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To cite this version:

José M. Cuezva, Álvaro D. Ortega, Imke Willers, Laura Sánchez-Cenizo, Marcos Aldea, et al.. The tumor suppressor function of mitochondria: Translation into the clinics. Biochimica et Biophysica Acta - Molecular Basis of Disease, 2009, 1792 (12), pp.1145. 10.1016/j.bbadis.2009.01.006 . hal-00537250

HAL Id: hal-00537250

https://hal.science/hal-00537250v1

Submitted on 18 Nov 2010

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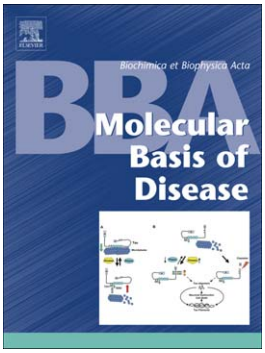

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PII: S0925-4439(09)00021-0
DOI: doi:10.1016/j.bbadis.2009.01.006
Reference: BBADIS 62916

To appear in: *BBA - Molecular Basis of Disease*

Received date: 23 September 2008
Revised date: 15 December 2008
Accepted date: 16 January 2009

Please cite this article as: José M. Cuezva, Álvaro D. Ortega, Imke Willers, Laura Sánchez-Cenizo, Marcos Aldea, María Sánchez-Aragó, The tumor suppressor function of mitochondria: Translation into the clinics, *BBA - Molecular Basis of Disease* (2009), doi:10.1016/j.bbadis.2009.01.006

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The Tumor Suppressor Function of Mitochondria: Translation into the Clinics.

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Keywords: Cancer, Cell cycle, Cell death, Glycolysis, H^+-ATP synthase, Markers of prognosis, Metabolic inhibitors, Oxidative phosphorylation, ROS.

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Summary

Recently, the inevitable metabolic reprogramming experienced by cancer cells as a result of the onset of cellular proliferation has been added to the list of hallmarks of the cancer cell phenotype. Proliferation is bound to the synchronous fluctuation of cycles of an increased glycolysis concurrent with a restrained oxidative phosphorylation. Mitochondria are key players in the metabolic cycling experienced during proliferation because of their essential roles in the transduction of biological energy and in defining the life-death fate of the cell. These two activities are molecularly and functionally integrated and are both targets of commonly altered cancer genes. Moreover, energetic metabolism of the cancer cell also affords a target to develop new therapies because the activity of mitochondria has an unquestionable tumor suppressor function. In this review, we summarize most of these findings paying special attention to the opportunity that translation of energetic metabolism into the clinics could afford for the management of cancer patients. More specifically, we emphasize the role that mitochondrial β-F1-ATPase has as a marker for the prognosis of different cancer patients as well as in predicting the tumor response to therapy.

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1. Introduction

Cancer is considered a highly heterogeneous and complex genetic disease that drives the progressive transformation of normal cells into malignancy by a multistep and sequential ordered process. However, and in addition to the contribution of genetic mutations in the well established cancer genes (oncogenes and tumor suppressors), the onset and progression of cancer is also bound to the cancer cell's microenvironment and to other epigenetic events that contribute to funnel the cell into malignancy. In this regard, large projects aimed at deciphering the genetic changes occurring in tumors are being questioned due to their limited applicability for the development of effective therapies [1]. These arguments [1,2] suggest that the development of an effective therapy against cancer will require the targeting of the biological pathways commonly altered in the cancer cell [1-5]. A starting point for this change of gear was the admirable summary of the phenotype of the cancer cell in the following six traits: an unlimited replicative potential, sustained angiogenesis, evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals and tissue invasion and metastasis [2]. Quite recently, the so-called "metabolic reprogramming" of the cancer cell has been added as a seventh hallmark of the cancer phenotype [3,5].

Indeed, the incidence of malignant disorders increases with age while the activity of mitochondrial oxidative metabolism diminishes in most tissues during the ageing process. The apparent inverse correlation that exists between mitochondrial activity and cellular transformation was first suggested by Otto Warburg back in the early days of the previous century (for review see [6]). He made the seminal observation that tumors have an abnormal high aerobic glycolysis and therefore proposed that the bioenergetic activity of mitochondria was impaired in the cancer cell [7,8]. However, with the advancement of molecular biology, Warburg's hypothesis was largely neglected [9-11] or considered an epiphenomenon of cell transformation [12] until the early days of the present century when it has been formally contrasted for a relevant part of it [13,14] (for review see [5]). Indeed, many biochemical, molecular, functional and clinical studies have confirmed that most human tumors and cancer cells display, even in the presence of oxygen, increased rates of glucose consumption and of lactate release when compared to normal tissues, extending the original Warburg observation to many different neoplasias [5]. However, the strongest backing to the so-called Warburg phenotype of the tumors came from the development and clinical application of tumor imaging by positron emission tomography (PET) using 2-deoxy-2-[^{18}F]fluoro-D-

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glucose (FDG) as a probe [15]. This technique, that actually measures the glucose analogue captured by the tumor relative to its surrounding non-tumor tissue, has proven to be one of the most effective techniques to detect tumors and its metastasis as well as for the staging, follow-up and prognosis of cancer patients [14,15]. In this regard, both FDG-PET and expression data of markers of mitochondrial bioenergetic function in tumors of lung cancer patients, as well as findings on the rates of glycolysis of cancer cells, have strongly supported that an altered oxidative phosphorylation is one of the determinants that underlies the abnormal aerobic glycolysis of the cancer cell [14].

The revitalization of energetic metabolism and hence, of the "mitochondrial interest" in cancer biology, has boosted in recent years because of the potential clinical and biotechnological interest that the peculiar metabolism of the cancer cell could afford in the combat of the disease. A significant number of studies devoted to understand the molecular basis of the enforced glycolytic phenotype of cancers have been developed. Most of them have reported links between oncogenes and/or tumor suppressors with the expression of enzymes of the glycolytic pathway and/or of mitochondrial oxidative metabolism (see Section 4). However, before summarizing these findings, aimed at unveiling the precise mechanisms that might underlie a particular transformation event, the simpler following scenario should be discussed: "an enforced glycolysis is a prerequisite for malignant transformation because it is the pathway that provides the metabolic precursors that could support cellular proliferation". In this review, we therefore summarize our present understanding of the metabolic trait of the cancer cell stressing the tumor suppressor function of mitochondrial oxidative phosphorylation. We further emphasize those aspects of energetic metabolism that might be exploited in the benefit of patients to facilitate the rapid translation of mitochondrial knowledge into the clinical setting. More detailed accounts of the alterations of energetic metabolism found in tumors have been recently summarized elsewhere [3,5].

2. The onset of cellular proliferation *per se* imposes a burst in aerobic glycolysis.

In non-proliferating cells the demand for glucose is low because the complete oxidation of glucose by mitochondrial activity coupled to respiration and oxidative phosphorylation provides a high yield of ATP (Fig. 1A). However, the efficient mitochondrial extraction of biological energy has the drawback that the glucose carbon skeleton is entirely oxidized to CO_2 and thus, provides no available matter for biosynthetic purposes (Fig. 1A). On the contrary, proliferating cells take up

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considerably much larger amounts of glucose than non-proliferating cells and partially oxidize the glucose carbon skeleton to lactate, which is excreted from the cell in order to regenerate the NAD^+ that is required for glycolysis to proceed (Fig. 1A). An increased glycolysis and truncated mitochondrial activity affords the proliferating cell with the large amounts of the precursors needed for the duplication of the cellular material as well as of the energy for the assembly of precursors into macromolecules (Fig. 1A). Indeed, highly proliferating cells have a truncated Krebs cycle linked to an enhanced citrate release from mitochondria for the purpose of supplying the cytoplasmic acetyl-CoA that is required for the biosynthesis of fatty acids [16,17] and cholesterol [18]. However, it should be noted that in cancer cells the mitochondrial oxidation of glutamine could replenish with carbon skeletons the Krebs cycle [19,20]. Besides, in the majority of cells mitochondrial respiration is also responsible for the production of reactive oxygen species (ROS) (Fig. 1A) that could become toxic molecules and eventually damage DNA if the ROS scavenging antioxidant defense is overwhelmed. A diminished mitochondrial activity in the proliferating cell (Fig. 1A) warranties less production of ROS at a time when DNA is most vulnerable because of its loosened and naked state. The largely divergent diagrams depicted on Fig. 1A illustrate two metabolic extreme situations for differentiated and proliferating cells and Figs. 1B and 1C provide some illustrative examples of these traits in non-tumor and breast cancer cells, respectively.

