



Review

Choosing between glycolysis and oxidative phosphorylation: A tumor's dilemma? ☆

Caroline Jose, Nadège Bellance, Rodrigue Rossignol *

MRGM, Laboratoire Maladies Rares: Génétique et Métabolisme, F-33076 Bordeaux, France
Université Victor Segalen Bordeaux 2, F-33076 Bordeaux, France

ARTICLE INFO

Article history:

Received 15 August 2010

Received in revised form 11 October 2010

Accepted 11 October 2010

Available online 16 October 2010

Keywords:

Cancer

Bioenergetics

Oxidative phosphorylation

Mitochondrion

Oncogene

ABSTRACT

A considerable amount of knowledge has been produced during the last five years on the bioenergetics of cancer cells, leading to a better understanding of the regulation of energy metabolism during oncogenesis, or in adverse conditions of energy substrate intermittent deprivation. The general enhancement of the glycolytic machinery in various cancer cell lines is well described and recent analyses give a better view of the changes in mitochondrial oxidative phosphorylation during oncogenesis. While some studies demonstrate a reduction of oxidative phosphorylation (OXPHOS) capacity in different types of cancer cells, other investigations revealed contradictory modifications with the upregulation of OXPHOS components and a larger dependency of cancer cells on oxidative energy substrates for anabolism and energy production. This apparent conflictual picture is explained by differences in tumor size, hypoxia, and the sequence of oncogenes activated. The role of p53, C-MYC, Oct and RAS on the control of mitochondrial respiration and glutamine utilization has been explained recently on artificial models of tumorigenesis. Likewise, the generation of induced pluripotent stem cells from oncogene activation also showed the role of C-MYC and Oct in the regulation of mitochondrial biogenesis and ROS generation. In this review article we put emphasis on the description of various bioenergetic types of tumors, from exclusively glycolytic to mainly OXPHOS, and the modulation of both the metabolic apparatus and the modalities of energy substrate utilization according to tumor stage, serial oncogene activation and associated or not fluctuating microenvironmental substrate conditions. We conclude on the importance of a dynamic view of tumor bioenergetics. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

© 2010 Elsevier B.V. All rights reserved.

1. Cancer cell variable bioenergetics

Cancer cells exhibit profound genetic, bioenergetic and histological differences as compared to their non-transformed counterpart. All these modifications are associated with unlimited cell growth, inhibition of apoptosis and intense anabolism. Transformation from a normal cell to a malignant cancer cell is a multi-step pathogenic process which includes a permanent interaction between cancer gene activation (oncogenes and/or tumor-suppressor genes), metabolic reprogramming and tumor-induced changes in microenvironment. As for the individual genetic mapping of human tumors, their metabolic characterization (metabolic-bioenergetic profiling) has evidenced a cancer cell-type bioenergetic signature which depends on the history of the tumor, as composed by the sequence of oncogenes activated and the confrontation to intermittent changes in oxygen, glucose and amino-acid delivery.

In the last decade, bioenergetic studies have highlighted the variability among cancer types and even inside a cancer type as regards to the mechanisms and the substrates preferentially used for

deriving the vital energy. The more popular metabolic remodeling described in tumor cells is an increase in glucose uptake, the enhancement of glycolytic capacity and a high lactate production, along with the absence of respiration despite the presence of high oxygen concentration (Warburg effect) [1]. To explain this abnormal bioenergetic phenotype pioneering hypotheses proposed the impairment of mitochondrial function in rapidly growing cancer cells [2].

Although the increased consumption of glucose by tumor cells was confirmed *in vivo* by positron emission tomography (PET) using the glucose analog 2-(18F)-fluoro-2-deoxy-D-glucose (FDG), the actual utilization of glycolysis and oxidative phosphorylation (OXPHOS) cannot be evaluated with this technique. Nowadays, Warburg's "aerobic-glycolysis" hypothesis has been challenged by a growing number of studies showing that mitochondria in tumor cells are not inactive *per se* but operate at low capacity [3] or, in striking contrast, supply most of the ATP to the cancer cells [4]. Intense glycolysis is effectively not observed in all tumor types. Indeed not all cancer cells grow fast and intense anabolism is not mandatory for all cancer cells. Rapidly growing tumor cells rely more on glycolysis than slowly growing tumor cells. This is why a treatment with bromopyruvate, for example is very efficient only on rapidly growing cells and barely useful to decrease the growth rate of tumor cells when their normal proliferation is slow. Already in 1979, Reitzer and colleagues published an article entitled

☆ This article is part of a Special Issue entitled: Bioenergetics of Cancer.

* Corresponding author. INSERM U688 - Laboratoire de Physiopathologie mitochondriale, Université Victor Segalen, 146 rue Léo Saignat, F-33076 Bordeaux cedex, France. Tel.: +33 5 57 57 13 79; fax: +33 5 57 57 17 03.

E-mail address: rossig@u-bordeaux2.fr (R. Rossignol).

“Evidence that glutamine, not sugar, is the major energy source for cultured Hela cells”, which demonstrated that oxidative phosphorylation was used preferentially to produce ATP in cervical carcinoma cells [5]. Griguer et al. also identified several glioma cell lines that were highly dependent on mitochondrial OXPHOS pathway to produce ATP [6]. Furthermore, a subclass of glioma cells which utilize glycolysis preferentially (i.e., glycolytic gliomas) can also switch from aerobic glycolysis to OXPHOS under limiting glucose conditions [7,8], as observed in cervical cancer cells, breast carcinoma cells, hepatoma cells and pancreatic cancer cells [9–11]. This flexibility shows the interplay between glycolysis and OXPHOS to adapt the mechanisms of energy production to microenvironmental changes as well as differences in tumor energy needs or biosynthetic activity. Herst and Berridge also demonstrated that a variety of human and mouse leukemic and tumor cell lines (HL60, HeLa, 143B, and U937) utilize mitochondrial respiration to support their growth [12]. Recently, the measurement of OXPHOS contribution to the cellular ATP supply revealed that mitochondria generate 79% of the cellular ATP in HeLa cells, and that upon hypoxia this contribution is reduced to 30% [4]. Again, metabolic flexibility is used to survive under hypoxia. All these studies demonstrate that mitochondria are efficient to synthesize ATP in a large variety of cancer cells, as reviewed by Moreno-Sanchez [13]. Despite the observed reduction of the mitochondrial content in tumors [3,14–19], cancer cells maintain a significant level of OXPHOS capacity to rapidly switch from glycolysis to OXPHOS during carcinogenesis. This switch is also observed at the level of glutamine oxidation which can occur through two modes, “OXPHOS-linked” or “anoxic”, allowing to derive energy from glutamine or serine regardless of hypoxia or respiratory chain reduced activity [20].

While glutamine, glycine, alanine, glutamate, and proline are typically oxidized in normal and tumor mitochondria, alternative substrate oxidations may also contribute to ATP supply by OXPHOS. Those include for instance the oxidation of fatty-acids, ketone bodies, short-chain carboxylic acids, propionate, acetate and butyrate (as recently reviewed in [21]).

2. Varying degree of mitochondrial utilization during tumorigenesis

In vivo metabolomic analyses suggest the existence of a continuum of bioenergetic remodeling in rat tumors according to tumor size and its rate of growth [22]. Peter Vaupel's group showed that small tumors were characterized by a low conversion of glucose to lactate whereas the conversion of glutamine to lactate was high. In medium sized tumors the flow of glucose to lactate as well as oxygen utilization was increased whereas glutamine and serine consumption were reduced. At this stage tumor cells started with glutamate and alanine production. Large tumors were characterized by a low oxygen and glucose supply but a high glucose and oxygen utilization rate. The conversion of glucose to glycine, alanine, glutamate, glutamine, and proline reached high values and the amino acids were released [22]. Certainly, in the inner layers constituting solid tumors, substrate and oxygen limitation is frequently observed. Experimental studies tried to reproduce these conditions *in vitro* and revealed that nutrients and oxygen limitation does not affect OXPHOS and cellular ATP levels in human cervix tumor [23]. Furthermore, the growth of HeLa cells, HepG2 cells and HTB126 (breast cancer) in aglycemia and/or hypoxia even triggered a compensatory increase in OXPHOS capacity, as discussed above. Yet, the impact of hypoxia might be variable depending on cell type and both the extent and the duration of oxygen limitation.

In two models of sequential oncogenesis, the successive activation of specific oncogenes in non-cancer cells evidenced the need for active OXPHOS to pursue tumorigenesis. Funes et al. showed that the transformation of human mesenchymal stem cells increases their

dependency on OXPHOS for energy production [24], while Ferbeyre et al. showed that cells expressing oncogenic RAS display an increase in mitochondrial mass, mitochondrial DNA, and mitochondrial production of reactive oxygen species (ROS) prior to the senescent cell cycle arrest [25]. Such observations suggest that waves of gene regulation could suppress and then restore OXPHOS in cancer cells during tumorigenesis [20]. Therefore, the definition of cancer by Hanahan and Weinberg [26] restricted to six hallmarks (1—self-sufficiency in growth signals, 2—insensitivity to growth-inhibitory (antigrowth) signals, 3—evasion of programmed cell death (apoptosis), 4—limitless replicative potential, 5—sustained angiogenesis, and 6—tissue invasion and metastases) should also include metabolic reprogramming, as the seventh hallmark of cancer. This amendment was already proposed by Tennant et al. in 2009 [27]. In 2006, the review Science published a debate on the controversial views of Warburg theory [28], in support of a more realistic description of cancer cell's variable bioenergetic profile. The pros think that high glycolysis is an obligatory feature of human tumors, while the cons propose that high glycolysis is not exclusive and that tumors can use OXPHOS to derive energy. A unifying theory closer to reality might consider that OXPHOS and glycolysis cooperate to sustain energy needs along tumorigenesis [20]. The concept of oxidative tumors, against Warburg's proposal, was introduced by Guppy and colleagues, based on the observation that breast cancer cells can generate 80% of their ATP by the mitochondrion [29]. The comparison of different cancer cell lines and excised tumors revealed a variety of cancer cell's bioenergetic signatures which raised the question of the mechanisms underlying tumor cell metabolic reprogramming, and the relative contribution of oncogenesis and microenvironment in this process. It is now widely accepted that rapidly growing cancer cells within solid tumors suffer from a lack of oxygen and nutrients as tumor grows. In such situation of compromised energy substrate delivery, cancer cell's metabolic reprogramming is further used to sustain anabolism (Fig. 1), through the deviation of glycolysis, Krebs cycle truncation and OXPHOS redirection toward lipid and protein synthesis, as needed to support uncontrolled tumor growth and survival [30,31]. Again, these features are not exclusive to all tumors, as Krebs cycle truncation was only observed in some cancer cells, while other studies indicated that tumor cells can maintain a complete Krebs cycle [13] in parallel with an active citrate efflux. Likewise, generalizations should be avoided to prevent over-interpretations.

