylation (Chan et al. 1993; Clark and Melki 2002; Gowher and

Jeltsch 2004; Cherepanova et al. 2011), two potent cytosine analogs have been synthesized to block O6-methylguanine-DNA transferase (MGT) or DNA cytosine C5 methyltransferase and tested as anticancer drugs. 5-Azacytidine (AzaC; Fig. 2) and its deoxy derivative 5-aza-2'-deoxycytidine (also called *decitabine*) are frequently used for treatment of acute myeloid leukemia; other carcinomas (breast, ovary) are also sensitive to these drugs (Xu et al. 2007; Meng et al. 2013).

Adriamycin and trichostatin A potentiate the antitumor effect of decitabine by inducing p53-mediated mitochondrial apoptosis in leukemia cells (Table 2) and pancreas carcinoma without apparent effects on normal cells (Schmelz et al. 2005; Cecconi et al. 2009). In some leukemia cells, decitabine alone increases ROS level and collapses mitochondrial $\Delta\psi$ leading to apoptosis (Table 2; Shin et al. 2012). In contrast, in some OxPhos proteins, such as the mitochondrial citrate carrier in metastatic brain cells and the adenine nucleotide translocator subtype 4 (ANT4) in murine fibroblast, specific proximal promoter regions (CpG) highly susceptible to be methylated have been described (Brower et al. 2009). Accordingly, AzaC or decitabine increases the mitochondrial citrate carrier mRNA and ANT protein contents (Table 2), suggesting that epigenetic abnormalities could negatively affect the OxPhos pathway or that these drugs directly interact with the OxPhos genes/proteins. In other metabolic disorders such as Parkinson's diseases, decitabine also increases the ROS level (which presumably derives from the mitochondrial metabolism) and the cytochrome c protein expression (Kong et al. 2012).

Table 1 Effect of first-line antineoplastic drugs affecting tumor glycolysis

Cancer	Anticancer drug	Target	Effect	Doses	References
<i>Protein kinase inhibitors</i>					
Leukemia BCR–ABL K562 positive	Imatinib	TK inhibitor	Diminution in ALDOIA and PFK1 mRNA contents; GLUT1 protein content and HK activity Diminished glucose uptake and glycolysis flux	0.1–1 μ M/24 or 48 h	Boren et al. (2001), Boros et al. (2002), *Gottschalk et al. (2004), Barnes et al. (2005), Hickey and Cotter (2006), Klawitter et al. (2009)
Leukemia BCR–ABL imatinib-resistant DA1-3b/M2 negative			Increment in glycolysis; increment in HKII, HPI, PFK1, PGK1, ALDO mRNA content	0.05 μ M/6 months plus 3 μ M/12 months more	Kluza et al. (2011)
Breast carcinoma MDA-MB468, BT474 breast carcinoma	Lapatinib		Diminution in ENO, PYK and PGK1 mRNA or protein contents	1 μ M/12–24 h	Hegde et al. (2007)
Pancreas adenocarcinoma and pancreas cancer lines in rodent xenografts	Temsirolimus	mTOR inhibitor	Increased GLUT1 protein content	20 mg/kg/day i.p. 2 weeks	Ma et al. (2009)
<i>Proteasome inhibitors</i>					
Glioblastoma multiforme KMS20BM	Bortezomib	26S proteasome inhibitor	No effect on GADH content	50–75 nM, 48 h	Yin et al. (2005)
<i>Monoclonal antibodies</i>					
Glioblastoma spheroids transplanted in rodents	Bevacizumab	Anti-VEGFR	Increased HIF-1 α protein and mRNA contents; lactate, alanine and creatinine production	10 mg/kg/week i.v. 3 weeks	Keunen et al. (2011)
Breast carcinoma BT474 and ZR-7530 cells	Trastuzumab	Anti-Her2	Diminution in glucose uptake and PGK1 protein levels	20 μ g/mL/48 h	Zhang et al. (2005)
Tumor xenografts of BT474 carcinoma			Diminution of LDH activity	10 mg/kg/3 weeks	Zhao et al. (2011)
Head and neck HN5 and FaDu carcinoma cells	Cetuximab	Anti-EGFR	Lowering in HIF-1 α and LDH-A protein contents; diminished glycolysis flux and glucose consumption	10–20 nM, 8–16 h	Lu et al. (2013)
B-NHL xenograft mice	Rituximab	Anti-CD20	Diminished ¹⁸ F-2DG uptake	50 mg/kg/day, i.v. 2 days	Kita et al. (2012)
<i>Others clinical drugs</i>					
Ehrlich rat hepatoma cells	Mitomycin c	DNA crosslinker	Diminished glycolysis rate (40–50 %)	0.75 mM	Saito and Kaneko (1968)