Aerobic glycolysis is sharply increased in lymphocytes [21,22] and thymocytes [23] when the cells are stimulated to proliferate (Fig. 1B). In lymphocytes, the burst of glycolysis coincides with the peak of synthesis of cellular DNA (Fig. 1B, upper panel) [21]. Concurrent with these changes, mitochondrial respiration (Fig. 1B) and the production of ROS are arrested in proliferating thymocytes [23]. Likewise, when breast cancer cells reach confluence cellular proliferation is diminished and the glycolytic flux is significantly reduced (Fig. 1C). Consistently, in the absence of growth factors that can stimulate cellular proliferation the expression of glucose and amino acid transporters are lost in human T-cells [24,25]. Conversely, when T-cells are stimulated with interleukin-7 the trafficking of GLUT1 to the plasma membrane, the uptake of glucose and the subsequent cell cycle progression are stimulated through a PI3K-Akt dependent pathway [22,25,26]. An immediate conclusion that stems from the particular metabolic trait of proliferating cells is that proliferation is only possible when there is a non-restrained availability of energetic substrates in the milieu because glycolysis is an

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energy- and matter-wasteful pathway (Fig. 1A) [5]. In any case, this "selfish" metabolic trait provides the cancer cell with the strategy that satisfies their matter and energy demands for tumor growth irrespective of host considerations (Fig. 1A).

3. Aerobic glycolysis controls progression through the cell cycle.

If proliferation is bound to aerobic glycolysis (Fig. 1) the relative activity of the metabolic pathways relevant for energy provision are also tightly regulated during the cell cycle of the eukaryotic cell. In fact, in *S. cerevisiae* a significant number of genes that have functions in energy metabolism are coordinately and periodically expressed during the cycle [27,28]. The yeast metabolic cycle can be divided into three different phases: (i) the oxidative, where the components of the translational machinery are transcribed and there is peak in RNA synthesis; (ii) the reductive/building, that comprises the expression of nuclear genes codifying for mitochondrial components; and (iii) the reductive/charging, that includes the expression of genes involved in glycolysis, breakdown of storage of carbohydrates and of the pentose phosphate pathway [27,28]. Consistently, human lymphocytes stimulated to proliferate show a peak in RNA synthesis that is previous to the triggering of aerobic glycolysis and the onset of DNA synthesis [21]. Moreover, it has been recently shown that the accretion of mitochondrial membranes, mitochondrial DNA (mtDNA) and of several mitochondrial proteins of the respiratory chain is carried out during the S phase of the cycle [29], temporally coinciding with the burst of glycolysis (Fig. 1B) that is in the reductive phase of the cycle. However, fully functional mitochondria are not achieved until the end of G2/M [29]. At this stage of the cycle a key protein of oxidative phosphorylation (β-F1-ATPase) is preferentially synthesized by translational activation of its mRNA [29].

Translation of β-F1-ATPase mRNA in the G2/M phase of the cycle, a time when *cap*-dependent translation is arrested [30], is possible because the 3' untranslated region (3'UTR) of the mRNA is endowed with an internal ribosome entry site (IRES)-like activity [31]. Changes in the translational efficiency of this mRNA have also been reported during liver development [32] and in cancer cells [33]. These findings suggest a relevant role for translation and specifically of *cap*-independent mechanisms of translation, in the control of the appropriate biogenesis of mitochondria both in normal and in cancer cells [29]. On the other hand, the full development of mitochondria at G2/M seems to be a likely mechanism to guarantee that the re-entrance into the oxidative phase of the cycle happens after the DNA is packaged, the cell cycle is concluded and

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the cell is bioenergetically ready to afford the energy demanding next step of active biosynthesis of the cellular constituents synthesized in G1 phase.

The supply of energy required to appropriately fulfill the tasks of the oxidative G1 phase of the cycle is stringently controlled before the cells could enter the reductive S phase. In fact, cells treated with oligomycin, a specific inhibitor of the mitochondrial H^+-ATP synthase, become reversibly arrested at the G1/S transition [34,35]. Similarly, *Drosophila* mutants on a gene that encodes subunit Va of cytochrome c oxidase, which limits cellular ATP availability, also arrests cell cycle progression at the same stage of the cycle [36]. Likewise, cells growing in a condition of low glucose availability also fall into cell cycle arrest at the G1/S boundary [37]. The G1/S arrest is achieved by the so-called metabolic stress checkpoint that is controlled by the activation of the AMP-activated protein kinase (AMPK) which triggers the phosphorylation of p53 and its subsequent cellular accumulation [37,38].

AMPK is a metabolic sensor of the cellular energy charge that controls the metabolic activity of the cell by inhibiting ATP-consuming anabolic processes upon nutrient deprivation [39]. In this regard, AMPK inactivates HMG-CoA reductase and acetyl-CoA carboxylase, thus arresting cholesterol and fatty acids biosynthesis, respectively [40]. Consistently, pharmacologic and/or genetic activation of AMPK bring about the inhibition of the proliferation of a number of cancer cells due to G1/S arrest [41]. More recently, it has been shown that disruption of complex I of the mitochondrial electron transport chain also specifically retards cell cycle progression during the G1-S transition [42]. In this case, the mechanism appears to involve the production of ROS, another retrograde signaling molecule originated in mitochondria [42]. In addition, active AMPK repress protein biosynthesis by the phosphorylation of both TSC2 and raptor, leading to the suppression of the rapamycin-sensitive mTOR pathway [43,44]. Intriguingly, it has been recently described that mTOR-raptor complex may control mitochondrial activity directly and independently of the previously identified targets of mTOR (S6K and 4EBPs), suggesting a reciprocal homeostatic loop of cross-regulation between mitochondria and mTOR activity during cell cycle progression [45,46]. It appears that cell cycle arrest is an emergency mechanism by which the cells enter a transient quarantine-like state to avoid energy depletion that would otherwise induce necrotic and/or apoptotic cell death. In any case, a sustained energy depletion stress, like a total glucose withdrawal from the culture media or a restrained blood flow, would induce apoptosis through the same AMPK-p53 signaling cascade [47].

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Once the cell overcomes the G1/S transition the mitochondrial bioenergetic activity is repressed as a result of cyclin D1 activity [48,49]. Cyclin D1 is involved in the phosphorylation and inactivation of the retinoblastoma protein, a process that marks the entry of cells into the S phase. Cyclin D1 is expected to control the metabolic switch from the oxidative to the reductive phase of the cycle to minimize the production of ROS at a time when the genetic material is most vulnerable [23,50]. Consistent with this suggestion, it has been shown that the quenching of ROS produced from dysfunctional mitochondria bypasses the G1/S checkpoint allowing cellular proliferation [42].

4. Cancer genes and energetic metabolism.

Diverse mechanisms have been proposed to explain the metabolic switch from mitochondrial respiration to glycolysis experienced by the cancer cell. In particular, those originating from genetic alterations in oncogenes and/or in tumor suppressors that impact on glycolysis or in mitochondrial activity and the biogenesis of the organelle.

The oncogene *c-myc*, which is frequently over-expressed in cancer cells, is involved in the transactivation of most of the glycolytic enzymes including lactate dehydrogenase A (LDHA) and the glucose transporter GLUT1 [51,52]. Thus, *c-myc* activation is a likely candidate to promote the enhanced glucose uptake and lactate release in the proliferating cancer cell (Fig. 2). More recently, and consistent with the relevant role of the oncogene in proliferation, *c-myc* has also been shown to control the transcriptional expression of essential genes required for the biogenesis of mitochondria [53] (Fig. 2) and to functionally coordinate both the metabolic and regulatory events required for entry and progression through the cell cycle [54]. In this regard, it has been reported that the *c-myc* dosage is important for sustaining tumorigenesis [55] because the expression of its targeted genes depends on *c-myc* expression levels [56].