The oncogene C-MYC participate to these changes via the stimulation of glutamine utilization through the coordinate expression of genes necessary for cells to engage in glutamine catabolism [30]. According to Newsholme EA and Board M [32] both glycolysis and glutaminolysis not only serve for ATP production, but also provide precious metabolic intermediates such as glucose-6-phosphate, ammonia and aspartate required for the synthesis of purine and pyrimidine nucleotides (Fig. 1). In this manner, the observed apparent excess in the rates of glycolysis and glutaminolysis as compared to the requirement for energy production could be explained by the need for biosynthetic processes. Yet, one should not reduce the shift from glycolysis to OXPHOS utilization to the sole activation of glutaminolysis, as several other energy substrates can be used by tumor mitochondria to generate ATP [21]. The contribution of these different fuels to ATP synthesis remains poorly investigated in human tumors.

In 1989, the group of Peter Vaupel proposed that tumor blood flow was the principal modulator of oxidative and glycolytic metabolism, and of the metabolic micromilieu of human tumor xenografts *in vivo* [33]. Likewise, a recent study evidenced that the sole *in vitro* growth environment generates abnormalities of lipid metabolism and respiratory chain activity in cultured non-tumorigenic astrocytes, similar to those associated with tumorigenicity. In particular, cultured cells switch their metabolism toward glycolysis when placed in culture dishes [34,35]. This seriously limits the impact of numerous studies performed on cultured cells. Lastly, apparent changes in

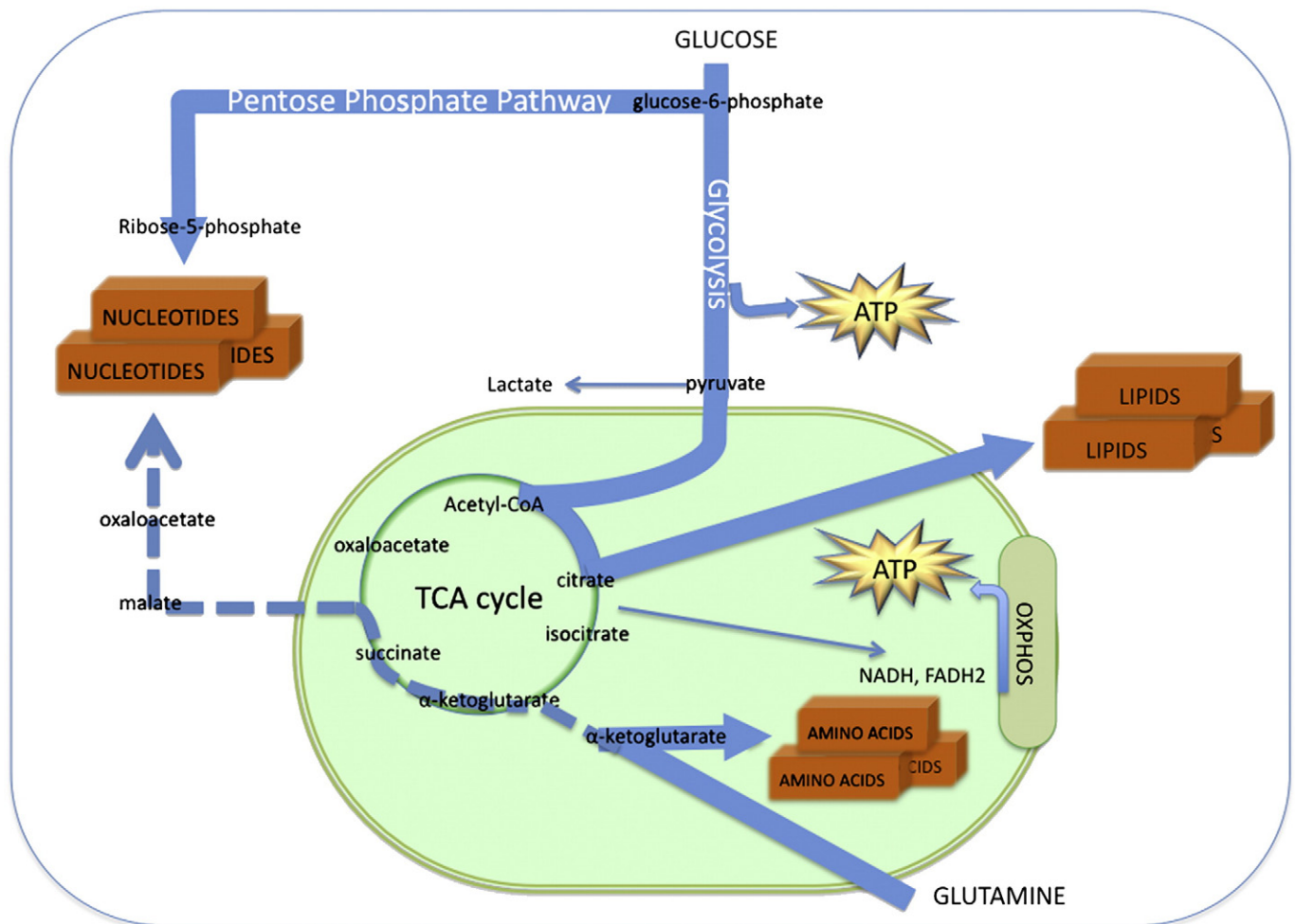


Fig. 1. Energy metabolism at the crossroad between catabolism and anabolism.

energy metabolism could also be explained by regulatory events in the control of apoptosis, as OXPHOS can be required for the activation of tumor suppressors Bax and Bak [36]. Given the numerous determinants of cancer cells energy features, a thorough description of the biochemical pathways used for energy production and branched anabolism in every tumor is requisite to derive adapted metabolic anti-cancer therapies.

3. The metabolism of pre-cancer cells and its ongoing modulation by carcinogenesis

At the beginning of cancer, there might have been a cancer stem cell hit by an oncogenic event, such as alterations in mitogen signaling to extracellular growth factor receptors (EGFR), oncogenic activation of these receptors, or oncogenic alterations of downstream targets in the pathways that leads to cell proliferation (RAS–Raf–ERK and PI3K–AKT, both leading to m-TOR activation stimulating cell growth). Alterations of checkpoint genes controlling the cell cycle progression like Rb also participate in cell proliferation (Fig. 2) and this re-entry in the cell cycle implies three major needs to fill in: 1) supplying enough energy to grow and 2) synthesize building blocks *de novo* and 3) keep vital oxygen and nutrients available. However, the bioenergetic status of the pre-cancer cell could determine in part the evolution of carcinogenesis, as shown on mouse embryonic stem cells. In this study, Schieke et al. showed that mitochondrial energy metabolism modulates both the differentiation and tumor formation capacity of mouse embryonic stem cells [37]. The idea that cancer

derives from a single cell, known as the cancer stem cell hypothesis, was introduced by observations performed on leukemia which appeared to be organized as origination from a primitive hematopoietic cell [38]. Nowadays cancer stem cells were discovered for all types of tumors [39–42], but little is known of their bioenergetic properties and their metabolic adaptation to the microenvironment. This question is crucial as regards the understanding of what determines the wide variety of cancer cell's metabolic profile. What is the impact of a given oncogene activation in pre-cancer cells of different metabolic background? The analysis of the metabolic changes that occur during the transformation of adult mesenchymal stem cells revealed that these cells did not switch to aerobic glycolysis, but their dependency on OXPHOS was even increased [24]. Hence, mitochondrial energy metabolism could be critical for tumorigenesis, in contrast with Warburg's hypothesis. As discussed above, the oncogene C-MYC also stimulates OXPHOS [30]. Furthermore, it was recently demonstrated that cells chronically treated with oligomycin repress OXPHOS and produce larger tumors with higher malignancy [19]. Likewise, alteration of OXPHOS by mutations in mtDNA increases tumorigenicity in different types of cancer cells [43–45]. Recently, it was proposed that mitochondrial energy metabolism is required to generate reactive oxygen species used for the carcinogenic process induced by the K-RAS mutation [46]. This could explain the large number of mitochondrial DNA mutations found in several tumors. The analysis of mitochondria in human embryonic cells which derive energy exclusively from anaerobic glycolysis have demonstrated an immature mitochondrial

