Chapter 11
The Relevance of the Mitochondrial H^+-ATP Synthase in Cancer Biology

Inmaculada Martínez-Reyes and José M. Cuezva

Contents

I. Martínez-Reyes
Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Centro de Investigación Biomédica en Red de Enfermedades Raras CIBERER-ISCIII, Instituto de Investigación Hospital 12 de Octubre, Universidad Autónoma de Madrid, Madrid 28049, Spain

J.M. Cuezva (✉)
Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Centro de Investigación Biomédica en Red de Enfermedades Raras CIBERER-ISCIII, Instituto de Investigación Hospital 12 de Octubre, Universidad Autónoma de Madrid, Madrid 28049, Spain

Centro de Biologia Molecular Severo Ochoa, Universidad Autonoma de Madrid, Nicolas Cabrera 1, Madrid 28049, Spain
e-mail: jmcuezva@cbm.csic.es

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11.1 Introduction

During the last decade, energy metabolism has become a central issue of investigation in cancer studies. Indeed, energy metabolism regulates the complex network of cellular processes that allow adaptation of the cell to different normal and pathological situations. Uncontrolled proliferation is not an exception because it is very much influenced by changes in the activity of the metabolic pathways of the cell. The dependency of cancer cells on aerobic glycolysis was basically neglected for many years due in part to the prevailing idea that cancer was considered only a genetic disease. Nowadays, thanks to the effort of many laboratories, cancer can also be regarded as a metabolic disease since its onset and progression cannot be understood without the reprogramming of energy metabolism of the cells.

One of the most powerful techniques available in oncology for the diagnosis, staging, and follow-up of most cancer patients is tumor imaging using ^{18}F-deoxyglucose positron emission tomography (^{18}FDG-PET) (Rigo et al. 1996). This technique is based on the high glucose avidity of cancer cells (Ortega et al. 2009), and it represents the translation to the clinics of the original Warburg (Warburg 1930) observation: "cancer cells have an enhanced aerobic glycolysis when compared to normal cells." In addition to the differential uptake of FDG, some human cancers also display a differential uptake of choline, acetate, and some amino acids, suggesting a heterogeneous alteration of the metabolic pathways as solution to cover the energy and building blocks demands of proliferating cancer cells (Cairns et al. 2011). In the present chapter, we will review (1) the overall changes of the metabolic pathways that support proliferation and (2) the findings that implicate the bioenergetic alteration of mitochondria in carcinogenesis. Other related topics that integrate mitochondrial bioenergetics with the cell death machinery and the mechanisms that trigger the silencing of oxidative phosphorylation (OXPHOS) in prevalent human carcinomas have been recently summarized elsewhere (Willers and Cuezva 2011; Sanchez-Arago et al. 2013a).

11.2 Overview of the Changes in Metabolic Pathways During Proliferation

Glycolysis and mitochondrial OXPHOS are the two main pathways of energy provision that are tightly and inversely regulated in the cell (Fig. 11.1). The control of the flux through these pathways is primarily exerted by regulation of the activity of key enzymes by the availability of metabolic intermediates such as ATP and NADH and/or by genetic regulation of the expression of the genes involved (Formentini et al. 2010). In normal differentiated aerobic cells, glucose is partially oxidized to pyruvate by glycolysis in the cytoplasm (Fig. 11.1). Pyruvate can be further oxidized in mitochondria to form the acetyl-CoA that enters the tricarboxylic acid cycle (TCA) for its complete oxidation to CO_2, or it is reduced and