At a certain stage of tumor development cancer cells grow in a hypoxic microenvironment. Hypoxia results in the stabilization of the transcription factor hypoxia inducible factor 1 alpha (HIF1α), which in turn induces the expression of GLUT1 and of many glycolytic enzymes as well as of the factors that trigger angiogenesis (VEGF) and cell growth and survival pathways (IGF-2) [57,58]. Recently, it has been reported that *c-myc* and HIF1α cooperate to induce the expression of pyruvate dehydrogenase kinase 1 (PDK1) and hexokinase II (HK-II) targeting in this way the two pathways of energetic metabolism and favoring the glycolytic phenotype of the cancer cell [59]. Indeed, the expression of PDK1, a protein that phosphorylates and

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inactivates the pyruvate dehydrogenase complex (PDH), inhibits the mitochondrial metabolism of pyruvate (Fig. 2) [53,60,61]. On the other hand, HIF1α also alters the activity of cytochrome c oxidase (COX) by changing the molecular composition of complex IV of the respiratory chain (Fig. 2). It seems that the preferred expression of COX4-2 in hypoxia (instead of COX4-1) contributes to a more efficient transfer of electrons to O_2 therefore limiting the amount of ROS generated in this situation [62]. In addition, it has been shown that a gain of function of HIF1α in renal cell carcinomas indirectly represses the biogenesis of mitochondria [63]. This situation originates from a deficient von Hippel-Lindau (VHL) factor that renders a constitutive activation of HIF1α under normoxic conditions (Fig. 2) [64-66]. HIF1α also induces the expression of MXI1, which inactivates *c-myc* and in turn could decrease the *c-myc* promoted mitochondrial biogenesis (Fig. 2) [63,67].

The oncogene Akt is a critical molecule in the transduction of signals that convey proliferation and apoptotic resistance [68]. An enhanced glycolytic metabolism seems to be essential for Akt-mediated cell survival upon growth factor withdrawal [69]. In addition, Akt promotes a dose-dependent stimulation of glycolysis (Fig. 2) that correlates with tumor aggressiveness *in vivo* [70]. In this regard, over-expression of Akt in non-invasive radial-growth melanomas induces the expression of glycolytic markers, stimulates glycolysis as well as transformation of the tumor to an invasive vertical-growth phenotype [71]. Recently, mitochondrial respiratory defects caused by genetic manipulation, chemical or hypoxic stresses have been shown to promote the inactivation of PTEN through a *redox* mechanism leading to Akt activation (Fig. 2) that results in drug resistance and survival advantage in hypoxia [72]. Furthermore, the PTEN/Akt/mTOR pathway also activates HIF1α (Fig. 2) thus reinforcing the shift from mitochondrial to glycolytic production of energy in cancer cells [73].

The tumor suppressor protein p53 also promotes the glycolytic phenotype of cancer cells. On the one hand, over-expression of a dominant negative mutant p53, a situation that is found in many tumors, leads to the induction of HK-II and to an increased glucose uptake (Fig. 2) [74,75]. Moreover, p53 induces the expression of TIGAR (TP53-induced glycolysis and apoptotic regulator), an inhibitor of fructose bisphosphatase-2, and down-regulates the expression of the glucose transporter GLUT1 [76,77]. Consequently, loss-of-function of p53 results in the down-regulation of TIGAR and the up-regulation of GLUT1, which in turn results in an enhancement of glycolysis (Fig. 2) [76-78]. On the other hand, it has been reported that p53 can regulate the

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activity of mitochondrial respiration through the activation of SCO2, which is a critical factor for assembling the cytochrome c oxidase (COX) complex (Fig. 2) [79]. Loss-of-function of either p53 or SCO2 results in a decreased rate of mitochondrial respiration and a metabolic shift toward glycolysis [79].

In addition to the metabolic trait imposed by proliferation and the effects of the hypoxic environment where the tumor develops it is also possible that the enforced aerobic glycolysis of tumors could result from mutations in nuclear and/or mitochondrial genes that impact on mitochondrial activity (Fig. 2). In this regard, it has been found that mutations in succinate dehydrogenase and fumarate hydratase predispose to different neoplasia syndromes (for review see [80]). Moreover, it has been reported that the accumulation of the Krebs cycle intermediates succinate and fumarate triggers the stabilization of HIF1α by inhibiting the prolyl-hydroxylase involved in the degradation of the transcription factor (Fig. 2) [81,82]. Consistent with the idea of retrograde signaling by mitochondria [83], it has been suggested that other metabolites such as pyruvate, oxaloacetate as well as ROS may stabilize HIF1α and thus provide in normoxic cells a positive feedback cycle of regulation [84,85].

Multiple mtDNA mutations that interfere with oxidative phosphorylation, increase ROS production and promote tumor cell proliferation have been described in tumors (Fig.2) [86]. For instance, complexes of the mitochondrial respiratory chain exhibit a diminished activity in cancer due to a decreased expression of the subunits encoded by mitochondrial DNA [87]. Cells totally depleted of mtDNA (ρ0 cells) have been used to asses the influence of mtDNA in tumor progression. However, these studies have yielded conflicting results [88,89]. It has been argued that ρ0 cells are not good representatives of tumor cells because cancer cells are not completely depleted of mtDNA. Therefore, studies with mtDNA-partially depleted cells (ρ- cells) or cells with defined mtDNA point mutations are nowadays being characterized to assess the contribution of mtDNA in cancer progression. The cybrid technology [90] developed in the laboratory of the late mitochondriologist Giuseppe Attardi (Pasadena, CA, 2008) has been especially useful in this regard. Using this model system different mtDNA mutations and partial mtDNA depletions have been related to tumorigenicity [91], tumor growth [92], metastasis [93] and invasion [94].

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5. Translation of mitochondria into the clinics: a protein signature of energetic metabolism.

In hepatomas, the complement of mitochondria is down-regulated when compared to paired normal hepatocytes [33,95]. Moreover, mitochondria in hepatomas have less *cristae* than in the normal cell [13], further supporting that the mitochondrial bioenergetic activity is compromised in cancer. Fetal rat hepatocytes very much resemble the mitochondrial phenotype of hepatomas, from the point of view of both the complement and activity of mitochondria ([96], for review see [97]) as well as of the mechanisms that regulate the expression of the catalytic β-F1-ATPase subunit of the mitochondrial H^+-ATP synthase ([32,98], for review see [6]). The metabolic and enzymatic analogies that exist between the phenotype of cancer cells and embryonic tissues have already been summarized [6]. However, it is worth mentioning here that the rapid changes that occur in the bioenergetic phenotype of liver mitochondria at the time of birth [96,98] mechanistically resemble very closely those that occur during cellular transformation [32,33]. Moreover, it has been documented that the expression of markers of mitochondrial energy metabolism during development of the liver inversely correlate with the expression of markers of the glycolytic pathway [6]. These observations prompted us to design a simple assay of the protein signature of energy metabolism [13] that could be translated into the cancer field with the purpose of contrasting the feasibility of the Warburg hypothesis [7,8].

With this aim, we studied in normal and tumor biopsies derived from the same patients the expression level of two mitochondrial proteins: β-F1-ATPase and Hsp60, respectively representing a bioenergetic and a structural mitochondrial protein. We completed the study by determining the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), used here as a representative of the glycolytic pathway [13,99]. This approach allows a protein estimation of both the bioenergetic competence of the organelle (assessed by the β-F1-ATPase/Hsp60 ratio) and of the overall mitochondrial potential of the cell (β-F1-ATPase/Hsp60/GAPDH ratio), the two levels that could reveal a compromised bioenergetic activity of mitochondria in the cancer cell [13]. This point is very relevant because, depending on the type of neoplasia being considered, a reduction of total mitochondrial mass or a specific reduction of the expression of β-F1-ATPase is observed in the tumors [13]. The ratio that estimated the overall mitochondrial potential of the cell was defined as the BioEnergetic Cellular

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(BEC) Index [13] which has been recently simplified to the β-F1-ATPase/GAPDH ratio [14].