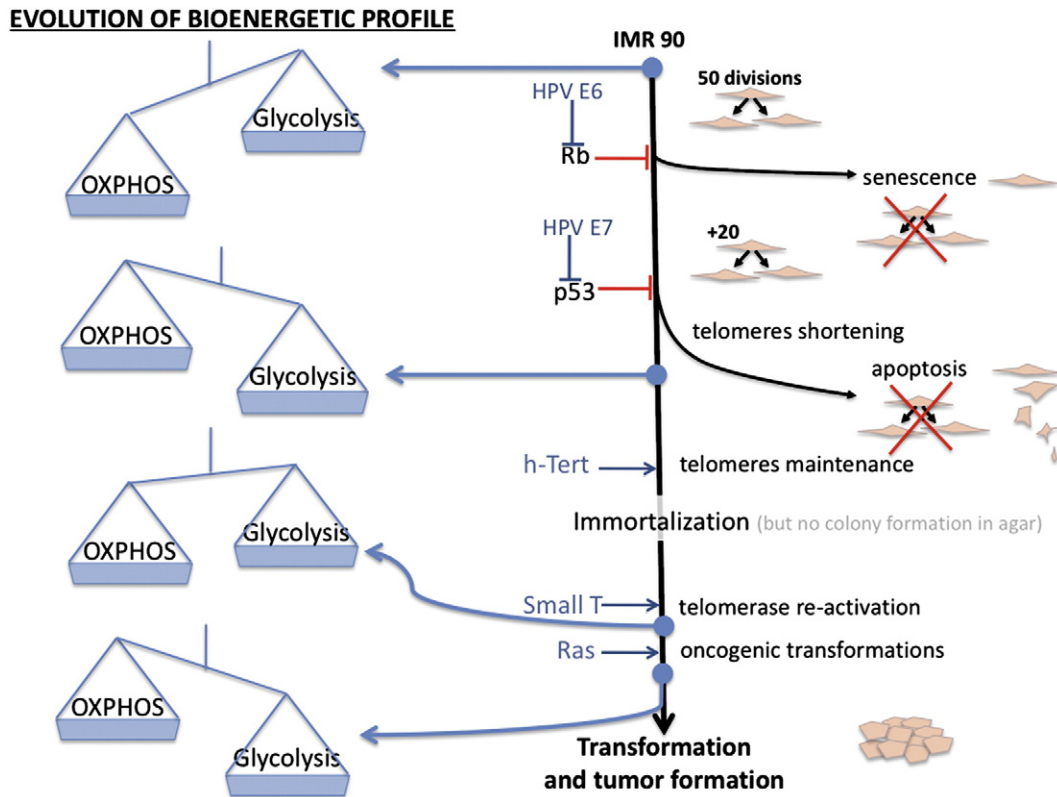


Fig. 2. Impact of different oncogenes on tumor progression and energy metabolism remodeling.

network characterized by few organelles with poorly developed cristae and peri-nuclear distribution [47,48]. The generation of human induced pluripotent stem cell by the introduction of different oncogenes as C-MYC and Oct4 reproduced this reduction of mitochondrial OXPHOS capacity [49,50]. This indicates again the impact of oncogenes on the control of OXPHOS and might explain the existence of pre-cancer stem cells with different bioenergetic backgrounds, as modeled by variable sequences of oncogene activation. Accordingly, the inhibition of mitochondrial respiratory chain has been recently found associated with enhancement of hESC pluripotency [51]. Based on the experimental evidence discussed above, one can argue that 1) glycolysis is indeed a feature of several tumors and associates with faster growth in high glucose environment, but 2) active OXPHOS is also an important feature of (other) tumors taken at a particular stage of carcinogenesis which might be more advantageous than a “glycolysis-only” type of metabolism in conditions of intermittent shortage in glucose delivery. The metabolic apparatus of cancer cells is not fixed during carcinogenesis and might depend both on the nature of the oncogenes activated and the microenvironment. It was indeed shown that cancer cells with predominant glycolytic metabolism present a higher malignancy when submitted to carcinogenetic induction and analysed under fixed experimental conditions of high glucose [19]. Yet, if one grows these cells in a glucose-deprived medium they shift their metabolism toward predominant OXPHOS, as shown in HeLa cells and other cell types [9]. Therefore, one might conclude that glycolytic cells have a higher propensity to generate aggressive tumors when glucose availability is high. However, these cells can become OXPHOS during tumor progression [24,52]. All these observations indicate again the importance of maintaining an active OXPHOS metabolism to permit evolution of both embryogenesis and carcinogenesis, which emphasizes the importance of targeting mitochondria to alter this malignant process.

4. Oncogenes and the modulation of energy metabolism

Mutations occurring in any genes along the MAPKKK/MAPK pathways (from EGFR to m-TOR) could at least partly engage the cell in a proliferative phase. Activation of oncogenes or inactivation of tumor suppressors are responsible for uncontrolled cell proliferation and tumor progression. The master regulators of the cell cycle, such as p53, Rb, p21, p27 and PTEN are typically mutated in several cancers and trigger cell cycle progression despite the presence of DNA damage or environmental stress. The overexpression of activated oncogenes can also overcome cell natural defenses by surpassing the capacity of the tumor suppressors. Several oncogenes and associated proteins such as HIF-1 α , RAS, C-MYC, SRC, and p53 can influence energy substrate utilization by affecting cellular targets, leading to metabolic changes that favor cancer cell survival, independently of the control of cell proliferation. These oncogenes stimulate the enhancement of aerobic glycolysis, and an increasing number of studies demonstrate that at least some of them can also target directly the OXPHOS machinery, as discussed in this article (Fig. 2). For instance, C-MYC can concurrently drive aerobic glycolysis and/or OXPHOS according to the tumor cell microenvironment, via the expression of glycolytic genes or the activation of mitochondrial oxidation of glutamine [53]. The oncogene RAS has been shown to increase OXPHOS activity in early transformed cells [24,52,54] and p53 modulates OXPHOS capacity via the regulation of cytochrome c oxidase assembly [55]. Hence, carcinogenic p53 deficiency results in a decreased level of COX2 and triggers a shift toward anaerobic metabolism. In this case, lactate synthesis is increased, but cellular ATP levels remain stable [56]. The p53-inducible isoform of phosphofructokinase, termed TP53-induced glycolysis and apoptotic regulator, TIGAR, a predominant phosphatase activity isoform of PFK-2, has also been identified as an important regulator of energy metabolism in tumors [57]. In non-cancer cells TIGAR inhibits glycolysis by lowering F2,6BP levels, and

upon mutation of p53 as occurs in several cancer cells, such inhibition is released and glycolysis can proceed [58]. Yet, FDG-6P can also be a substrate for G6PDH and PGM. Lastly, a recent study described the role of a tumor-specific peptide (p32) that is differentially expressed during the progression of epidermal carcinoma, which regulates the balance between OXPHOS and glycolysis [59]. p32 sustains OXPHOS by playing a role in the synthesis of mitochondrial-DNA-encoded genes. All the above listed studies delineate the role of different oncogenes in OXPHOS regulation.

5. Tumor specific isoforms (or mutated forms) of energy genes

Tumors are generally characterized by a modification of the glycolytic system where the level of some glycolytic enzymes is increased, some fetal-like isozymes with different kinetic and regulatory properties are produced, and the reverse and back-reactions of the glycolysis are strongly reduced [60]. The GAPDH marker of the glycolytic pathway is also increased in breast, gastric, lung, kidney and colon tumors [18], and the expression of glucose transporter GLUT1 is elevated in most cancer cells. The group of Cuezva J.M. developed the concept of cancer bioenergetic signature and of bioenergetic index to describe the metabolic profile of cancer cells and tumors [18,61–65]. This signature describes the changes in the expression level of proteins involved in glycolysis and OXPHOS, while the BEC index gives a ratio of OXPHOS protein content to glycolytic protein content, in good correlation with cancer prognostic [61]. Recently, this group showed that the beta-subunit of the mitochondrial F_1F_0 -ATP synthase is downregulated in a large number of tumors, thus contributing to the Warburg effect [64,65]. It was also shown that IF1 expression levels were increased in hepatocellular carcinomas, possibly to prevent the hydrolysis of glycolytic ATP [66]. Numerous changes occur at the level of OXPHOS and mitochondrial biogenesis in human tumors, as we reviewed previously [67]. Yet the actual impact of these changes in OXPHOS protein expression level or catalytic activities remains to be evaluated on the overall fluxes of respiration and ATP synthesis. Indeed, the metabolic control analysis and its extension indicate that it is often required to inhibit activity beyond a threshold of 70–85% to affect the metabolic fluxes [68,69]. Another important feature of cancer cells is the higher level of hexokinase II bound to mitochondrial membrane (50% in tumor cells). A study performed on human gliomas (brain) estimated the mitochondrial bound HK fraction (mHK) at 69% of total, as compared to 9% for normal brain [70]. This is consistent with the 5-fold amplification of the type II HK gene observed by Rempel et al. in the rapidly growing rat AS-30D hepatoma cell line, relative to normal hepatocytes [71]. HK_{II} subcellular fractionation in cancer cells was described in several studies [72–74]. The group led by Pete Pedersen explained that mHK contributes to (i) the high glycolytic capacity by utilizing mitochondrially regenerated ATP rather than cytosolic ATP (nucleotide channelling) and (ii) the lowering of OXPHOS capacity by limiting Pi and ADP delivery to the organelle [75,76]. Recently, at the European Bioenergetic Conference held in Varsaw, P. Pedersen proposed a novel therapeutic strategy aiming at the blockade of HK_{II} supported glycolysis with bromopyruvate [77]. Yet, the actual site of action of this drug remains to be clarified as one study established that OXPHOS is also a 3-bromopyruvate target [78].