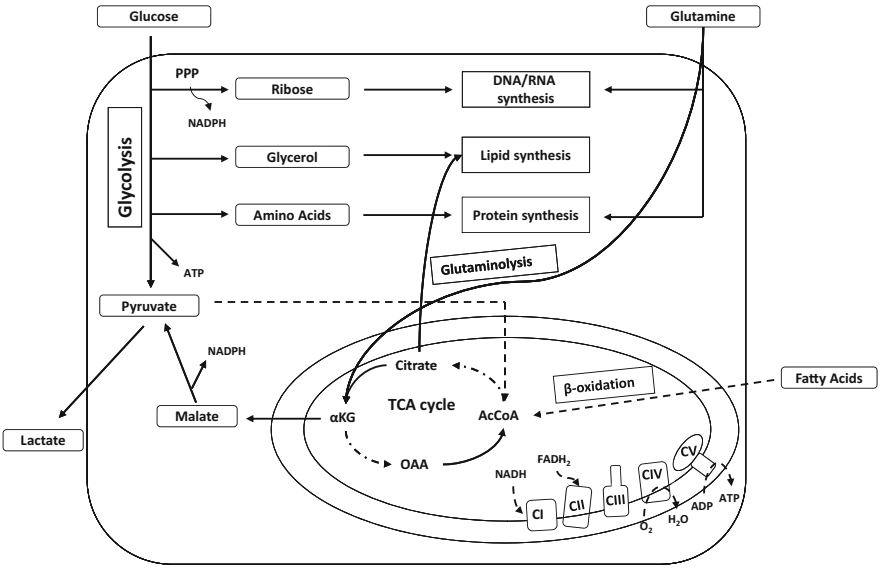

Fig. 11.1 Metabolic pathways in tumor cells. The scheme shows some of the relevant aspects of the metabolism of cancer cells. After entering the cell by specific transporters, glucose enters the glycolytic pathway, and it can be (1) catabolized by the pentose phosphate pathway (PPP) to obtain ribose required for the synthesis of nucleotides and reducing power in the form of NADPH, (2) used for the synthesis of glycerol and amino acid precursors that are needed for the synthesis of lipid and proteins, respectively, or (3) utilized to generate pyruvate. In the cytoplasm, the generated pyruvate can be reduced to lactate and further excreted from the cell or oxidized in the mitochondria by pyruvate dehydrogenase to generate acetyl-CoA. The operation of the tricarboxylic acid cycle (TCA cycle) and the activity of oxidative phosphorylation (OXPHOS) complete the oxidation of mitochondrial pyruvate. The transfer of electrons obtained in biological oxidations ($NADH/FADH_2$) to molecular oxygen by respiratory complexes (CI to CIV) of the inner mitochondrial membrane and the synthesis of ATP by the H^+-ATP synthase (CV) are also represented. Different pathways that drain intermediates of the TCA cycle (oxaloacetate (OAA), α-ketoglutarate (αKG), malate, and citrate), for biosynthetic purposes are shown. The incorporation of glutamine carbon skeletons into the TCA cycle by glutaminolysis and its diversion to other biosynthetic pathways is depicted as well as the conversion of fatty acids into acetyl-CoA by β-oxidation. Continuous arrows represent activated pathways in cancer cells, whereas discontinuous lines represent repressed pathways in cancer cells

excreted from the cell as lactate if oxygen availability is limited and/or mitochondria have an impaired function (Fig. 11.1). The oxidation of fatty acids in mitochondrial β-oxidation is an additional pathway that supplies energy and acetyl-CoA in many normal aerobic cells (Fig. 11.1). Under normal oxygenated conditions, the electrons obtained in the oxidation of acetyl-CoA are collected in the *redox* coenzymes NADH and $FADH_2$. These electrons are funneled into the respiratory chain which is placed in the inner membrane of mitochondria to reduced O_2 and generate the water of respiration (Fig. 11.1). Electron transfer in the respiratory chain promotes proton pumping from the matrix interior to the intermembrane space to generate the proton gradient that is used as intermediate for the synthesis of