Fig. 3A shows a representative example of these determinations by western blotting in surgical specimens of colon cancer patients. It is of interest noting the variability that could be observed for the expression of β-F1-ATPase between the tumors of the two patients depicted in the almost complete absence of changes for the expression of Hsp60 (Fig. 3A). As a result of these changes, colon cancers show a significant reduction of the β-F1-ATPase/Hsp60 ratio when compared to paired normal colon tissue (Fig. 3A), suggesting a deficit in the bioenergetic competence of the mitochondrion [13]. Remarkably, and consistent with Warburg's postulates, these changes in the mitochondrial proteome of the tumor were accompanied by the concurrent sharp increase of the glycolytic GAPDH marker (Fig. 3A). Consistently, the estimation of the overall mitochondrial activity of the cell relative to its glycolytic potential (BEC index), revealed larger differences between normal and tumor tissue (Fig. 3A), supporting a bioenergetic deficit of mitochondria in colon cancer [13]. This analysis, that was defined as *the bioenergetic signature* of cancer [13], was extended to most prevalent human tumors [99]. The results obtained revealed that the down-regulation of the β-F1-ATPase protein provides a proteomic feature of many different types of human carcinomas [13,14,99-101] with the exception of prostate cancer [99]. These findings have been largely confirmed in other laboratories ([102] and see [5] for extensive review).

The reported changes in the bioenergetic signature of colon, lung, breast, gastric, esophageal and renal cancer suggested, at the protein level, a bioenergetic impediment of mitochondria of the cancer cell in these tissues. Functional demonstration of this point came when the expression level of β-F1-ATPase and the bioenergetic signature of lung cancer were shown to correlate inversely with the rate of glucose capture of the tumors as assessed *in vivo* by FDG-PET imaging and with the rates of aerobic glycolysis of cancer cells [14], strongly supporting that the down-regulation of the bioenergetic function of mitochondria is a hallmark of cancer and part of the mechanism that triggers the increased glucose avidity of the cancer cell. Therefore, the bioenergetic signature also informs of the functional activity of energy provision pathways.

The transfer of results obtained at the bench-side in benefit of the management and treatment of patients is often hampered by the lack of clinically oriented studies and by the availability of the adequate tools required to implement such findings in the

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patient's setting. A paradigm of this desperately common and frustrating situation is the discovery of many cancer biomarkers and its rare translational application. To bridge this gap, we have studied the bioenergetic signature of tumors in large cohorts of different cancer patients [13,14,100,101]. Fig. 3B and Fig. 3C provide a representative example of this sort of study with a cohort of stage II colon cancer patients [13]. In this case, the expression of markers of the bioenergetic signature was carried out by immunohistochemistry using colon-tissue microarrays (Fig. 3B). The results confirmed previous findings obtained by other approaches in a reduced cohort of the same type of cancer patients (Fig. 3A) [13]. Indeed, it was observed that the expression of β-F1-ATPase and the BEC index were significantly reduced in colon cancer when compared to paired normal tissue (Fig. 3B). However, it was outstanding that the expression of β-F1-ATPase in tumors of the patients that had recurrence of the disease was much less than that in tumors of patients that survive colon cancer (Fig. 3B) [13]. Consistent with this observation, it was found that tumor β-F1-ATPase expression afforded an excellent marker of both the overall and disease-free survival (Fig. 3C) of colon cancer patients [13]. In fact, the tumor expression of β-F1-ATPase allows the discrimination of the patients that have a higher risk of disease recurrence (Fig. 3C). Similarly, the bioenergetic signature has also been shown to provide relevant markers of disease progression in lung [14,100] and breast [101] cancer patients, strongly supporting the relevant role that the mitochondrial impairment of the cancer cell plays in progression of the disease. Very recently, our findings on the clinical utility of β-F1-ATPase as marker of colon cancer prognosis have been confirmed in a different large cohort of colon cancer patients [102]. Interestingly, in that study it is shown that neither mtDNA content nor the tumor expression of TFAM provided markers of prognosis in colon cancer [102].

The specific molecular mechanisms that promote the down-regulation of the mitochondrial β-F1-ATPase in the cancer cell remain to be determined. However, as indicated previously, the expression of β-F1-ATPase during development of the liver [32,98,103,104], progression through the cell cycle [29] and in liver carcinogenesis [6,33] is regulated by post-transcriptional mechanisms that affect the subcellular localization, the stability and the translation of the mRNA. The 3'UTR of β-F1-ATPase mRNA is essential for efficient translation of the transcript [31,32,105]. It appears that translation masking of the mRNA is due to the binding of proteins (β-mRNABPs) to the 3'UTR of the transcript which presumably hamper its role in translation [6,32,33].

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Within this context, we have pursued the identification of the β-mRNABPs that interact and could define the bioenergetic phenotype of the cancer cell in order to establish its relevance as markers of breast cancer progression.

The AU-rich element (ARE) binding protein HuR, a central regulator of post-transcriptional gene expression [106] whose activity and sub-cellular localization is tightly regulated during the cell cycle [107,108] and that controls the expression of cell cycle relevant proteins [107-109], has been identified as a 3'UTR β-mRNA interacting protein [110]. Mechanistically, HuR over-expression and shRNA-mediated silencing in different human cells indicate that the protein is not regulating β-F1-ATPase expression [110]. However, the analysis of the expression of HuR in a large cohort of breast carcinomas has pointed out its relevance as an independent marker of breast cancer prognosis [110]. In fact, when HuR expression is studied in combination with the bioenergetic signature of the tumor it allows the identification of breast cancer patients at higher risk of disease recurrence [110]. These results strongly encourage the incorporation of HuR as an additional protein marker of the bioenergetic signature for the follow-up of breast cancer patients.

Mitochondrial activity and specifically oxidative phosphorylation play a relevant role in facilitating the execution of cell death (see section 6) [34]. In this regard, it appears that the bioenergetic signature of the cell or tumor also affords a marker of the cellular response to chemotherapy in colon [111] and liver [34] cancer cells. Recently, the down regulation of β-F1-ATPase expression in colon cancer patients has been shown to provide a significant predictive marker of the response of the tumors to the treatment with 5-fluorouracil [102]. Overall, the relevance of protein markers of energetic metabolism in the prognosis and eventual treatment of cancer patients provide strong support to push forward the translation of mitochondria into the cancer field. The development of high affinity and specific monoclonal antibodies against these markers, as well as the methodology to quantify its expression in tumor biopsies (Acebo *et al*, unpublished data), has represented an additional step forward of our lab in bridging mitochondrial research to the bedside of cancer patients.

6. The tumor suppressor function of mitochondrial activity.

Deregulated cell division and cell death are hallmarks of cancer acquired by defects in signaling pathways [2]. Mitochondria play an essential role in cell death

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[112,113]. Different stimuli that converge at the mitochondrial level provoke the permeabilization of its outer membrane and the subsequent release of different proteins that participate in the execution of cell death [114]. Mitochondrial geared cell death is regulated by members of the Bcl-2 family of proteins [115-117]. To evade apoptosis cancer cells develop different strategies such as the inactivation of p53 [118,119], induction of senescence [120], disruption of the Fas-death receptor pathway [121], upregulation of inhibitors of apoptosis (IAPS) [122], down-regulation of caspases [123,124], the activation of the PI3K/Akt/PTEN pathway [125] and the restriction of the bioenergetic activity of mitochondria [34,113]. Indeed, the generation of ROS that mostly depends on the activity of mitochondrial respiration is known to promote changes in the signal transduction pathways that control the expression of genes required to execute cell death [126,127].

Cellular metabolism is molecularly and functionally integrated with cell death [128-131] and both oxidative phosphorylation and the H^+-ATP synthase itself [34,132-136] are required for the efficient execution of death. In this regard, Bax induced toxicity in yeast cells has been shown to require oxidative phosphorylation [137]. Screens aimed at the identification of genes that could confer a Bax-resistance phenotype have identified a subunit of the H^+-ATP synthase critical for Bax-mediating killing of *S. cerevisiae* [134]. Moreover, Bax-mediated killing has been shown to be strictly dependent upon the nuclear encoded β-subunit of the H^+-ATP synthase (β-F1-ATPase) and mitochondrial genome-encoded proteins [138]. Recent studies in mammalian cells have further provided an insight on the mechanism of participation of the H^+-ATP synthase in the execution of cell death [34]. It has been shown that this complex is involved in the generation of ROS in the early induction phase of programmed cell death [34]. ROS are further responsible for the oxidation and covalent modification of mitochondrial constituents [34] facilitating in this way the release of the mitochondrial proteins [34] that effectively swamp the cells into death. Indeed, inhibition of ROS production with oligomycin prevented these modifications [34], strongly supporting the role of the H^+-ATP synthase in controlling the extent of mitochondrial damage and the execution of cell death.