All these observations are consistent with the increased rate of FDG uptake observed by PET in living tumors which could result from both an increase in glucose transport, and/or an increase in hexokinase activity. However, FDG is not a complete substrate for glycolysis (it is only transformed into FDG-6P by hexokinase before to be eliminated) and cannot be used to evidence a general increase in the glycolytic flux. Moreover, FDG-PET scan also gives false positive and false negative results, indicating that some tumors do not depend on, or do not have, an increased glycolytic capacity. The fast glycolytic

system described above is further accommodated in cancer cells by an increase in the lactate dehydrogenase isoform A (LDH-A) expression level. This isoform presents a higher V_{max} useful to prevent the inhibition of high glycolysis by its end product (pyruvate) accumulation. Recently, Fantin et al. showed that inhibition of LDH-A in tumors diminishes tumorigenicity and was associated with the stimulation of mitochondrial respiration [79]. The preferential expression of the glycolytic pyruvate kinase isoenzyme M2 (PKM2) in tumor cells, determines whether glucose is converted to lactate for regeneration of energy (active tetrameric form, Warburg effect) or used for the synthesis of cell building blocks (nearly inactive dimeric form) [80]. In the last five years, mutations in proteins of the respiratory system (SDH, FH) and of the TCA cycle (IDH1,2) leading to the accumulation of metabolite and the subsequent activation of HIF-1 α were reported in a variety of human tumors [81–83]. Recent mechanism linking energetics and epigenetics have also been discovered by the group of Thompson. They include the phosphorylation of Histone H2B by AMPK [84]. The same group showed that citrate also regulate energy gene expression by histone acetylation [85]. Lastly, the group of Singh was the first to demonstrate that changes in mitochondrial activity can influence the methylation of nuclear DNA and to regulate gene expression [86]. All these mechanisms describe novel and powerful mechanisms linking energy metabolism and the regulation of gene expression.

6. Tumor microenvironment modulates cancer cell's bioenergetics

It was extensively described how hypoxia activates HIF-1 α which stimulates in turn the expression of several glycolytic enzymes such as HK2, PFK, PGM, enolase, PK, LDH-A, MCT4 and glucose transporters Glut 1 and Glut 3. It was also shown that HIF-1 α can reduce OXPHOS capacity by inhibiting mitochondrial biogenesis [14,15], PDH activity [87] and respiratory chain activity [88]. The low efficiency and uneven distribution of the vascular system surrounding solid tumors can lead to abrupt changes in oxygen (intermittent hypoxia) but also energy substrate delivery. The response of HeLa cells to an abrupt change in extracellular energy source was analysed by Brand et al. [89] using a microperfusion system. They observed that the removal of glucose, or the inhibition of glycolysis by iodoacetate led to a switch toward glutamine utilization without delay followed by a rapid decrease in acid release. This illustrates once again how tumors and human cancer cell lines can utilize alternative energy pathway such as glutaminolysis to deal with glucose limitation, provided the presence of oxygen. It was also observed that in situations of glucose limitation, tumor derived-cells can adapt to survive by using exclusively an oxidative energy substrate [9,10]. This is typically associated with an enhancement of the OXPHOS system, as observed in these two studies [9,10]. Likewise, cultivation of HepG2 hepatoma cells in a glucose-deprived medium led to the stimulation of both mitochondrial biogenesis and the OXPHOS system with a 2-fold increase in COX, an elevated level of mt-DNA, mRNAs, mt-DNA encoded proteins, and mitochondrial transcription factor A. Associated with this features, lactate production was decreased and glutamine oxidation increased [90]. In summary, cancer cells can survive by using exclusively OXPHOS for ATP production, by altering significantly mitochondrial composition and form to facilitate optimal use of the available substrate (Fig. 3). Yet, glucose is needed to feed the pentose phosphate pathway and generate ribose essential for nucleotide biosynthesis. This raises the question of how cancer cells can survive in the growth medium which do not contain glucose (so-called “galactose medium” with dialysed serum [9]). In the OXPHOS mode, pyruvate, glutamate and aspartate can be derived from glutamine, as glutaminolysis can replenish Krebs cycle metabolic pool and support the synthesis of alanine and NADPH [31]. Glutamine is a major source for oxaloacetate (OAA) essential for citrate synthesis. Moreover, the conversion of glutamine to pyruvate is associated with the reduction of NADP⁺ to NADPH by malic

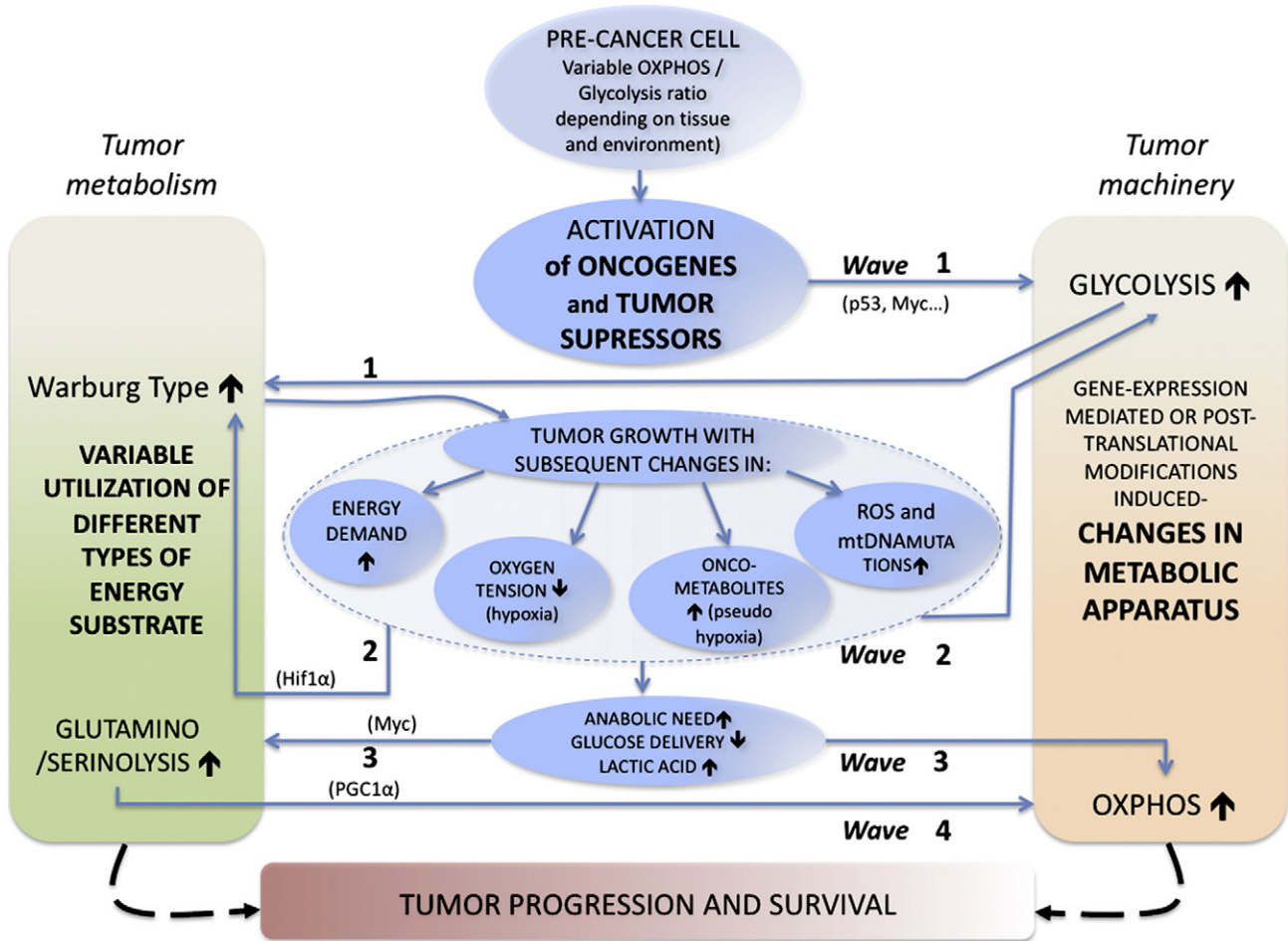


Fig. 3. Interplay between energy metabolism, oncogenes and tumor microenvironment during tumorigenesis (the “metabolic wave model”).

enzyme. Such NADPH is a required electron donor for reductive steps in lipid synthesis, nucleotide metabolism and GSH reduction. In glioblastoma cells the malic enzyme flux was estimated to be high enough to supply all of the reductive power needed for lipid synthesis [31]. Phospholipids can be generated in the absence of glycolysis from the acetyl-coA generated from the citrate deviated from the Krebs cycle. Glutamine is also used as nitrogen donor for the *de novo* synthesis of both purines and pyrimidines.

The signaling pathways by which the metabolic shift toward OXPHOS is accomplished in cancer cells must include transcription factors that alter expression of OXPHOS components, such as PGC1 α or CREB. While the mechanisms leading to the enhancement of glycolytic capacity in tumors are well documented, less is known about the parallel OXPHOS changes. Both phenomena could result from a selection of pre-malignant cells forced to survive under hypoxia and limited glucose delivery, followed by an adaptation to intermittent hypoxia, pseudo-hypoxia, substrate limitation and acidic environment. This hypothesis was first proposed by Gatenby and Gillies to explain the high glycolytic phenotype of tumors [91–93], but several lines of evidence suggest that it could also be used to explain the mitochondrial modifications observed in cancer cells. The rationale for this “micro-environmental hypothesis” is supported by the topological observation of tumors in formation that typically takes place at a particular distance from blood vessels, at the boundary between viable and necrotic cells, where oxygen is no longer available. Beyond this “diffusion limit” the pre-malignant cells are submitted to important fluctuations in oxygen delivery. This suggests again a role for hypoxia and/or pseudo-hypoxia in tumor initiation.