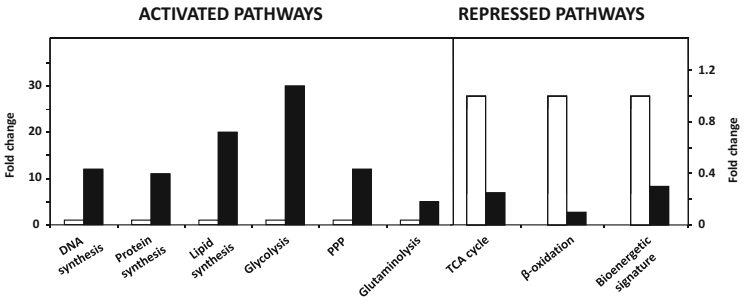

Fig. 11.2 Metabolic reprogramming in proliferating cells. *Plots* represent changes in the activity of metabolic pathways when comparing proliferating and nonproliferating cells. Increased rates of DNA, lipid, and protein synthesis support high rates of cellular proliferation. Highly proliferative cells are dependent on enhanced activities of glycolysis, glutaminolysis, and the pentose phosphate pathway (PPP). The activities of the TCA cycle and β-oxidation are repressed in proliferative cells when compared to quiescent cells. A change in the bioenergetic signature between normal (quiescent) and tumor (proliferative) tissues is also shown. Data taken from (Wang and Green 2012b)

ATP in OXPHOS. The reentrance of H^+ through the H^+-ATP synthase (CV in Fig. 11.1) harnesses most of the ATP that is utilized by normal aerobic differentiated cells.

Proliferative cells exhibit different metabolic requirements when compared to normal differentiated cells (Fig. 11.2) (Vander Heiden et al. 2013; DeBerardinis et al. 2008; Wang and Green 2012a, b; Brand and Hermfisse 1997). Highly proliferative cells have an increased demand of carbon skeletons, nitrogen, and reducing equivalents to duplicate the mass of the cell that supports cell division in addition to the requirements needed to maintain housekeeping functions. This situation makes necessary the consumption of more nutrients and the diversion of metabolic precursors into the pathways of macromolecular synthesis (Figs. 11.1 and 11.2). Thus, proliferating cancer cells experience the reprogramming of metabolism to balance biosynthetic pathways with those that supply ATP to assure cellular growth and survival (Sanchez-Arago et al. 2013a; Cuezva et al. 2009). However, it has been reported that ATP demand is not significantly different between proliferating and differentiated cells (Vander Heiden et al. 2013; Kilburn et al. 1969; Locasale and Cantley 2010; Sanchez-Arago et al. 2013b), suggesting that maintenance of the differentiated state is also a very energy demanding process.

As mentioned above, a fundamental feature of proliferating cancer cells is the increased uptake of glucose and its conversion to lactate in the presence of oxygen (Fig. 11.1), the so-called enhanced aerobic glycolysis of carcinomas. The abnormal rate of glycolysis of cancer cells was ascribed to an altered mitochondrial function as a compensatory mechanism to supply the energy required for proliferation (Warburg 1956a, b). It is well established that the rate of ATP production by OXPHOS determines the rate of glucose consumption by the cell (Sanchez-Arago et al. 2010). However, Warburg's hypothesis was taken with suspicion by the

scientific community because it appeared paradoxical that cancer cells that theoretically have an increased need for ATP relied on the less efficient glycolytic pathway for ATP provision. It is now clear that the catabolism of glucose through glycolysis provides cancer cells with other metabolic needs in addition to ATP (Fig. 11.1). In fact, glucose carbon skeletons provide ribose and NADPH for nucleotide biosynthesis through the pentose phosphate pathway (Fig. 11.1), glycerol and citrate for the synthesis of lipids and cholesterol (Fig. 11.1), and other intermediates for the synthesis of nonessential amino acids (Fig. 11.1). Many cancer cells have an increased flux from glucose into serine and glycine (de Koning et al. 2003) that represent important intermediates in the building of proteins, lipids, and nucleic acids (Possemato et al. 2011; Locasale et al. 2011) and also help cells to mitigate oxidative stress (Mullen and DeBerardinis 2012). These amino acids are generated by the glycolytic enzyme phosphoglycerate dehydrogenase (PHGDH) that has been found to be activated in tumors (Locasale et al. 2011; Pollari et al. 2011) and by an alternative glycolytic pathway that seems to be controlled by the inhibition of PKM2 (Vander Heiden et al. 2013). It has been suggested that aerobic glycolysis could provide an additional advantage to proliferative cells because it allows cells to use glucose at a faster rate when compared to OXPHOS (Pfeiffer et al. 2001; Koppenol et al. 2011).