In all described studies, the activity, content or functionality of the mAb-targeted proteins, as well as the effect of the DNA-destabilizing inhibitors, on tumor growth was not assayed in parallel to the determination of the drug-induced metabolic effects, except for imatinib (*) where the same drug concentration used to induce inhibition of glycolytic enzymes, transporters and fluxes also diminished the TK content by 80 % (Gottschalk et al. 2004)

B-NHL B-cell non-Hodgkin lymphoma, *CD20* cluster of differentiation 20, *EGFR* epithelial growth factor receptor, ¹⁸F-*FDG* 2-deoxy-2-¹⁸F-fluoro-D-glucose, *GLUT* glucose transporter, *HK* hexokinase, *Her2* human epidermal growth factor receptor 2, *PFK1* phosphofructokinase type 1, *ALDO* aldolase, *PGK* phosphoglycerate kinase, *ENO* enolase, *PYK* pyruvate kinase, *LDH* lactate dehydrogenase, *VEGFR* vascular endothelial growth factor receptor

Table 2 Effect on mitochondrial function of first-line antineoplastic drugs

Cancer type	Anticancer drug	Target	Effect	Doses	References
<i>Protein kinase inhibitors</i>					
Leukemia BCR–ABL K562-positive cells	Imatinib	TK inhibitor	Increased [Glu] and NADH/NAD ⁺ and ATP/ADP, UTP/ADP, CTP/CDP and GTP/GDP ratios Decreased PCr and acetate contents	0.1 μM at week/5 weeks; 0.1–2.5 μM/96 h	*Gottschalk et al. (2004), Klawitter et al. (2009)
Leukemia BCR–ABL HC1 or DA1-3b/M2 negative cells			Increased [Glu, Mal, Fum, Succ] concentrations and NADH/NAD ⁺ , ATP/ADP, UTP/ADP, CTP/CDP and GTP/GDP ratios; low O ₂ consumption	0.1 μM at week/5 weeks; 50 μM/6 months; 3 μM/12 months	*Gottschalk et al. (2004), Kluza et al. (2011)
Hepatocellular carcinoma PLC/PRF/5 and SNU-449 cells	Sorafenib	Raf kinase, PDGF and VEGF receptor	Decreased ATP content	4–16 μM/1–3 h	Fiume et al. (2011)
<i>Epigenetic modifiers</i>					
Acute myeloid leukemia EOL, HL60, U937, K-562 and MEG-01 cells	Dacitabine	DNA hypomethylating agent	Cytochrome <i>c</i> release Collapse of Δψ Autophagy activation	1–5 μM for 3 days 15 μM for 72 h 2 μM for 4 days	Schmelz et al. (2005) Shin et al. (2012) Schnekenburger et al. (2011)
Bone marrow-derived metastatic brain cell line SK-N-SH	Azaeytidine		Increased mitochondrial citrate carrier mRNA	5–50 μM	Iacobazzi et al. (2008)
<i>Proteasome inhibitors</i>					
Glioblastoma multiforme KMS20BM cells	Bortezomib	26S proteasome inhibitor	Mitochondrial Ca ²⁺ overloading; collapse of Δψ; low PDH-E1α and CyP contents. Increment ROS level	50–75 nM, 48 h	Yin et al. (2005)
Melanoma A375 cells			Autophagy activation, increased cellular ROS levels; collapse of Δψ	10 nM, 24 h	Selimovic et al. (2013)
<i>Monoclonal antibodies</i>					
Human colorectal carcinoma xenograft	Bevacizumab	Anti-VEGFR	Mitochondrial biogenesis suppression; low NADH dehydrogenase content	5 mg/kg/twice a week for 3 months	Xu et al. (2013)
Human biopsy glioblastoma spheroids transplanted in rodents			Autophagy activation	10 mg/kg/week; i.v. 3 weeks	Keunen et al. (2011)
Head and neck HN5 and FaDu carcinoma cells	Cetuximab	Anti-EGFR	Increased OxPhos flux; diminution in ATP content	10–20 nM, 4–16 h	Lu et al. (2013)
CD20-positive Burkitt lymphoma 2F7 cells; follicular lymphoma HF1A3 cells	Rituximab	Anti-CD20	Cytochrome <i>c</i> release; ROS overproduction, collapse of Δψ	10–20 μg/mL, 3–24 h	Alas et al. (2002), Eeva et al. (2009)