7. Aerobic glycolysis and mitochondria cooperate during cancer progression

Metabolic flexibility considers the possibility for a given cell to alternate between glycolysis and OXPHOS in response to physiological needs. Louis Pasteur found that in most mammalian cells the rate of glycolysis decreases significantly in the presence of oxygen (Pasteur effect). Moreover, energy metabolism of normal cell can vary widely according to the tissue of origin, as we showed with the comparison of five rat tissues [94]. During stem cell differentiation, cell proliferation induces a switch from OXPHOS to aerobic glycolysis which might generate ATP more rapidly, as demonstrated in HepG2 cells [95] or in non-cancer cells [96,97]. Thus, normal cellular energy metabolism can adapt widely according to the activity of the cell and its surrounding microenvironment (energy substrate availability and diversity). Support for this view came from numerous studies showing that *in vitro* growth conditions can alter energy metabolism contributing to a dependency on glycolysis for ATP production [98]. Yet, Zu and Guppy analysed numerous studies and showed that aerobic glycolysis is not inherent to cancer but more a consequence of hypoxia [99]. They concluded that weakness in experimental design or cell lines/tumors comparisons brought mistaken conclusions on commonly Warburg-oriented agreements of cancer metabolism. Moreno-Sanchez and colleagues also demonstrated that numerous cancer cells use OXPHOS to supply ATP [4]. Another emerging hypothesis linking metabolic remodeling variability and tumorigenesis is the reverse Warburg effect. The originality of this hypothesis lies on the assumption of a cooperativity between fibroblasts and epithelial cells in tumor

Table 1
Impact of different oncogenes on energy metabolism.

ONCOGENE OR ENERGY PROTEIN ACTIVATION	Glycolysis	Krebs-Cycle	PDH	OXPHOS	Ref.
<i>Mutated allele or altered expression level</i>					
K-RAS		Increased conversion of glucose carbons into glutamate/glutamine aspartate and uridine		Generation of (ROS) by complex III	[46]
H-RASV12				Increase in oxygen consumption	[54]
H-RASV12/E1A	Late increase in glycolysis and lactate production			Early increase in oxygen consumption and superoxide production	[52]
C-MYC	Decreased contribution of glycolysis to phospholipids synthesis	Stimulation of mitochondrial glutaminolysis		Stimulation of mitochondrial glutaminolysis	[30, 53]
Hif 1α Overexpressed in cancer	Activation of glycolytic genes	Inhibition of PDH and subsequent limitation of the Krebs cycle		Repression of OXPHOS proteins. Expression of COX4-2 (low rate)	[14, 88, 115]
STAT3 (activated in some tumors such as those bearing H-RasV12)	Increased LDH activity			Activation of complex II and complex V.	[116]
Oct1 Overexpressed in cancer	Glucose metabolism is reduced			Mitochondrial activity and amino acid oxidation are increased	[117]
P53/SCO2 Mutated p53 decreases SCO2 expression				Inhibition of complex IV	[55]
P53/Tigar Mutated p53 decreases TIGAR expression	Activation of glycolysis (Tigar normally inhibits glycolysis)				[57, 58]
PK1 Overexpressed in some tumors		Inhibition of PDH and subsequent Krebs cycle		Subsequent reduction of respiration	[87]
PI3/AKT Hyperactivated in tumors	Activates the expression of glycolytic enzymes				[118]

Refs. [115–118].

progression. Here, epithelial cells induce aerobic glycolysis to neighbouring stromal fibroblasts that then produce lactate and pyruvate that will enter TCA cycle in epithelial cells, thereby promoting efficient ATP production via mitochondrial oxidation [100].

Some studies have highlighted a tight cooperation among certain oncogenes and tumor-suppressor genes in cancer progression and metabolic remodeling which might explain the variety of metabolic profiles observed in different tumors. For example, C-MYC and RAS are frequent genes known to be mutated in human cancer. These genes are also the pioneering example for oncogene cooperation during tumorigenesis, whereby the anti-cancer effects of C-MYC deregulation (apoptosis) and oncogenic RAS (senescence) are antagonized and therefore cancelled out by each other [101,102]. Hahn and colleagues evidenced that human transformation from a normal cell to a cancer cell is only possible through the coexpression of three cooperative oncogenes, telomerase catalytic subunit (hTert), the simian virus 40 large T and H-RAS (i.e. the so-called “Weinberg model” [103]). Reactivation of telomerase maintains telomere length and permits immortality of cell. SV40 large T is implicated in the inactivation of tumor-suppressor proteins (p53 and p105), causing the cells to leave G1 phase and enter into S phase. H-RAS is known to procure a tumorigenic shift of transformed cells (Fig. 2). Undeniably, multiple genetic alterations are necessary for tumorigenesis, and as discussed above, all these event trigger the inhibition or the activation of OXPHOS, thus possibly explaining the variety of metabolic profiles observed in cancer cells. Surprisingly, the inactivation of a single oncogene can induce tumor repression, a phenomenon called by Weinstein “the oncogene addiction” [104,105]. They explained this

addiction by the often multiple roles in complex and interactive networks played by the proteins encoded by most oncogenes. Therefore, in cancer cells, an oncogene may play a more essential and qualitatively different role in a given pathway compared with its role in normal cells. One may also propose that during activation of sequential oncogenes, the metabolism is reprogrammed at each step as we have seen before with C-MYC, HIF and RAS for instance (Table 1). The final metabolic steady-state is adapted to the cancer cell requirements and suppression of one oncogene can unbalance this bioenergetic program and lead to energy supply impairment and tumor repression.

8. Conclusion

Energy metabolism is deviated in human tumors, so that it remains a therapeutic target of choice. The way it is altered can differ widely among tumor but if the metabolic profile is well established, targeting the predominant bioenergetic pathway altered could improve significantly the prognostic of patients. As recently pointed out by Gogvadze and colleagues [106], combined strategies involving modulation of both glycolytic and mitochondrial pathways might be required for more efficient elimination of malignant cells. Therapeutic strategies able to interfere with the specificities of cancer cell's bioenergetics could ideally permit to reduce tumor growth [107,108]. As cancer cells present a variety of energy metabolism deviations [2,20,67,109], pharmacological attempts to interfere with distinctive steps of cancer energy production pathways could provide drug specificity. The first step is the determination of cancer cell's

bioenergetic profile to decipher the biochemical pathway primarily used for energy production [3,4,9,10,61,62]. As discussed in this review article, the bioenergetic type of a given tumor can vary widely from glycolytic to oxidative, according to the oncogenes activated and the microenvironment [13,18,20,99,110]. The typical “glycolytic” type of cancer cells includes enhanced glycolytic machinery confronted to a low efficiency OXPHOS system, while the “OXPHOS” type of cancer cells relies mainly on mitochondrial respiration to produce ATP from glucose and glutamine oxidation [4,5,29]. It was demonstrated that mitochondrial oxidative phosphorylation is low efficiency in glycolytic tumors, notably through a reduction of the cellular mitochondrial content [3,14,15,19,111]. Hence, a potential therapeutic strategy might consist in the re-activation of mitochondrial oxidative metabolism in glycolytic tumors with altered mitochondria, as obtained by the stimulation of PDH activity with sodium-dichloroacetate [112] or the overexpression of frataxin [113]. Another possibility could consist in the global stimulation of mitochondrial biogenesis in cancer cells with a reduced mitochondrial content and OXPHOS capacity [3]. Strategies aiming at mimicking a low-energy state in cancer cells to trigger cell proliferation arrest and apoptosis are also valid, and the research for identifying novel energy restriction-mimetic agents (ERMAs) [114] capable of reducing human tumor growth will undoubtedly benefit from a better bioenergetic characterization of the different cancer cell lines, in particular for what concern their response to challenging energy conditions, as occurs in hypoxia and aglycemia.

Acknowledgements

We thank the French National Institute for Scientific and Medical Research (INSERM), Université Victor Segalen Bordeaux 2, Région Aquitaine, Ammi, and Cancéropôle Grand Sud-Ouest for financial support. C. Jose was supported by a grant from the FQRNT (Fond Québécois de la Recherche sur la Nature et les Technologies), and N. Bellance by a Grant from INSERM/Région Aquitaine.