Moreover, it appears that the dependence on oxidative phosphorylation for cellular ATP provision defines the susceptibility of a cell to execute ROS-dependent cell death by the mitochondrial geared pathway [34], in agreement with other reports [132,133,135,137] and with the role of the H^+-ATP synthase in cell death [134,138]. In

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line with these findings, we have recently verified that cells that rely heavily on glycolysis for energy provision display an apoptotic resistant phenotype [34] similar to ρ^o cells [132,133,135] because ROS signaling after chemotherapeutic targeting of the mitochondria is blunted [34]. Therefore, it is not surprising that the expression of the H^+-ATP synthase, and perhaps of other OXPHOS complexes, is down-regulated in most human tumors (for review see [5]).

The metabolism of cancer cells is relevant for cancer treatment and could define the pathway and response of the tumor to chemotherapy and/or radiotherapy (Fig. 4). In cancer cells with an active oxidative phosphorylation ROS production after therapy can engage cells into any of the pathways of programmed cell death (Fig. 4) [113]. In contrast, cancer cells with a predominant aerobic glycolysis will become resistant to the same therapy (Fig. 4). Therefore, the strategy of changing the metabolism of the cancer cell to oxidative phosphorylation may restore the cell sensitivity to apoptotic inducers and promote arrest of tumor growth. Alternatively, the strategy in glycolytic tumors might be the employment of alkylating agents that kill cells by programmed necrosis (Fig. 4) [139].

The renaissance of energetic metabolism of cancer cells has encouraged investigations aimed at establishing a link between this metabolic trait and tumor growth. Different experimental approaches, from cell lines to animal models aside from those already discussed, have been used to address this issue and are summarized in the following. Frataxin, a mitochondrial protein defective in Friedreich ataxia, is known to increase oxidative metabolism and ATP synthesis [140] probably by interacting with respiratory chain components [141] and/or by regulating the synthesis of Fe/S clusters [142]. Patients suffering from Friedreich ataxia, (i.e., with low frataxin levels) have a defective ATP synthesis and occasionally develop tumors at an unusual young age. Recently, a model of down-regulation of mitochondrial function was created by disrupting the frataxin gene in the liver of mice [142]. Consistent with Warburg's postulates, the frataxin knockout mice had a shortened life span and the mitochondrial impairment displayed in the hepatocytes was accompanied by tumor development [142]. Conversely, the same group has shown that the over-expression of frataxin in colon cancer cells triggers an increase in oxidative metabolism concurrent with a decrease in cellular growth rates, colony forming capacity as well as the tumorigenicity of the cells when injected into nude mice [143]. Taken together, the frataxin models

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support that interfering with mitochondrial function predisposes to cancer development and tumor growth.

Another experimental system linking defective oxidative phosphorylation and tumor growth is provided by studies with the von Hippel-Lindau (VHL) tumor suppressor. When VHL deficient cells ectopically expressing a functional pVHL protein are injected into nude mice, tumor formation and growth is retarded [144]. At that time VHL was described as a key player in the regulation of tumor glycolysis due to its ability to bind and promote the oxygen dependent degradation of HIF1α (Fig. 2) [145]. In recent years, VHL has also been described as an activator of oxidative phosphorylation because VHL deficient cells ectopically expressing wild-type pVHL triggered the biogenesis of mitochondria and a concurrent increase in the functional activity of oxidative phosphorylation [146]. Therefore, it seems reasonable to conclude that VHL deficiency is one of the factors responsible for the down-regulation of the biogenesis of mitochondria while promoting the activation of glycolysis via HIF1α in some tumors (Fig. 2).

Genetic and metabolic stresses of mitochondria have been used to assess that a dysfunctional oxidative phosphorylation is also associated with the trait of invasion and metastasis of the cancer phenotype. In this regard, it has been shown that the non-invasive tumor A549 cells become highly invasive when subjected to the above mentioned manipulations [147,148]. Likewise, a genetic stress in mitochondria of C2C12 cells also results in an invasive phenotype of the manipulated cells [148]. The mechanism appears to involve the over-expression of cathepsin L (an extracellular matrix protease) and TGFβ, which are relevant markers of invasive tumors [148]. These results have been further confirmed in osteosarcoma cells, in which defects in oxidative phosphorylation induced by similar approaches (mtDNA depletion and/or mutations, exposure to KCN and hypoxia) promote cellular invasiveness as well as changes in the expression of genes involved in the remodeling of the extracellular matrix [94]. Overall, these findings support that the activity of mitochondrial oxidative phosphorylation might also play a role in cell migration and tissue invasion.

The mitochondrial PDH complex is a key enzyme that regulates the flux between glycolysis and oxidative phosphorylation due to its positioning at the cross-road of both metabolic pathways [5]. The activity of the complex is inhibited by pyruvate dehydrogenase kinases (PDKs) that phosphorylate the pyruvate dehydrogenase E1α subunit of the complex. Inhibition of PDK1 expression in human carcinoma cells

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diverts pyruvate to mitochondrial metabolism promoting the activity of oxidative phosphorylation [85]. The activation of PDH also has an effect in cell proliferation and invasion. The knockdown of PDK1 in metastatic cells reveals that they are more prone to die in hypoxic conditions, release less VEGF and form tumors with a reduced growth rate [85], highlighting the beneficial effect that restoring mitochondrial metabolism have in tumor progression.

Finally, glycolysis interfering models have also been developed to study the link between aerobic glycolysis and tumor growth. In this regard, it has been shown that knocking down the glycolytic enzyme LDHA in mouse mammary tumor cells trigger a reduction in the tumorigenicity and tumor growth rate of these cells when transplanted into mice [149]. Moreover, LDHA knockdown cells exhibited increased mitochondrial function and compromised ability to proliferate under hypoxia [149]. These results show the tight connection that exists between glycolysis and oxidative phosphorylation in this specific case mediated by the cytoplasmic enzyme placed at the branch point of pyruvate metabolism [5]. These findings also confirm that inhibition of glycolysis can restrict the ability to proliferate of cancer cells. Overall, all these findings with different cellular and animal models strongly support and confirm Warburg's postulates, i.e., the bioenergetic activity of mitochondria of the cancer cell must be impaired. Mitochondrial oxidative phosphorylation has an undisputable tumor suppressor function.

7. Energetic metabolism provides a promising target for cancer treatment

Nowadays, it is accepted that the peculiar metabolism of the cancer cell provides a target to develop strategies that could halt disease progression [4,12]. In this regard, several small molecules interfering with cancer specific metabolic pathways are being tested either alone or in combined chemotherapy. Table I summarizes some of the compounds that are currently being evaluated for clinical use in the treatment of cancer and are discussed in the following.

The non-metabolizable glucose analogue *2-deoxyglucose* (2DG) blocks glycolysis [150,151] because 2-DG is phosphorylated by hexokinase and cannot be further metabolized, leading to the exhaustion of cellular ATP and cell death [152]. 2-DG treatment sensitizes gliomas to radiation therapy [153] and increases the efficacy of several chemotherapies in osteosarcomas, head and neck, and colon cancer cells [154-156]. Like 2-DG, *3-bromopyruvate* (3-BrPA) has been shown to abolish the production of ATP by inhibiting the glycolytic enzyme hexokinase [157-159]. Hexokinase II, that

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is over-expressed in carcinomas [160-162] bound to the mitochondria [163], has a central role in the regulation of cell death [164]. The efficacy of the small alkylating agent 3-BrPA in the eradication of liver-implanted tumors and in the induction of apoptosis has been successfully tested *in vivo* without apparent toxicity [157,158,165]. Recent studies using FDG-PET have evaluated the antitumor activity and biodistribution of 3-BrPA in a rabbit tumor model [166,167].