Table 2 continued

Cancer type	Anticancer drug	Target	Effect	Doses	References
<i>Others clinical drugs</i>					
SMP of hepatoma cells	Metformin	Antidiabetic drug	Inhibition of CI activity	79 μ M at 24 h	Owen et al. (2000)
Rat hepatoma cells			Inhibition of total oxygen consumption and collapse of $\Delta\psi$	50–100 μ M/24–60 h	Owen et al. (2000), El-Mir et al. (2000)
Murine leukemia isolated mitochondria (L1210)	Phen formin		Inhibition of state 3 respiration	0.1–0.3 mM	Byczkowski et al. (1982)
Breast carcinoma MCF-7 cells	Mitomycin C	DNA crosslinker	Collapse of $\Delta\psi$	45 μ M/4 h	Lee et al. (2004)
Ehrlich rat hepatoma cells			Inhibition of total cellular respiration	0.75 mM	Saito and Kaneko (1968)
Human breast biopsies	Letrozole	Non-steroidal aromatase inhibitor	Down-regulation of ATP synthase, cytochrome c oxidase and CI genes	2.5 mg/3 months, daily	Miller and Larionov (2011), Miller et al. (2007, 2012)
Human breast biopsies	Valproic acid	Histone deacetylase inhibitor	High microarray gene expression of NADH dehydrogenase, ubiquinol cytochrome c reductase, cytochrome c oxidase subunit VIIc	30 mg/kg daily during 7 days + 21 days more with doxo plus CP, 4 treatments per day	Arce et al. (2006)

In all described studies the activity, content or functionality of the mAb-targeted proteins, as well as the effect of DNA-destabilizing inhibitors, on tumor growth was not assayed in parallel to the determination of the drug-induced metabolic effects, except for imatinib (*) where the same drug concentration used to induce inhibition of OxPhos, metabolite contents and O_2 consumption also diminished the TK content by 80 % (Gottschalk et al. 2004)

$\Delta\psi$ mitochondrial membrane potential, CyP cyclophilin, the membrane permeability transition regulator, *glut* glutamate, *mal* malate, *CD20* cluster of differentiation 20, *EGFR* endothelial growth factor receptor, *PCr* phosphocreatine, *PDGF* platelet-derived growth factor, *PDH-E1 α* pyruvate dehydrogenase subunit E1 alpha, *ROS* reactive oxygen species, *succ* succinate, *VEGF* vascular endothelial growth factor receptor, *doxo* doxorubicin, *CP* cyclophosphamide

Proteasome inhibitors

The ubiquitin–26S proteasome pathway is responsible for degradation of the majority of regulatory proteins in mammalian cells including those that control cell cycle progression, apoptosis induction and DNA repair. Proteasomal activity is higher in B-cell chronic lymphocytic leukemia (Masdehors et al. 2000) than in normal lymphocytes promoting an accelerated degradation of key proteins. Therefore, the disruption of proteasomal activity leads to growth arrest and cell death. *Bortezomib* was the first-generation proteasome inhibitor used in the clinical treatment of multiple myeloma and non-Hodgkin lymphoma (Piperdi et al. 2011). The drug mode of action derives from its reversible binding to the B1 subunit of the catalytic site of 26S proteasome (Shah et al. 2009), leading to inhibition of its proteolytic activity (Blackburn et al. 2010).

Although bortezomib has been considered as a suitable drug for targeted therapy based on its high potency and selectivity for the proteasome, it also has multiple effects in tumor cells. For example, in prostate cancer (LNCaP-Pro5) and malignant B cells, it has been demonstrated that relatively low doses (nanomolar range) of bortezomib stabilizes p53 and Bax (pro-apoptotic proteins), whereas in glioblastoma multiforme the drug decreases the mRNA and protein contents of the anti-apoptotic protein Bcl2 (Williams and McConkey 2003; Yin et al. 2005; Liu et al. 2008), leading to tumor arrest. In several carcinomas, bortezomib promotes a severe impairment of OxPhos and autophagy activation prompting a significant mitochondrial and cellular ATP depletion (Table 2). The content of some glycolytic enzymes was unaltered by bortezomib, although determination of the glycolysis flux was not performed (Yin et al. 2005; Selimovic et al. 2013). Bortezomib (0.2 mg/kg weight for 1–3 weeks) induces in rat heart mitochondrial ultrastructural abnormalities and a decrease in cellular respiration (47 %) and, in consequence, a significant decrease in cellular ATP (Nowis et al. 2010).

For other TK small-molecule inhibitors (dasatinib, erlotinib, axitinib, nilotinib, busotinib; see Fig. 2 for chemical structures), information on their effects at the energy metabolism level is not yet available, although significant effects may be expected given their structural analogy with imatinib.

Antibody-based tumor targeting affecting glycolytic and mitochondrial enzymes

The use of monoclonal antibodies (mAb) directed at a particular protein over-expressed in cancer cells has become a common clinical strategy for identification and pharmacological intervention. However, although

immunotherapy may result in a more selective strategy than other approaches, its use is associated with undesirable side effects such as cardiac failure (Baldo 2013). In addition, most of the mAbs certainly bind to their extracellular targeted proteins with high affinity, but some of them may cross the plasma membrane and exert their effects on intracellular targets (Dao et al. 2013).