References

- [1] Warburg, Metabolism of tumors, Arnold Constable, London, 1930.
- [2] P.L. Pedersen, Tumor mitochondria and the bioenergetics of cancer cells, *Prog. Exp. Tumor Res.* 22 (1978) 190–274.
- [3] N. Bellance, G. Benard, F. Furt, H. Begueret, K. Smolkova, E. Passerieux, J.P. Delage, J.M. Baste, P. Moreau, R. Rossignol, Bioenergetics of lung tumors: alteration of mitochondrial biogenesis and respiratory capacity, *Int. J. Biochem. Cell Biol.* 41 (2009) 2566–2577.
- [4] S. Rodriguez-Enriquez, L. Carreno-Fuentes, J.C. Gallardo-Perez, E. Saavedra, H. Quezada, A. Vega, A. Marin-Hernandez, V. Olin-Sandoval, M.E. Torres-Marquez, R. Moreno-Sanchez, Oxidative phosphorylation is impaired by prolonged hypoxia in breast and possibly in cervix carcinoma, *Int. J. Biochem. Cell Biol.* 42 (10) (Oct 2010) 1744–1751.
- [5] L. Reitzer, B. Wice, D. Kennel, Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells, *JBC* 254 (1979) 2669–2676.
- [6] C.E. Griguer, C.R. Oliva, G.Y. Gillespie, Glucose metabolism heterogeneity in human and mouse malignant glioma cell lines, *J. Neurooncol.* 74 (2005) 123–133.
- [7] M.E. Beckner, G.T. Gobbel, R. Abounader, F. Burovic, N.R. Agostino, J. Laterra, I.F. Pollack, Glycolytic glioma cells with active glycogen synthase are sensitive to PTEN and inhibitors of PI3K and gluconeogenesis, *Lab. Invest.* 85 (2005) 1457–1470.
- [8] A.K. Bouzier, P. Voisin, R. Goodwin, P. Canioni, M. Merle, Glucose and lactate metabolism in C6 glioma cells: evidence for the preferential utilization of lactate for cell oxidative metabolism, *Dev. Neurosci.* 20 (1998) 331–338.
- [9] R. Rossignol, R. Gilkerson, R. Aggeler, K. Yamagata, S.J. Remington, R.A. Capaldi, Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells, *Cancer Res.* 64 (2004) 985–993.
- [10] K. Smolkova, N. Bellance, F. Scandurra, E. Genot, E. Gnaiger, L. Plecita-Hlavata, P. Jezek, R. Rossignol, Mitochondrial bioenergetic adaptations of breast cancer cells to aglycemia and hypoxia, *J. Bioenerg. Biomembr.* 42 (2010) 55–67.
- [11] L. Plecita-Hlavata, M. Lessard, J. Santorova, J. Bewersdorf, P. Jezek, Mitochondrial oxidative phosphorylation and energetic status are reflected by morphology of mitochondrial network in INS-1E and HEP-G2 cells viewed by 4Pi microscopy, *Biochim. Biophys. Acta* 1777 (2008) 834–846.
- [12] P.M. Herst, M.V. Berridge, Cell surface oxygen consumption: a major contributor to cellular oxygen consumption in glycolytic cancer cell lines, *Biochim. Biophys. Acta* 1767 (2007) 170–177.
- [13] R. Moreno-Sanchez, S. Rodriguez-Enriquez, A. Marin-Hernandez, E. Saavedra, Energy metabolism in tumor cells, *FEBS J.* 274 (2007) 1393–1418.
- [14] E. Hervouet, A. Cizkova, J. Demont, A. Vojtiskova, P. Pecina, N.L. Franssen-van Hal, J. Keijer, H. Simonnet, R. Ivanek, S. Kmoch, C. Godinot, J. Houstek, HIF and reactive oxygen species regulate oxidative phosphorylation in cancer, *Carcinogenesis* 29 (2008) 1528–1537.
- [15] E. Hervouet, J. Demont, P. Pecina, A. Vojtiskova, J. Houstek, H. Simonnet, C. Godinot, A new role for the von Hippel-Lindau tumor suppressor protein: stimulation of mitochondrial oxidative phosphorylation complex biogenesis, *Carcinogenesis* 26 (2005) 531–539.
- [16] J.M. Cuezva, L.K. Ostronoff, J. Ricart, M. Lopez de Heredia, C.M. Di Liegro, J.M. Izquierdo, Mitochondrial biogenesis in the liver during development and oncogenesis, *J. Bioenerg. Biomembr.* 29 (1997) 365–377.
- [17] L. Formentini, I. Martinez-Reyes, J.M. Cuezva, The mitochondrial bioenergetic capacity of carcinomas, *IUBMB Life* 62 (2010) 554–560.
- [18] A. Isidoro, M. Martinez, P.L. Fernandez, A.D. Ortega, G. Santamaria, M. Chamorro, J.C. Reed, J.M. Cuezva, Alteration of the bioenergetic phenotype of mitochondria is a hallmark of breast, gastric, lung and oesophageal cancer, *Biochem. J.* 378 (2004) 17–20.
- [19] M. Sanchez-Arago, M. Chamorro, J.M. Cuezva, Selection of cancer cells with repressed mitochondria triggers colon cancer progression, *Carcinogenesis* 31 (2010) 567–576.
- [20] K. Smolkova, L. Plecita-Hlavata, N. Bellance, G. Benard, R. Rossignol, P. Jezek, Waves of gene regulation suppress and then restore oxidative phosphorylation in cancer cells, *Int. J. Biochem. Cell Biol.* (May 10 2010) (in press). doi:10.1016/j.biocel.2010.05.003.
- [21] S.J. Ralph, S. Rodriguez-Enriquez, J. Neuzil, R. Moreno-Sanchez, Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger, *Mol. Aspects Med.* 31 (2010) 29–59.
- [22] E. Eigenbrodt, F. Kallinowski, M. Ott, S. Mazurek, P. Vaupel, Pyruvate kinase and the interaction of amino acid and carbohydrate metabolism in solid tumors, *Anticancer Res.* 18 (1998) 3267–3274.
- [23] S. Rodriguez-Enriquez, L. Carreno-Fuentes, J.C. Gallardo-Perez, E. Saavedra, H. Quezada, A. Vega, A. Marin-Hernandez, V. Olin-Sandoval, M.E. Torres-Marquez, R. Moreno-Sanchez, Oxidative phosphorylation is impaired by prolonged hypoxia in breast and possibly in cervix carcinoma, *Int. J. Biochem. Cell Biol.* 42 (2010) 1744–1751.
- [24] J.M. Funes, M. Quintero, S. Henderson, D. Martinez, U. Qureshi, C. Westwood, M.O. Clements, D. Bourbouli, R.B. Pedley, S. Moncada, C. Boshoff, Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 6223–6228.
- [25] O. Moiseeva, V. Bourdeau, A. Roux, X. Deschenes-Simard, G. Ferbeyre, Mitochondrial dysfunction contributes to oncogene-induced senescence, *Mol. Cell Biol.* 29 (2009) 4495–4507.
- [26] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, *Cell* 100 (2000) 57–70.
- [27] D.A. Tennant, R.V. Duran, H. Boulahbel, E. Gottlieb, Metabolic transformation in cancer, *Carcinogenesis* 30 (2009) 1269–1280.
- [28] K. Garber, Energy deregulation: licensing tumors to grow, *Science* 312 (2006) 1158–1159.
- [29] M. Guppy, P. Leedman, X. Zu, V. Russell, Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells, *Biochem. J.* 364 (2002) 309–315.
- [30] D.R. Wise, R.J. DeBerardinis, A. Mancuso, N. Sayed, X.Y. Zhang, H.K. Pfeiffer, I. Nissim, E. Daikhin, M. Yudkoff, S.B. McMahon, C.B. Thompson, Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 18782–18787.
- [31] R.J. DeBerardinis, A. Mancuso, E. Daikhin, I. Nissim, M. Yudkoff, S. Wehrli, C.B. Thompson, Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 19345–19350.
- [32] E.A. Newsholme, M. Board, Application of metabolic-control logic to fuel utilization and its significance in tumor cells, *Adv. Enzyme Regul.* 31 (1991) 225–246.
- [33] F. Kallinowski, K.H. Schlenger, M. Kloes, M. Stohrer, P. Vaupel, Tumor blood flow: the principal modulator of oxidative and glycolytic metabolism, and of the metabolic microclimate of human tumor xenografts *in vivo*, *Int. J. Cancer* 44 (1989) 266–272.
- [34] M.A. Kiebish, X. Han, H. Cheng, T.N. Seyfried, *In vitro* growth environment produces lipidomic and electron transport chain abnormalities in mitochondria from non-tumorigenic astrocytes and brain tumours, *ASN Neuro* 1 (2009).
- [35] G. Gstraunthaler, T. Seppi, W. Pfaller, Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? *Cell. Physiol. Biochem.* 9 (1999) 150–172.
- [36] A. Tomiyama, S. Serizawa, K. Tachibana, K. Sakurada, H. Samejima, Y. Kuchino, C. Kitanaka, Critical role for mitochondrial oxidative phosphorylation in the activation of tumor suppressors Bax and Bak, *J. Natl. Cancer Inst.* 98 (2006) 1462–1473.
- [37] S.M. Schieke, M. Ma, L. Cao, J.P. McCoy Jr., C. Liu, N.F. Hensel, A.J. Barrett, M. Boehm, T. Finkel, Mitochondrial metabolism modulates differentiation and teratoma formation capacity in mouse embryonic stem cells, *J. Biol. Chem.* 283 (2008) 28506–28512.
- [38] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, *Nat. Med.* 3 (1997) 730–737.