Several other small compounds have also been tested as chemotherapeutic agents. In fact, the anticancer activity of *iodoacetic acid* (IAA), a well known inhibitor of glycolysis that targets GAPDH [168], has shown anti-tumor activity in an ascites tumor model [169]. *Lonidamine*, represses glycolysis by inactivation of hexokinase [170] and enhances the radiation effects on mice tumor models [171]. The ability of lonidamine to suppress the energy metabolism of cancer cells and to improve the activity of other anticancer agents has led to phase II–III clinical trials of this compound in combination with other anticancer agents for the treatment of glioblastomas, breast, ovarian, and lung cancers [172-175]. The glycolytic inhibitor, *5-thioglucose* (5-TH), has also been classified as an antineoplastic agent because it promotes a drop in glucose uptake and under hypoxic conditions triggers the induction of cell death [176-178]. *Somatostatin* and its structural analogue, TT-232, have been found to interact with type M2 of pyruvate kinase (PKM2). PKM2 is over-expressed during oncogenesis and presumably responsible for diverting glycolytic pyruvate away from mitochondria [179]. It appears that the translocation of PKM2 to the nucleus in response to TT-232 treatment is sufficient to induce cell death [180]. *Mannoheptulose* is another non-metabolizable glucose analog with known anticancer effects that inhibits glucokinase activity, decreases glucose uptake and suppresses tumor cell growth in an ascites tumor model [181,182]. *Glufosfamide*, a new alkylating agent that uses the transport system of glucose [183], has been successfully tested in pancreatic and other cancer cells [184-186]. Glufosfamide treatment also enhances the reduction of pancreatic tumors when used in combination with gemcitabine [187]. There are several other compounds that inhibit glycolytic metabolism although their effects as anticancer agents remain to be studied. Of note, *α-chlorohydrin* and *ornidazole*, that inhibit GAPDH [188,189] and *oxalate*, that inhibits pyruvate kinase (PK) [190].

Another plausible approach to exploit the energetic metabolism with anticancer purposes is the enhancement of mitochondrial activity [85,143,149]. In this regard, *dichloroacetate* (DCA), a small molecule used in the past to treat metabolic disorders

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[191] has proven its utility in promoting apoptosis and halting tumor progression in mice models without apparent toxicity [192]. DCA is a well-characterized inhibitor of PDKs [193] that shifts the metabolism of pyruvate from glycolysis to its terminal oxidation. A recent study has also demonstrated that DCA is effective in sensitizing invasive endometrial cancer cells to apoptosis [194].

Molecules promoting the pro-apoptotic activity of mitochondria that trigger outer membrane permeabilization (MOMP) have also been proposed as anticancer agents (For review see [195]). In this regard, *Arsenite* has been shown to sensitize human melanomas to apoptosis via tumor necrosis factor-alpha (TNF-α)-mediated pathway [196,197]. *Betulinic acid*, a natural pentacyclic triterpenoid, has proven its ability to induce apoptosis in tumor cells via the mitochondrial pathway and also to stimulate the effect of several anticancer drugs [198,199]. *Jasmonates,* which are plant stress hormones, induce cytocrome c release and mitochondrial swelling in cancer cells but not in their normal cells counterparts [200,201].

The pentose phosphate pathway (PPP) rate-limits cell proliferating because it provides the ribose required for nucleic acid biosynthesis [5]. It has been suggested that the inhibition of transketolase (TK) would cause deprivation of glyceraldehyde-3-phosphate and hence limit ATP generation, as well as the NADPH and ribose required for macromolecule biosynthesis [202,203]. Indeed, *oxythiamine*, an irreversible inhibitor of TK, decreases tumor growth both *in vitro* and *in vivo* [202,203]. The PPP can also be interfere by *6-aminonicotinamide* (6-AN) that inhibits the activity of glucose-6-phosphate dehydrogenase (G6PD). The anticancer activity of 6-AN is mediated through oxidative stress and in combined therapy contributes to sensitize the cells to radiation and other anticancer agents [204-206].

Cancer progression requires the biosynthesis of essential lipid components of biological membranes. The fatty acid synthase (FAS), a multifunctional enzyme, is over-expressed in most human cancers [207-211] and the inhibition of FAS activity by *cerulenin* promotes apoptosis in several cancer cell lines [208,212,213]. Moreover, *soraphen A*, an inhibitor of acetyl-CoA carboxylase (ACC), has been shown to induce growth arrest in cancer cells [214]. *Etomoxir*, the specific inhibitor of carnitine palmitoyl transferase 1 (CPT1), which is a bottleneck for the entrance and oxidation of fatty acids in the mitochondria, might as well have some beneficial effects in the treatment of cancer [154]. Therefore, it appears that the peculiar lipid metabolism of cancer cells also affords an attractive target for cancer therapy.

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8. Concluding remarks.

The genetic and epigenetic changes that trigger cellular transformation are invariably bound to an increase in the glycolytic flux and a restrain in the bioenergetic function of mitochondria. This is so, because cellular proliferation is controlled by the synchronous changes in the activity of the two energy provision pathways as the cells progress through the different phases of the cycle. The "metabolic cycle" is expressed in tumors at the proteome level by significant reductions in the relative expression of β-F1-ATPase when compared to paired normal tissue. The "abnormal" expression of this relevant marker of mitochondrial energy transduction further provides a bioenergetic signature of the tumor with potential clinical utility for the prognosis and eventual treatment of cancer patients. Moreover, the metabolic hallmark of cancer further affords a target for the development of new anti-tumor therapies. It is obvious that during the last decade we have experienced a boost in the interest of mitochondria because of its relevance in programmed cell death and in a growing number of human diseases. However, our knowledge of the mitochondria in the different human tissues that could develop a neoplasia is negligible; mostly, because the molecular composition, the number and functional relevance of the organelle is very different from one tissue to another. Therefore, we urgently need to study the specific mechanisms that control the biogenesis of mitochondria in the different mammalian tissues in order to understand the real contribution of mitochondria to the different types of cancers. Overall, there is much to be done ahead in this field and the laboratories traditionally involved in mitochondrial research are excellently suited and socially compelled to embark in the struggle against cancer and to promote the basic and clinical advances required to definitively transform cancer into a chronic disease.

Acknowledgments

The authors gratefully acknowledge the work, support and ideas of many colleagues and collaborators who contributed in the past to characterize the role of mitochondria in cancer progression. Work in the authors' laboratory was supported by grants from the Ministerio de Educación y Ciencia BFU2007-65253, by the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII and by Comunidad de Madrid (S-GEN-0269), Spain. The CBMSO receives an institutional grant from Fundación Ramón Areces. We apologize to authors whose work or primary references could not be cited owing to space limitations.

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Legend to Figures.

Figure 1. The glycolytic phenotype of proliferation. (A) Schemes highlighting the metabolic phenotype of quiescent and proliferating cells. In quiescent cells, most of the glucose taken up by the cell is completely oxidized within mitochondria generating CO_2 and a high yield of ATP by oxidative phosphorylation. Mitochondrial activity also generates reactive oxygen species (ROS) in the respiratory chain. In contrast, proliferating cells take up much larger amounts of glucose that is partially oxidized in the cytoplasm by the glycolytic pathway to generate lactate that is further excreted from the cell. The high glycolytic flux generates most of cellular ATP requirements and provides the metabolic intermediates that become precursors for the biosynthesis of the macromolecules required for proliferation. Mitochondria in proliferating cells are not committed to energy generation and therefore produce little CO_2 and ROS and export to the cytoplasm the precursors required for lipid biosynthesis. **(B)** Proliferation in normal cells is bound to the sharp increase in aerobic glycolysis. Upper panel represents the time course of the glycolytic flux (blue) and synthesis of DNA (red) in lymphocytes stimulated to proliferate. These data have been taken and redrawn from reference [21]. The lower panel illustrates the origin of ATP generated in quiescent (Q, red bars) and proliferating (P, blue bars) thymocytes. The represented data have been taken and redrawn from reference [23]. **(C)** Glycolytitc flux measured in different breast cancer cells during proliferating (blue bars) and at confluent stage (red bars). The results shown are means ± s.e.m. of 3 independent experiments. *, $p<0.05$ when compared to confluent by Student's t test.