Anti-angiogenic humanized mAbs

Bevacizumab (Avastin®) exerts its effects through different mechanisms including inhibition of the de novo growth of blood vessels along with the regression of newly formed tumor vasculature, both of which altering vascular function. This mAb also has a direct effect on the tumor cells. The molecular mechanism for the direct effect is associated with its extracellular binding ($K_d = 1.8$ nM) (Papadopoulos et al. 2012) to the vascular endothelium growth factor (VEGF) subtype A, thus blocking the subsequent signal transduction pathways PI3K/MAPK, AKT/PKB, RAS/ERK coupled to TK receptors (VEGFR). This mAb has been employed in the treatment of several cancers including breast, colorectal, lung, brain, kidney and ovarian carcinomas. At the metabolic level, bevacizumab increases the transcription factors associated with glycolysis activation (Table 1) and lowers the mitochondria content in MCTSs derived from human glioblastoma biopsies transplanted in nude rats (Table 2) (Keunen et al. 2011). Also in bevacizumab-resistant tumor cells such as xenograft colorectal cancer, the drug suppresses mitochondrial biogenesis and the content of the OxPhos flux-controlling enzyme, NADH dehydrogenase or respiratory complex I (Xu et al. 2013).

Trastuzumab shows high efficacy in the treatment of breast HER-2 neu -positive patients and is potentially effective on other carcinomas (non-small cell lung cancer) (Mazieres et al. 2013). However, many patients eventually develop resistance to the drug after continuous treatment. The TK receptor ERB2B2 (HER-2 neu) is 30 % over-expressed in breast cancer (Yu and Hung 2000) and is involved in the activation of several intracellular pathways associated with proliferation (RAS–RAF–MAP kinases), tumor survival (PI3K/AKT kinase) and apoptosis (AKT kinase and NF κ B transcription factor activation) (Prenzel et al. 2001; Mendelsohn and Baselga 2000; Papanikolaou et al. 2011). Trastuzumab binds to the extracellular domain of ERB2B2 TK receptor diminishing tumor proliferation, transformation and metastatic potential. At the therapeutic doses used in the clinical treatment (Chan 2007), trastuzumab also affects tumor energy metabolism (Tables 1, 2). Regarding mitochondrial metabolism, it has been reported in ERBB2-positive breast cancer (MCF-7/ERBB2 and MDA-MB-231/ERBB2) that trastuzumab (100 μ g/ml)

promotes the translocation of the ERBB2 receptor that usually localizes to the plasma membrane and cytosol fractions, to tumor mitochondrial inner membrane (Ding et al. 2012). Although OxPhos performance in the presence of this mAb has not been assessed in tumor cells, in cardiomyocytes exposed to the selective ERBB2 clone 9G6 mAb (5–7.5 $\mu\text{g}/\text{mL}$) for 24 h, OxPhos (measured as $\Delta\psi$, ATP intracellular content and redox capacity) was partially impaired (Grazette et al. 2004).

A “bifunctional peptide” composed by anti-HER-2 peptide (to neutralize HER-2) fused to a mitochondriotoxic peptide (to impair mitochondrial function) has been engineered against HER-2-overexpressing tumors (Fantin et al. 2005). Presumably, the mitochondriotoxic peptide named BHAP acts at two levels (1) as a lipophilic cation having selectivity for mitochondria with high $\Delta\psi$ (negative inside) and (2) as HER-2 analog blocking the ERBB2 receptor. This peptide (10 μM for 3 h) altered mitochondrial morphology and induced cytochrome c release, the main trigger of apoptosis (Von Ahlsen et al. 2000; Ott et al. 2002) in ERBB2 over-expressing BT474 and MDA-MB-453 breast cancer cells (Fantin et al. 2005).

Cetuximab is another mAb that blocks the over-expressed EGF receptor in patients with head and neck as well as colorectal cancers. Cetuximab binding to EGFR blocks the ligand-induced activation of EGFR downstream cell signaling promoting G1-phase arrest and apoptosis. Low doses of cetuximab down regulate HIF-1 α , the key transcriptional regulator of tumor glycolysis in epidermoid carcinoma (Luwor et al. 2005). As a consequence, the content of some glycolytic enzymes, glucose consumption and lactate production decreases significantly (Table 1). Although cetuximab induces a significant increase in OxPhos (oligomycin-sensitive respiration), the levels of intracellular ATP diminish by 50 % which probably contribute to the apoptosis induction observed in cetuximab-treated tumor cells.