- [39] M. Wang, J. Xiao, M. Shen, Y. Yahong, R. Tian, F. Zhu, J. Jiang, Z. Du, J. Hu, W. Liu, R. Qin, Isolation and characterization of tumorigenic extrahepatic cholangiocarcinoma cells with stem cell-like properties, *Int. J. Cancer* 128 (2011) 72–81.
- [40] D. Feng, C. Peng, C. Li, Y. Zhou, M. Li, B. Ling, H. Wei, Z. Tian, Identification and characterization of cancer stem-like cells from primary carcinoma of the cervix uteri, *Oncol. Rep.* 22 (2009) 1129–1134.
- [41] Z. Zhu, X. Hao, M. Yan, M. Yao, C. Ge, J. Gu, J. Li, Cancer stem/progenitor cells are highly enriched in CD133+CD44+ population in hepatocellular carcinoma, *Int. J. Cancer* 126 (2010) 2067–2078.
- [42] N. Guzman-Ramirez, M. Voller, A. Wetterwald, M. Germann, N.A. Cross, C.A. Rentsch, J. Schalken, G.N. Thalmann, M.G. Cecchini, *In vitro* propagation and characterization of neoplastic stem/progenitor-like cells from human prostate cancer tissue, *Prostate* 69 (2009) 1683–1693.
- [43] J.A. Petros, A.K. Baumann, E. Ruiz-Pesini, M.B. Amin, C.Q. Sun, J. Hall, S. Lim, M.M. Issa, W.D. Flanders, S.H. Hosseini, F.F. Marshall, D.C. Wallace, mtDNA mutations increase tumorigenicity in prostate cancer, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 719–724.
- [44] K. Ishikawa, K. Takenaga, M. Akimoto, N. Koshikawa, A. Yamaguchi, H. Imanishi, K. Nakada, Y. Honma, J. Hayashi, ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis, *Science* 320 (2008) 661–664.
- [45] J.S. Park, L.K. Sharma, H. Li, R. Xiang, D. Holstein, J. Wu, J. Lechleiter, S.L. Naylor, J.J. Deng, J. Lu, Y. Bai, A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis, *Hum. Mol. Genet.* 18 (2009) 1578–1589.
- [46] F. Weinberg, R. Hamanaka, W.W. Wheaton, S. Weinberg, J. Joseph, M. Lopez, B. Kalyanaraman, G.M. Mutlu, G.R. Budinger, N.S. Chandel, Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 8788–8793.
- [47] H. Sathananthan, M. Pera, A. Trounson, The fine structure of human embryonic stem cells, *Reprod. Biomed. Online* 4 (2002) 56–61.
- [48] J.M. Facucho-Oliveira, J.C. St John, The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation, *Stem Cell Rev.* 5 (2009) 140–158.
- [49] A. Prigione, B. Fauler, R. Lurz, H. Lehrach, J. Adjaye, The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells, *Stem Cells* 28 (2010) 721–733.
- [50] L. Armstrong, K. Tilgner, G. Saretzki, S.P. Atkinson, M. Stojkovic, R. Moreno, S. Przyborski, M. Lako, Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells, *Stem Cells* 28 (2010) 661–673.
- [51] S. Varum, O. Momcilovic, C. Castro, A. Ben-Yehudah, J. Ramalho-Santos, C.S. Navara, Enhancement of human embryonic stem cell pluripotency through inhibition of the mitochondrial respiratory chain, *Stem Cell Res.* 3 (2009) 142–156.
- [52] A.J. de Groof, M.M. te Lindert, M.M. van Dommelen, M. Wu, M. Willemsse, A.L. Smift, M. Winer, F. Oerlemans, H. Pluk, J.A. Fransen, B. Wieringa, Increased OXPHOS activity precedes rise in glycolytic rate in H-RasV12/E1A transformed fibroblasts that develop a Warburg phenotype, *Mol. Cancer* 8 (2009) 54.
- [53] P. Gao, I. Tchernyshyov, T.C. Chang, Y.S. Lee, K. Kita, T. Ochi, K.I. Zeller, A.M. De Marzo, J.E. Van Eyk, J.T. Mendell, C.V. Dang, c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism, *Nature* 458 (2009) 762–765.
- [54] S. Telang, A.N. Lane, K.K. Nelson, S. Arumugam, J. Chesney, The oncoprotein H-RasV12 increases mitochondrial metabolism, *Mol. Cancer* 6 (2007) 77.
- [55] S. Matoba, J.G. Kang, W.D. Patino, A. Wragg, M. Boehm, O. Gavrilova, P.J. Hurler, F. Bunz, P.M. Hwang, p53 regulates mitochondrial respiration, *Science* 312 (2006) 1650–1653.
- [56] S. Zhou, S. Kachhap, K.K. Singh, Mitochondrial impairment in p53-deficient human cancer cells, *Mutagenesis* 18 (2003) 287–292.
- [57] K. Bensaad, E.C. Cheung, K.H. Vousden, Modulation of intracellular ROS levels by TIGAR controls autophagy, *EMBO J.* 28 (2009) 3015–3026.
- [58] K. Bensaad, A. Tsuruta, M.A. Selak, M.N. Vidal, K. Nakano, R. Bartrons, E. Gottlieb, K.H. Vousden, TIGAR, a p53-inducible regulator of glycolysis and apoptosis, *Cell* 126 (2006) 107–120.
- [59] V. Fogal, A.D. Richardson, P.P. Karmali, I.E. Scheffler, J.W. Smith, E. Ruoslahti, Mitochondrial p32 protein is a critical regulator of tumor metabolism via maintenance of oxidative phosphorylation, *Mol. Cell. Biol.* 30 (2010) 1303–1318.
- [60] A. Marin-Hernandez, J.C. Gallardo-Perez, S.J. Ralph, S. Rodriguez-Enriquez, R. Moreno-Sanchez, HIF-1 α modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms, *Mini Rev. Med. Chem.* 9 (2009) 1084–1101.
- [61] J.M. Cuezva, G. Chen, A.M. Alonso, A. Isidoro, D.E. Misk, S.M. Hanash, D.G. Beer, The bioenergetic signature of lung adenocarcinomas is a molecular marker of cancer diagnosis and prognosis, *Carcinogenesis* 25 (2004) 1157–1163.
- [62] J.M. Cuezva, M. Krajewska, M.L. de Heredia, S. Krajewski, G. Santamaria, H. Kim, J.M. Zapata, H. Marusawa, M. Chamorro, J.C. Reed, The bioenergetic signature of cancer: a marker of tumor progression, *Cancer Res.* 62 (2002) 6674–6681.
- [63] J.M. Cuezva, A.D. Ortega, I. Willers, L. Sanchez-Cenizo, M. Aldea, M. Sanchez-Arago, The tumor suppressor function of mitochondria: translation into the clinics, *Biochim. Biophys. Acta* 1792 (2009) 1145–1158.
- [64] L. Sanchez-Cenizo, L. Formentini, M. Aldea, A.D. Ortega, P. Garcia-Huerta, M. Sanchez-Arago, J.M. Cuezva, The up-regulation of the ATPase Inhibitory Factor 1 (IF1) of the mitochondrial H⁺-ATP synthase in human tumors mediates the metabolic shift of cancer cells to a Warburg phenotype, *J. Biol. Chem.* 285 (33) (Aug 13 2010) 25308–25313.
- [65] I.M. Willers, A. Isidoro, A.D. Ortega, P.L. Fernandez, J.M. Cuezva, Selective inhibition of beta-F1-ATPase mRNA translation in human tumours, *Biochem. J.* 426 (2010) 319–326.
- [66] F. Capuano, F. Guerrieri, S. Papa, Oxidative phosphorylation enzymes in normal and neoplastic cell growth, *J. Bioenerg. Biomembr.* 29 (1997) 379–384.
- [67] N. Bellance, P. Lestienne, R. Rossignol, Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis, *Front. Biosci.* 14 (2009) 4015–4034.
- [68] R. Rossignol, M. Malgat, J.P. Mazat, T. Letellier, Threshold effect and tissue specificity. Implication for mitochondrial cytopathies, *J. Biol. Chem.* 274 (1999) 33426–33432.
- [69] R. Rossignol, T. Letellier, M. Malgat, C. Rocher, J.P. Mazat, Tissue variation in the control of oxidative phosphorylation: implication for mitochondrial diseases, *Biochem. J.* 347 (Pt 1) (2000) 45–53.
- [70] S. Oudard, E. Boitier, L. Miccoli, S. Rousset, B. Dutrillaux, M.F. Poupon, Gliomas are driven by glycolysis: putative roles of hexokinase, oxidative phosphorylation and mitochondrial ultrastructure, *Anticancer Res.* 17 (1997) 1903–1911.
- [71] A. Rempel, S.P. Mathupala, C.A. Griffin, A.L. Hawkins, P.L. Pedersen, Glucose catabolism in cancer cells: amplification of the gene encoding type II hexokinase, *Cancer Res.* 56 (1996) 2468–2471.
- [72] D.M. Parry, P.L. Pedersen, Intracellular localization and properties of particulate hexokinase in the Novikoff ascites tumor. Evidence for an outer mitochondrial membrane location, *J. Biol. Chem.* 258 (1983) 10904–10912.
- [73] K.K. Arora, P.L. Pedersen, Functional significance of mitochondrial bound hexokinase in tumor cell metabolism. Evidence for preferential phosphorylation of glucose by intramitochondrially generated ATP, *J. Biol. Chem.* 263 (1988) 17422–17428.
- [74] A. Marin-Hernandez, S. Rodriguez-Enriquez, P.A. Vital-Gonzalez, F.L. Flores-Rodriguez, M. Macias-Silva, M. Sosa-Garrocho, R. Moreno-Sanchez, Determining and understanding the control of glycolysis in fast-growth tumor cells. Flux control by an over-expressed but strongly product-inhibited hexokinase, *FEBS J.* 273 (2006) 1975–1988.
- [75] P.L. Pedersen, Warburg, me and Hexokinase 2: multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen, *J. Bioenerg. Biomembr.* 39 (2007) 211–222.
- [76] P.L. Pedersen, S. Mathupala, A. Rempel, J.F. Geschwind, Y.H. Ko, Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention, *Biochim. Biophys. Acta* 1555 (2002) 14–20.
- [77] S.P. Mathupala, Y.H. Ko, P.L. Pedersen, The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies, *Biochim. Biophys. Acta* 1797 (2010) 1225–1230.
- [78] A.P. Pereira da Silva, T. El-Bacha, N. Kyaw, R.S. dos Santos, W.S. da-Silva, F.C. Almeida, A.T. Da Poian, A. Galina, Inhibition of energy-producing pathways of HepG2 cells by 3-bromopyruvate, *Biochem. J.* 417 (2009) 717–726.
- [79] V.R. Fantin, J. St-Pierre, P. Leder, Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance, *Cancer Cell* 9 (2006) 425–434.
- [80] S. Mazurek, Pyruvate kinase type M2: A key regulator of the metabolic budget system in tumor cells, *Int. J. Biochem. Cell Biol.* (2010) (in press). doi:10.1016/j.biocel.2010.02.005.
- [81] S. Niemann, U. Muller, Mutations in SDHC cause autosomal dominant paraganglioma, type 3, *Nat. Genet.* 26 (2000) 268–270.
- [82] P.J. Pollard, J.J. Briere, N.A. Alam, J. Barwell, E. Barclay, N.C. Wortham, T. Hunt, M. Mitchell, S. Olpin, S.J. Moat, I.P. Hargreaves, S.J. Heales, Y.L. Chung, J.R. Griffiths, A. Dalgleish, J.A. McGrath, M.J. Gleeson, S.V. Hodgson, R. Poulosom, P. Rustin, I.P. Tomlinson, Accumulation of Krebs cycle intermediates and over-expression of HIF1 α in tumours which result from germline FH and SDH mutations, *Hum. Mol. Genet.* 14 (2005) 2231–2239.
- [83] L. Dang, D.W. White, S. Gross, B.D. Bennett, M.A. Bittinger, E.M. Driggers, V.R. Fantin, H.G. Jang, S. Jin, M.C. Keenan, K.M. Marks, R.M. Prins, P.S. Ward, K.E. Yen, L.M. Liao, J.D. Rabinowitz, L.C. Cantley, C.B. Thompson, M.G. Vander Heiden, S.M. Su, Cancer-associated IDH1 mutations produce 2-hydroxyglutarate, *Nature* 465 (2010) 966.
- [84] D. Bungard, B.J. Fuerth, P.Y. Zeng, B. Faubert, N.L. Mass, B. Viollet, D. Carling, C.B. Thompson, R.G. Jones, S.L. Berger, Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation, *Science* 329 (2010) 1201–1205.
- [85] K.E. Wellen, G. Hatzivassiliou, U.M. Sachdeva, T.V. Bui, J.R. Cross, C.B. Thompson, ATP-citrate lyase links cellular metabolism to histone acetylation, *Science* 324 (2009) 1076–1080.
- [86] D.J. Smiraglia, M. Kulawiec, G.L. Bistulfi, S.G. Gupta, K.K. Singh, A novel role for mitochondria in regulating epigenetic modification in the nucleus, *Cancer Biol. Ther.* 7 (2008) 1182–1190.
- [87] S.M. Wigfield, S.C. Winter, A. Giatromanolaki, J. Taylor, M.L. Koukourakis, A.L. Harris, PDK-1 regulates lactate production in hypoxia and is associated with poor prognosis in head and neck squamous cancer, *Br. J. Cancer* 98 (2008) 1975–1984.
- [88] R. Fukuda, H. Zhang, J.W. Kim, L. Shimoda, C.V. Dang, G.L. Semenza, HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells, *Cell* 129 (2007) 111–122.
- [89] R.M. Brand, R.H. Lyons, A.R. Midgley, Understanding the dynamics of cellular responsiveness to modifications of metabolic substrates in perfusion, *J. Cell. Physiol.* 160 (1994) 10–16.