Figure 2. Cancer genes and energetic metabolism. The diagram shows several pathways that could promote the glycolytic phenotype of cancer cells. Proliferation favors the glycolytic production of ATP and restrains the bioenergetic activity of mitochondria. Gain-of-function mutations in the oncogenes Akt and *c-myc* lead to an increased glycolysis through induction of the glucose transporter GLUT1 and/or of glycolytic enzymes. Loss-of-function mutations in the tumor suppressor p53 cause the simultaneous induction of glycolysis and an impairment of mitochondrial respiration, thus promoting aerobic glycolysis. The hypoxic environment of tumor cells induces the stabilization of HIF1α, which enforces glycolysis by promoting the concurrent expression of glycolytic enzymes and restraining oxidative phosphorylation. In addition, mtDNA and nDNA mutations that limit mitochondrial activity could favor the

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glycolytic pathway in cancer cells. Deficiencies in mitochondrial metabolic and respiratory functions could cause both an increase in ROS and of the metabolic intermediates succinate and fumarate, which in turn could provoke the stabilization of HIF1α leading to a positive feed-forward cycle of glycolytic enforcement. Loss-of-function mutations of VHL cause a failed degradation of HIF1α that could inhibit mitochondrial biogenesis by activation of the *c-myc* inhibitor MXL1. For other details, see the text.

Figure 3. The bioenergetic signature of colon cancer. (**A**) Expression of β-F1-ATPase, Hsp60 and GAPDH in normal (N) and tumor (T) biopsies of two different colorectal cancer patients (X, Y). Histograms to the right illustrate the drop in β-F1-ATPase/Hsp60 ratio and the concurrent increase in the expression of the glycolytic GAPDH in colon cancer. Consistent with these changes, the BEC index of the tumors is sharply diminished when compared to paired normal colon. For other details see the text. These data has been taken and redrawn from reference [13]. *, illustrate significant differences when compared to normal. (**B**) Immunohistochemical analysis of the expression of β-F1-ATPase, Hsp60 and GAPDH in colorectal carcinomas using colon-tissue microarrays. Histograms to the right illustrate the absolute amount (a.u.) of the expression of β-F1-ATPase and GAPDH (O. D.) and of the BEC index in normal (N) and tumor (T) samples derived from patients with progressive disease (blue bars) and no-evidence of disease (red bars) after a median of 60 months clinical follow up. These data has been taken and redrawn from reference [13]. * and #, illustrate significant differences when compared to normal or no-evidence of disease group, respectively. (**C**) Kapplan-Meier disease-free survival (DFS) analysis of colon cancer patients [13] reveals the significant association of the expression level of β-F1-ATPase with metastatic disease. High and low expression levels of β-F1-ATPase for red and blue curves, respectively. Log-rank significance is indicated. Cox regression analysis revealed a significant higher relative risk of disease recurrence in patients expressing low levels of β-F1-ATPase.

Figure 4. Energetic metabolism and cell death. Cancer cells have different susceptibility to death induced by chemotherapeutic drugs as a result of their metabolic phenotype. A first event after priming the cell to death is the rapid dismantling and fragmentation of the mitochondrial tubular network into small mitochondria (in green).

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Tumor cells relying on oxidative phosphorylation (OXPHOS) for energy provision show a rapid burst in ROS production that oxidizes mitochondrial proteins (red dots) favoring in this way the release of mitochondrial proteins that will effectively swamp the cell into programmed cell death (fragmented nucleus in blue). Cancer cells that have a high glycolytic metabolism have a diminished and/or blunted ROS response to chemotherapy and experience no relevant cell death. In this situation it is suggested that metabolic inhibitors targeting energetic metabolism could kill the cancer cell by programmed necrosis (blast of cell in light blue).

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Table I. Small compounds targeting the metabolism of the cancer cell with potential clinical utility. GAPDH, glyceraldhyde 3-P-dehydrogenase; PK, pyruvate kinase; PDK1, pyruvate dehydrogenase kinase 1; G6PD, glucose-6-phosphate dehydrogenase; TK, transketolase; MOMP, mitochondrial outer membrane permeabilization; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; CPT1, carnitine palmitoyl transferase 1; PPP, pentose phosphate pathway.

#	Compound	Metabolic Process	Target	Clinical trials
1	2-deoxyglucose	Glycolysis	Hexokinase	Phase I/II
2	3-bromopyruvate	Glycolysis	Hexokinase	Pre-clinical
3	lonidamine	Glycolysis	Hexokinase	Phase II/III
4	5-thioglucose	Glycolysis	Hexokinase	Pre-clinical
5	mannoheptulose	Glycolysis	Glucokinase	Pre-clinical
6	iodoacetic acid	Glycolysis	GAPDH	Pre-clinical
7	α-chlorohydrin	Glycolysis	GAPDH	---
8	ornidazole	Glycolysis	GAPDH	---
9	oxalate	Glycolysis	PK	Pre-clinical
10	somatostatin	Glycolysis	PK	Phase I-III
11	dichloroacetate	Pyruvate oxidation	PDK1	Phase I/II
12	glufosfamide	Glycolysis/ PPP	Glucose transporter	Phase I/II
13	6-aminonicotinamide	PPP	G6PD	Pre-clinical
14	oxythiamine	PPP	TK	Pre-clinical
15	arsenite	apoptosis	MOMP	Pre-clinical
16	betulinic acid	apoptosis	MOMP	Phase I/II
17	jasmonates	apoptosis	MOMP	Pre-clinical
18	soraphen A	Fatty acid synthesis	ACC	Pre-clinical
19	cerulenin	Fatty acid synthesis	FAS	Pre-clinical
20	etomoxir	β-oxidation	CPT1	Pre-clinical

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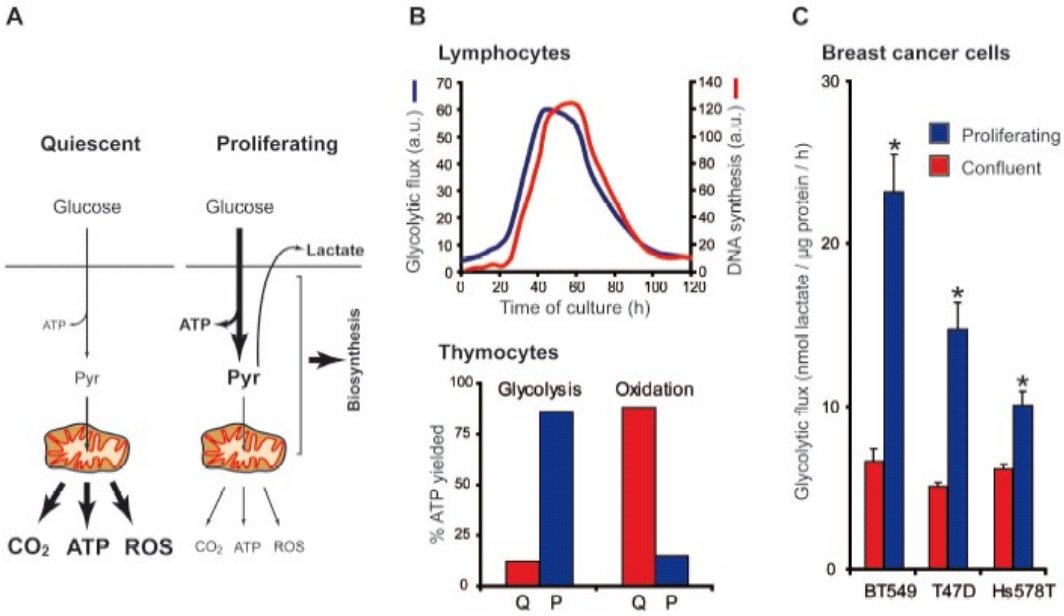