mAbs directed to tumor over-expressed proteins

Several cancer cells such as type B lymphomas, leukemias and some melanomas maintain highly expressed CD20 in their plasma membrane, which is required for an optimal immune evasive response. CD20 is also involved in the Ca²⁺ influx across the plasma membrane allowing lymphocyte B-cell activation (Tedder and Engel 1994). *Rituximab* is a chimeric mAb that recognizes, binds and destroys CD20. However, severe adverse effects such as heart failure, pulmonary toxicity or hepatitis B reactivation leading to fatal outcomes may develop in rituximab-treated patients. Rituximab combined with the bromide analog YM155 (a surviving suppressor) significantly diminishes FDG uptake in WSU-DLC lymphoma (Table 1), which correlates with

a marked decrease in the proliferation marker Ki67 (Kita et al. 2012). These results suggest that rituximab interferes with tumor glycolytic metabolism. Furthermore, rituximab alone, or combined with other canonical drugs (cisplatin at 3.3 μM ; or cyclophosphamide + bortezomib, 10 nM), induces cytochrome c release and caspase-9 and caspase-3 activation for apoptosis onset, ROS generation and collapse of mitochondrial $\Delta\psi$ (Table 2; Alas et al. 2002; Wang et al. 2008; Eeva et al. 2009).

Phase I, II and III clinical trial new anticancer drugs with severe effects on energy metabolism

The antidiabetes drug *metformin* (1,1-dimethylbiguanide hydrochloride) has shown potent anti-tumor response in breast, prostate, pancreas and lung carcinomas at similar doses to those required for diabetic patients to reestablish their blood glucose homeostasis (Blandino et al. 2012). Its anti-tumoral effect is related to LKB1/AMPK activation and the subsequent inhibition of mTOR, which in turn suppresses protein synthesis and cell proliferation (Viollet et al. 2012). In several isolated carcinoma cells from breast (MCF-7) and colon (HCT116) and in the human carcinoma-derived cell line (KB cells), metformin at micromolar doses and short-term exposure diminishes total O₂ consumption (Table 2; Fig. 4) (Guigas et al. 2004; Buzzai et al. 2007).

Although OxPhos has not been directly evaluated (i.e., measuring the oligomycin-sensitive cellular respiration), it is likely that metformin may affect mitochondrial metabolism as a consequence of its cationic lipophilic chemical nature (Owen et al. 2000). *Phenformin* (dimethylbiguanide) also affects tumor respiration at micromolar doses. Both phenformin and metformin partially inhibit the activities of the NADH and 2OG dehydrogenases in hepatoma submitochondrial particles without apparent effect on SDH, bc-1, ANT and ATP synthase at similar doses (Gettings et al. 1988; El-Mir et al. 2000; Owen et al. 2000). To explain the selective inhibition of metformin on NAD⁺-linked dehydrogenases, several reports indicate that the drug (50 μM) diminishes the NADH/NAD⁺ ratio, compromising the NAD⁺-dependent enzyme activities. However, the total inhibition of the NADH dehydrogenase activity was observed at 30 mM metformin concentration (Owen et al. 2000; El-Mir et al. 2000; Brunmair et al. 2004), suggesting that the principal target of metformin could occur at the level of the Krebs cycle NADH-producing enzymes. In addition, it has been observed in human fibroblasts and rodent heart myocytes that metformin (0.6–5 mM) increases glycolysis and blocks gluconeogenesis by affecting AMPK and mTOR signaling pathways (Hamann et al. 1993; Fischer et al. 1995).

Mitomycin C is a natural antibiotic used in the treatment of breast and lung cancers (Ardizzoni et al. 1995; Sculier et al. 2001; Mrozek et al. 2008). In established tumor cell lines, mitomycin C diminishes growth of different carcinomas such as lung (SCLC), squamous carcinomas (Ca9-22, HSC-2) and leukemias (HL-60) at relatively low doses ($IC_{50} = 2\text{--}37 \mu\text{M}$) (Sasaki et al. 2006). Its mechanism of action involves the alkylation of DNA and ROS generation (Paz et al. 2012). Mitomycin C is reduced by the NADPH-cytochrome P-450 reductase in anaerobic conditions (Spanwick et al. 1996); therefore, the drug has been proposed to kill selectively hypoxic tumor cells (Keyes et al. 1985; Cummings et al. 1995). Mitomycin C also collapses mitochondrial $\Delta\psi$ in breast cancer MCF-7 cells, disrupts the mtDNA in rodent carcinoma and inhibits the cellular respiration in Ehrlich ascites cells (Table 2) (Pritsos et al. 1997; Lee et al. 2004). However, in human HL-60 leukemia, similar doses of mitomycin C do not alter mitochondrial morphology (Lee et al. 2004; Sasaki et al. 2006). Also, the drug inhibits tumor glycolysis at micromolar doses (Saito and Kaneko 1968; Table 1).