- [90] K. Weber, D. Ridderskamp, M. Alfert, S. Hoyer, R.J. Wiesner, Cultivation in glucose-deprived medium stimulates mitochondrial biogenesis and oxidative metabolism in HepG2 hepatoma cells, *Biol. Chem.* 383 (2002) 283–290.
- [91] R.A. Gatenby, E.T. Gawlinski, The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models, *Cancer Res.* 63 (2003) 3847–3854.
- [92] R.A. Gatenby, R.J. Gillies, Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer* 4 (2004) 891–899.
- [93] R.A. Gatenby, R.J. Gillies, A microenvironmental model of carcinogenesis, *Nat. Rev. Cancer* 8 (2008) 56–61.
- [94] G. Benard, B. Faustin, E. Passerieux, A. Galinier, C. Rocher, N. Bellance, J.P. Delage, L. Casteilla, T. Letellier, R. Rossignol, Physiological diversity of mitochondrial oxidative phosphorylation, *Am. J. Physiol. Cell Physiol.* 291 (2006) C1172–C1182.
- [95] R. Nakashima, M. Paggi, P. Pedersen, Contributions of glycolysis and oxidative phosphorylation to adenosine 5'-triphosphate production in AS-30D hepatoma cells, *Cancer Res.* 44 (1984) 5702–5706.
- [96] J.M. Nascimento, L.Z. Shi, J. Tam, C. Chandsawangbhuwana, B. Durrant, E.L. Botvinick, M.W. Berns, Comparison of glycolysis and oxidative phosphorylation as energy sources for mammalian sperm motility, using the combination of fluorescence imaging, laser tweezers, and real-time automated tracking and trapping, *J. Cell. Physiol.* 217 (2008) 745–751.
- [97] T. Pfeiffer, S. Schuster, S. Bonhoeffer, Cooperation and competition in the evolution of ATP-producing pathways, *Science* 292 (2001) 504–507.
- [98] W.W. Ma, H. Jacene, D. Song, F. Vilardell, W.A. Messersmith, D. Laheru, R. Wahl, C. Endres, A. Jimeno, M.G. Pomper, M. Hidalgo, [18F]fluorodeoxyglucose positron emission tomography correlates with Akt pathway activity but is not predictive of clinical outcome during mTOR inhibitor therapy, *J. Clin. Oncol.* 27 (2009) 2697–2704.
- [99] X.L. Zu, M. Guppy, Cancer metabolism: facts, fantasy, and fiction, *Biochem. Biophys. Res. Commun.* 313 (2004) 459–465.
- [100] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros, N. Flomenberg, A.K. Witkiewicz, P.G. Frank, M.C. Casimiro, C. Wang, P. Fortina, S. Addya, R.G. Pestell, U.E. Martinez-Outschoorn, F. Sotgia, M.P. Lisanti, The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma, *Cell Cycle* 8 (2009) 3984–4001.
- [101] A. Trumpp, c-Myc and activated Ras during skin tumorigenesis: cooperation at the cancer stem cell level? *Ernst Schering Found. Symp. Proc.* (2006) 13–26.
- [102] L.G. Pedraza-Farina, Mechanisms of oncogenic cooperation in cancer initiation and metastasis, *Yale J. Biol. Med.* 79 (2006) 95–103.
- [103] W.C. Hahn, C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks, R.A. Weinberg, Creation of human tumour cells with defined genetic elements, *Nature* 400 (1999) 464–468.
- [104] I.B. Weinstein, A. Joe, Oncogene addiction, *Cancer Res.* 68 (2008) 3077–3080 discussion 3080.
- [105] I.B. Weinstein, A.K. Joe, Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy, *Nat. Clin. Pract. Oncol.* 3 (2006) 448–457.
- [106] V. Gogvadze, B. Zhivotovsky, S. Orrenius, The Warburg effect and mitochondrial stability in cancer cells, *Mol. Aspects Med.* 31 (2010) 60–74.
- [107] L. Galluzzi, N. Larochette, N. Zamzami, G. Kroemer, Mitochondria as therapeutic targets for cancer chemotherapy, *Oncogene* 25 (2006) 4812–4830.
- [108] S. Rodriguez-Enriquez, A. Marin-Hernandez, J.C. Gallardo-Perez, L. Carreno-Fuentes, R. Moreno-Sanchez, Targeting of cancer energy metabolism, *Mol. Nutr. Food Res.* 53 (2009) 29–48.
- [109] S. Rodriguez-Enriquez, J.C. Gallardo-Perez, A. Aviles-Salas, A. Marin-Hernandez, L. Carreno-Fuentes, V. Maldonado-Lagunas, R. Moreno-Sanchez, Energy metabolism transition in multi-cellular human tumor spheroids, *J. Cell. Physiol.* 216 (2008) 189–197.
- [110] F. Weinberg, N.S. Chandel, Mitochondrial metabolism and cancer, *Ann. N. Y. Acad. Sci.* 1177 (2009) 66–73.
- [111] P. Acebo, D. Giner, P. Calvo, A. Blanco-Rivero, A.D. Ortega, P.L. Fernandez, G. Roncador, E. Fernandez-Malave, M. Chamorro, J.M. Cuezva, Cancer abolishes the tissue type-specific differences in the phenotype of energetic metabolism, *Transl. Oncol.* 2 (2009) 138–145.
- [112] L.H. Stockwin, S.X. Yu, S. Borgel, C. Hancock, T.L. Wolfe, L.R. Phillips, M.G. Hollingshead, D.L. Newton, Sodium dichloroacetate selectively targets cells with defects in the mitochondrial ETC, *Int. J. Cancer* 127 (2010) 2510–2519.
- [113] T.J. Schulz, R. Thierbach, A. Voigt, G. Drewes, B. Mietzner, P. Steinberg, A.F. Pfeiffer, M. Ristow, Induction of oxidative metabolism by mitochondrial frataxin inhibits cancer growth: Otto Warburg revisited, *J. Biol. Chem.* 281 (2006) 977–981.
- [114] S. Wei, S.K. Kulp, C.S. Chen, Energy restriction as an antitumor target of thiazolidinediones, *J. Biol. Chem.* 285 (2010) 9780–9791.
- [115] J.W. Kim, I. Tchernyshyov, G.L. Semenza, C.V. Dang, HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia, *Cell Metab.* 3 (2006) 177–185.
- [116] D.J. Gough, A. Corlett, K. Schlessinger, J. Wegrzyn, A.C. Larner, D.E. Levy, Mitochondrial STAT3 supports Ras-dependent oncogenic transformation, *Science* 324 (2009) 1713–1716.
- [117] A. Shakya, R. Cooksey, J.E. Cox, V. Wang, D.A. McClain, D. Tantin, Oct1 loss of function induces a coordinate metabolic shift that opposes tumorigenicity, *Nat. Cell Biol.* 11 (2009) 320–327.
- [118] R.L. Elstrom, D.E. Bauer, M. Buzzai, R. Karnauskas, M.H. Harris, D.R. Plas, H. Zhuang, R.M. Cinali, A. Alavi, C.M. Rudin, C.B. Thompson, Akt stimulates aerobic glycolysis in cancer cells, *Cancer Res.* 64 (2004) 3892–3899.