FIGURE 1

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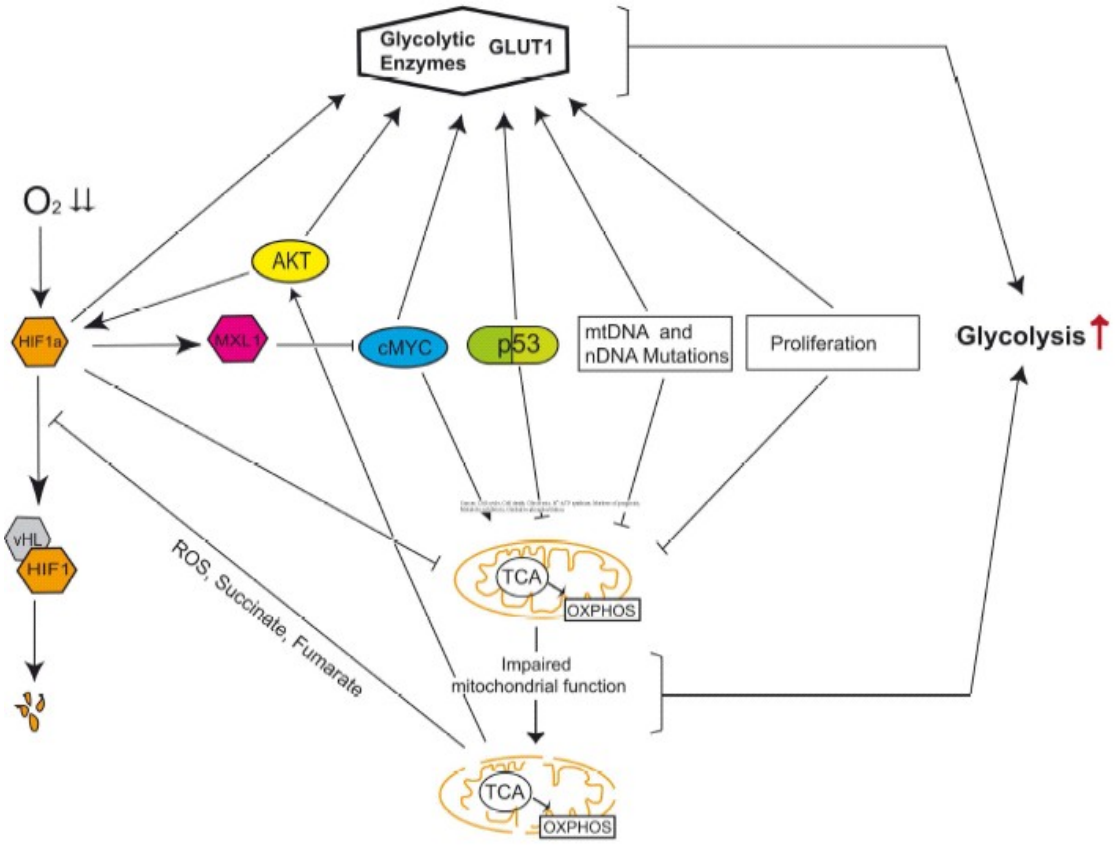

FIGURE 2

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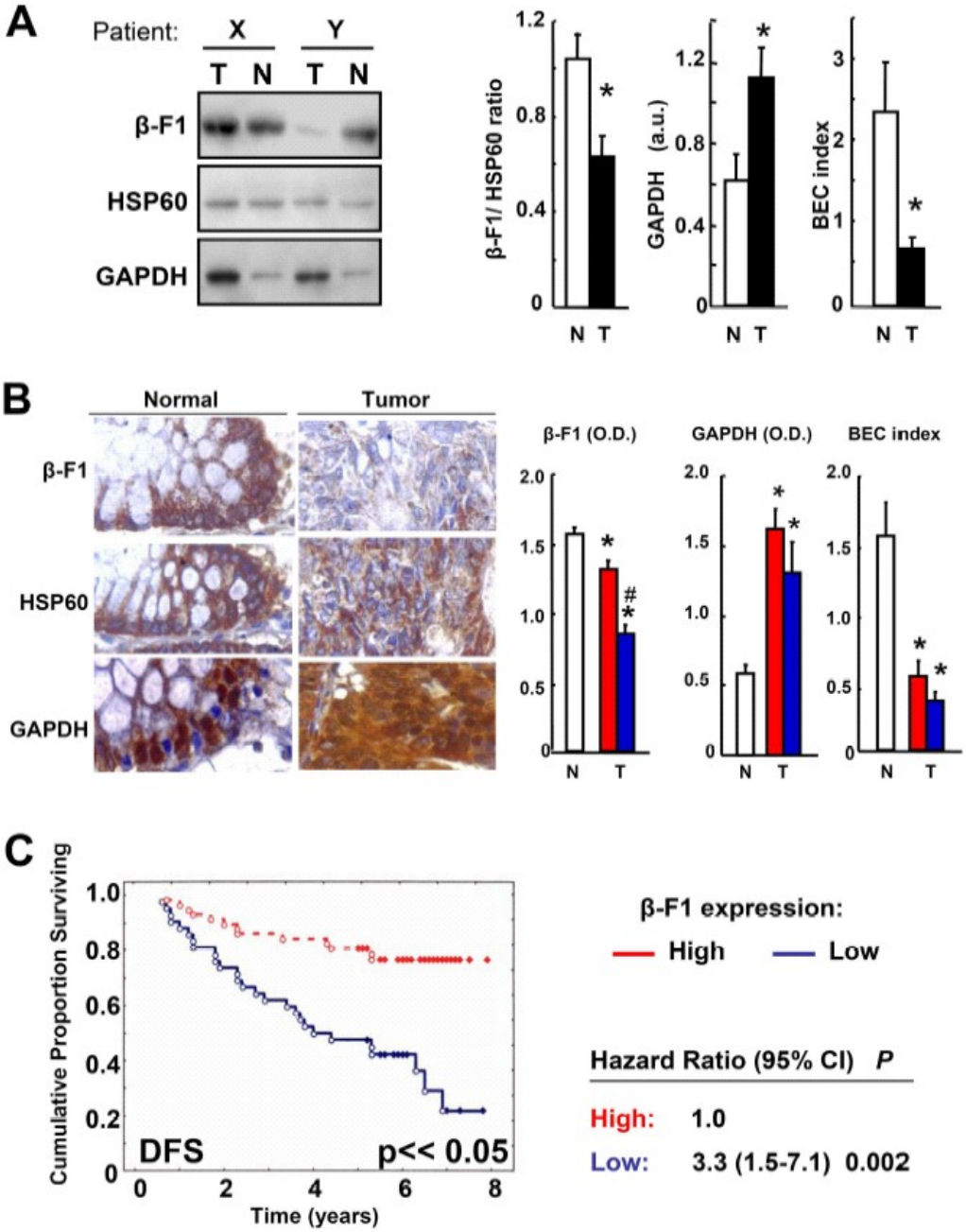
A
Patient: X Y
T N T N
β-F1
HSP60
GAPDH
β-F1/ HSP60 ratio
GAPDH (a.u.)
BEC index
N T
B
Normal
Tumor
β-F1
HSP60
GAPDH
β-F1 (O.D.)
GAPDH (O.D.)
BEC index
C
Cumulative Proportion Surviving
DFS
p<< 0.05
Time (years)
β-F1 expression:
High
Low
Hazard Ratio (95% CI) P
High: 1.0
Low: 3.3 (1.5-7.1) 0.002

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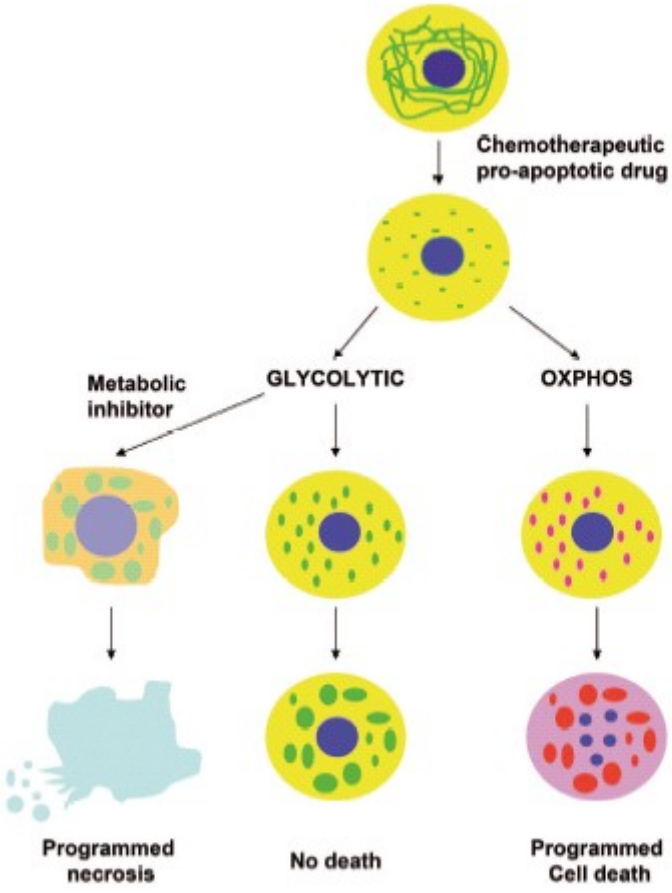

FIGURE 4