Letrozole is a third-generation nonsteroidal aromatase inhibitor with, presumably, high specificity for blocking estrogen biosynthesis (den Hollander et al. 2013) and displaying low side effects (Lake and Hudis 2002). This drug is currently used to treat estrogen-receptor-positive breast cancer patients (Haynes et al. 2003; Miller et al. 2012). Letrozole also affects the expression of genes related to the onset of proliferation such as CDC2, CKS2, NUSAP1, PCNA and thymidylate synthetase (Mackay et al. 2007; Miller and Larionov 2011). Chronic treatment (3 months) of letrozole induces severe down-regulation of several OxPhos genes (Table 2). However, there are no data available yet documenting the direct effects of letrozole on protein contents, enzyme activities and flux of OxPhos in cancer cells.

Valproic acid and some of their analogs such as DP-VPA (2-isopropylpentanoic acid-isovaleramide, heptyl-4-yn-valproic acid), inhibit proliferation, increase apoptosis and decrease metastasis and angiogenesis in glioma, breast, colon, prostate and ovarian tumor cells (Takai et al. 2004; Zgouras et al. 2004; Blaheta et al. 2005; Angelucci et al. 2006; Hodges-Gallagher et al. 2007). At the clinical level, valproic acid, in combination with decitabine, cisplatin or topotecan, has demonstrated an effective response in colorectal, melanoma and cervical cancer treatments (Trojnar et al. 2004; Braiteh et al. 2008; Coronel et al. 2011). At higher doses ($>200 \mu\text{M}$), valproic acid inhibits the ADP-stimulated state three mitochondrial respiration (i.e., OxPhos) in rat liver and behaves as a potent OxPhos uncoupler (Haas et al. 1981). In biopsies from breast cancer patients treated with valproic acid (30 mg/kg daily during 7 days + 21 days more with doxorubicin

plus cyclophosphamide, four treatments per day), the drug promoted the up-regulation of at least 89 genes involved in the tumor metabolism; of these, 20 % corresponded to OxPhos genes including flux-controlling OxPhos enzymes such as NADH dehydrogenase (Arce et al. 2006). These observations suggested that epigenetic modification may activate mitochondrial gene expression and OxPhos flux. Alternatively, the drug may directly interact with OxPhos enzymes. Interestingly, opposing effects of valproic acid on energy metabolism in non-tumor cells have been described. In isolated rat hepatocytes, the drug diminished the ATP synthesis (Silva et al. 1997) compromising cellular viability.

Effects of first-line anticancer drugs on cells lacking mtDNA and isolated mitochondria

As discussed throughout this review, it appears that the abrogation of the mitochondrial function is a parallel primary target of several antineoplastic drugs currently used in the clinical practice. In this regard, it has been demonstrated in different tumor (leukemia MOLT-4, fibrosarcoma HT1080 and cervix HeLa) and non-tumor (intestinal epithelium IE6) rho (0) cells that their viability sensitivity toward *adaphostin*, paclitaxel, cisplatin and adriamycin is lower than in their rho (+) counterparts (Singh et al. 1999; Qian et al. 2005; Le et al. 2007; Patenaude et al. 2007). In MOLT-4 cells, the treatment (10 μM , 30 min) with the preclinical anti-leukemia drug *adaphostin*, whose action mechanism is associated with ROS production, doubled the mitochondrial ROS production, whereas in rho (0) MOLT-4, *adaphostin* was unable to increase ROS production (Le et al. 2007). For HT1080 cells treated with paclitaxel (20 nM/16 h), 5-fluorouracil (67 μM /48 h) or vinblastin (20 nM/16 h), 90 % cellular mortality was attained, whereas for rho (0) HT1080 cells the same drug doses induced <30 % cell mortality (Patenaude et al. 2007). In rho (0) HeLa (*adriamycin*) and in normal rho (0) IE6 cells (*cisplatin*), the antineoplastic drug was less toxic ($IC_{90} = 1.6 \mu\text{M}$ and $IC_{50} > 50 \mu\text{M}$ for HeLa and IE6, respectively) than in their counterparts HeLa ($IC_{90} = 0.75 \mu\text{M}$) or IE6 cells ($IC_{50} = 10 \mu\text{M}$) (Singh et al. 1999; Qian et al. 2005). In 2008 (ovarian carcinoma) cells rho (0) cytotoxicity to cisplatin was lower ($IC_{50} = 3.5 \mu\text{M}$) than in their parental counterparts ($IC_{50} = 0.72 \mu\text{M}$) (Montopoli et al. 2011). In contrast, sensitivity to cisplatin ($IC_{50} = 5\text{--}6 \mu\text{M}$) in tumor cell lines highly dependent on glycolytic metabolism (and presumably with low mitochondrial activity) such as C13 ovarian carcinoma and their rho (0) counterparts was similar (Montopoli et al. 2011). These data strongly suggest that drug cytotoxicity depends on the presence of functional mitochondria.

Table 3 Effect on isolated mitochondria of first-line antineoplastic drugs

Mitochondrial origin	Drug	Doses	Effect	References
Rat adrenal	Adriamycin	$K_i = 100 \mu\text{M}$	P450 inhibition	Cuellar et al. (1984)
Rat heart and liver		0.5–2 mM; 50 μM /20 min; 80–200 μM	50–70 % Palmitate and palmitoyl-carnitine oxidation lowering. 16-times superoxide increment in Succ or GM-supported state 3 respiration; uncoupling of state 3 respiration	Nicolay et al. (1984), Abdel-Aleem et al. (1997)
Rat liver mitoplast		Rat injected i.p. with drug 2 mg/kg/3 times per week, 3 weeks or with 22.5 mg/kg after 24 h 40–250 μM	28–50 % Pyr or GM-supported state 3 respiration inhibition; 25 % ATP production decrement Binding to the mitochondrial membrane cardiolipin	Sviriaeva et al. (2007), Diotte et al. (2009), Bugger et al. (2011) Nicolay et al. (1984)
Rat kidney and liver; human head and neck squamous carcinoma	Cisplatin	Rat injected i.p. with drug 7.5–25 mg/kg/72 h or direct mitochondrial exposure to 10–100 μM ; 15 μM /1–4 h; 12–100 μM /40 min; 50 μM /1 h; 0.17–0.7 mM/1–3 h	Diminution in the COX and ATP activities; increment in mitochondrial Ca^{2+} efflux; uncoupling effect; cytochrome c release; altered mitochondrial morphology; binding to mitochondrial DNA and to the voltage anion channel. 50–60 % inhibition GM or Succ-supported state 3 respiration and ADP/O; inhibition of mtDNA synthesis	Aggarwal (1993), Yang et al. (2006), Garrido et al. (2008), Guerrero-Beltrán et al. (2010), Gaona-Gaona et al. (2011), Zsengellér et al. (2012)
Rat or sheep liver	Tamoxifen +E2	1–2 min/25–200 μM ; 1–2 min/10–75 μM ; 500 μM /2 h 25–30 μM /1–2 min	State 3 respiratory inhibition; complex I, III and IV respiratory chain inhibition; 90 % mitochondrial swelling, 10-times lowering in GSH levels; 63 % Ca^{2+} efflux 50 % Respiratory state 3 inhibition; 50 % lowering $m\Delta\Psi$; 50 % increment mitochondrial ROS production and 160 % respiratory State 4	Tuquet et al. (2000), Surendran and Krishnamurthy (2012) Moreira et al. (2006)
Rat heart	Sorafenib Dasatinib Sunatinib	100 nmol/mg mitochondrial protein $\text{IC}_{50} = 3 \mu\text{M}$ $\text{IC}_{50} = 5 \mu\text{M}$ $\text{IC}_{50} = 31 \mu\text{M}$ $\text{IC}_{50} = 124 \mu\text{M}$ $\text{IC}_{50} = 90 \mu\text{M}$	*State 3 respiration inhibition Complex II + III ATP synthase activity Complex I Complex IV (COX) activity ATP synthase activity Complex I activity ATP synthase activity	Will et al. (2008)
Rat brain, human neuroblastoma; human ovarian A2780 cancer Ehrlich ascites	Paclitaxel Lonidamine	10–100 μM ; 1 mM/1–4 h 160 μM <i>plus gossypol (10 μM)</i>	40 % Lowering in mitochondrial $\Delta\Psi$; 27–72 % cytochrome c release Altered mitochondrial morphology	André et al. (2000), Ferlini et al. (2003), Mironov et al. (2005) Floridi et al. (1984)

Table 3 continued

Mitochondrial origin	Drug	Doses	Effect	References
Rat liver	Etoposide	1 $\mu\text{mol}/2\text{--}10\text{ min}$; 10–25 μM ; 17 nM/1 h	MPT induction; uncoupling of Ca^{2+} -stimulated respiration; cytochrome c release	Robertson et al. (2000), Custódio et al. (2002), Park and Kim (2005)
Rat liver; digitonin permeabilized hepatocytes	Phenformin	1–6 mM; 100 nmol/mg mitochondrial protein/40 min	70–100 % Diminution in GM-supported state 3 respiration; 90 % Complex I inhibition; 50 % Complex II and III inhibition	Dykens et al. (2008), Drahota et al. (2013)
Rat liver, bovine heart	Buformin	100 nmol/mg mitochondrial protein/40 min $\text{IC}_{50} = 1.2\text{--}11\text{ mM}$	Full inhibition of GM-supported state 3 respiration Complex I inhibition	Dykens et al. (2008)
Rat liver, bovine heart Permeabilized rat hepatoma	Metformin	500 nmol/mg mitochondrial protein/40 min; 10 mM $\text{IC}_{50} = 66\text{ mM}$ 50–100 $\mu\text{M}/24\text{--}60\text{ h}$	33–100 % Glut/Mal-supported state 3 respiration diminution Complex I inhibition 40–100 % Inhibition of state 3 respiration with GM	Owen et al. (2000) Carvalho et al. (2008), Dykens et al. (2008)
Rat liver, heart and brain	Valproic acid	Rat injected i.p. with VA 500 mg/kg/4 days twice at day; Rat injected i.p. with VA 200–600 mg/kg; 0.24–2.4 mM	2-Times OGT increment; 6-times decreased 3-hydroxybutyrate content; 1.2–1.7-times short-, medium- and long-chain acyl-CoA dehydrogenase activities diminution; 20–80 % Succ or GM-supported state 3 respiration inhibition; uncoupling OxPhos effect	Haas et al. (1981), Rumbach et al. (1983), Kibayashi et al. (1999)
Permeabilized Myeloma KMS Cells	Bortezomib	50–75 nM, 48 h	Inhibition of state 3 respiration; lowering of intracellular ATP content	Song et al. (2013)
Rat liver	Adaphostin	25–100 $\mu\text{M}/2\text{--}3\text{--}5\text{ min}$	45–63 % State 3 respiration inhibition with Succ or GM; inhibition of complex III	Le et al. (2007)

State 3 respiration is the rate of oxygen consumption induced by adding ADP to isolated mitochondria incubated with oxidizable substrate and Pi. Therefore, the rate of state 3 respiration (multiplied by the P/O or ADP/O ratio) provides the rate of OxPhos (i.e., the rate of mitochondrial ATP synthesis). State 4 respiration is the rate of oxygen consumption by isolated mitochondria incubated with no ADP

E2 estradiol, COX cytochrome c oxidase, GM glutamate/malate, i.p. intraperitoneal, MPT membrane permeability transition, OGT oxaloacetate/glutamate aminotransferase, Pyr pyruvate, ROS reactive oxygen species, Succ succinate, $m\Delta\psi$ mitochondrial electrochemical H^+ potential

* No effect on state 3 respiration with imatinib, dasatinib and sumitinib was observed at concentrations up to 250 nmol/mg mitochondrial protein

Furthermore, we have evaluated the effect of the anti-mitochondrial drug Casiopeina II-gly (CasII-gly) (Marín-Hernández et al. 2003, 2012; Hernández-Esquível et al. 2006; Kachadourian et al. 2010) in combination with first-line canonical antineoplastic drugs in an effort to find chemotherapeutic synergism. Initial results in 24 h drug-treated HeLa cells have revealed that three out of 17 first-line antineoplastic drugs conventionally used at the Instituto Nacional de Cancerología de México induce a synergistic response with CasII-gly, diminishing viability and proliferation rates at nanomolar doses and without apparent effect on normal proliferating cells.

Another set of evidence clearly indicating that OxPhos is a direct target of several antineoplastic drugs is summarized in Table 3. Exposure of isolated mitochondria or permeabilized cells to chemotherapeutic drugs, at similar doses in which their protein- or DNA targets are inhibited, inhibit/activate within seconds or minutes different mitochondrial functions including OxPhos (state 3 respiration), Ca^{2+} homeostasis, generation and maintenance of mitochondrial $\Delta\Psi$, ROS generation, heat generation (i.e., uncoupling) and cytochrome c release (i.e., the initial step for apoptosis). In some of these experiments (Table 3), animals or cells were previously treated with anticancer drug for the indicated times and afterward, mitochondria were isolated or mitochondrial function was determined in permeabilized cells. The results of these last experiments are more difficult to interpret as a direct effect on mitochondrial function, because the long-term interaction between the anticancer drug and its primary target may also induce, indirectly, alterations of the mitochondrial functions. Nevertheless, these observations indicate that the anti-mitochondrial combination therapy (i.e., OxPhos inhibitor plus other canonical or non-canonical anticancer drug) arises as a promising alternative for cancer treatment.

Concluding remarks

Thorough analysis of the information available indicates that the effect of several canonical antineoplastic drugs used as first-line treatment certainly involves inhibition of a particular single specific complex signaling network, but it also involves the strong blocking of OxPhos and, to a lesser extent, glycolysis in tumors (Rodríguez-Enríquez et al. 2000; Marín-Hernández et al. 2006), which in turn could be associated with the severe side effects observed in patients. Therefore, strategies could be redirected to the design of combination therapy including selective OxPhos or/and glycolytic anticancer inhibitors and conventional drug therapy at nanomolar doses to avoid the undesirable side effects. Therefore, the identification of the principal ATP supplier (OxPhos and/or glycolysis) in tumors as the

relevant regulator of cancer growth/development emerges as a suitable and strong alternative in the design of new anticancer strategy therapies. This alternative metabolic therapy combined with first-line canonical anticancer drugs may help to improve the traditional therapies.

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