Anabolic processes also require a source of reducing power in the form of NADPH that, similar to ATP, must be constantly generated to support the biosynthetic processes. The catabolism of glucose by the pentose phosphate pathway (PPP) is a major source of NADPH production in cancer cells (Fig. 11.1). It has been reported that the flux through the oxidative part of the PPP is not activated in some proliferating cells (Boros et al. 2000), suggesting that other pathways might also participate in the replenishment of the NADPH required for synthetic purposes (Fig. 11.1). The generation of NADPH also contributes to the maintenance of the cellular *redox* state by keeping glutathione in the reduced state.

The activity and purpose of the TCA cycle is an important aspect that differentiates quiescent and proliferative cells (Fig. 11.2). Whereas in quiescent cells the TCA cycle is mainly used to maximize the production of ATP, in proliferative cells the TCA cycle plays a role as a source of intermediates for biosynthetic purposes in processes that consume ATP. For example, mitochondrial citrate is exported out to the cytoplasm (Fig. 11.1) where it is converted to oxaloacetate (OAA) and acetyl-CoA. This process is essential for de novo synthesis of fatty acids and cholesterol required for the building up of membranes of the proliferating cells (Kannan et al. 1980; Ookhtens et al. 1984). Tumor cells and hematopoietic cells display an upegulated expression of the lipogenic enzymes ATP citrate lyase and fatty acid synthase which are both important to stimulate proliferation (Bauer et al. 2005; Hatzivassiliou et al. 2005). OAA and α-KG are also involved in the biosynthesis of macromolecules as they constitute an intracellular pool of precursors required for the synthesis of nonessential amino acids and nucleotides.

The catabolism of glutamine, the most abundant amino acid in mammals, is also relevant during proliferation (Figs. 11.1 and 11.2) (DeBerardinis et al. 2008; Cuezva et al. 2009). In proliferating cells, glutamine is metabolized by multiple

pathways for different purposes (Fig. 11.1) (Eagle et al. 1956; Kovacevic and McGivan 1983). Glutamine is the nitrogen donor for several metabolic enzymes and for de novo synthesis of purines and pyrimidines (Fig. 11.1). In addition, glutamine can be partially oxidized by glutaminolysis providing NADPH and lactate to the cell (Fig. 11.1) (Reitzer et al. 1979). Glutamine is also essential to reload the TCA cycle with carbon skeletons as a result of citrate being exported from mitochondria for lipid synthesis (Fig. 11.1). Glutamine anaplerosis of the TCA cycle favors the biosynthesis of many precursors that derive from the intermediates of the cycle (DeBerardinis et al. 2008; Mullen and DeBerardinis 2012). The noncanonical conversion of glutamine into α-ketoglutarate (α-KG) that is directed to the synthesis of isocitrate and finally citrate is especially important for de novo lipogenesis to support tumor growth when the mitochondrial function is impaired or in hypoxic conditions (Mullen et al. 2011; Wise et al. 2011; Metallo et al. 2011). The reductive glutamine metabolic pathway has been found in adipose cells, in T cells, and in transformed cancer cells (Metallo et al. 2011; Des Rosiers et al. 1994; Yoo et al. 2008; Le et al. 2012). Recently, glutamine has been shown to be essential to support pancreatic cancer growth by a series of metabolic reactions that are signaled by the oncogene KRAS (Son et al. 2013). Alanine is another important amino acid involved in transaminations and in the synthesis of new proteins (Moreadith and Lehninger 1984). Alanine has been shown to be more abundant in prostate cancer tissue providing a biomarker of the disease (Costello and Franklin 2005; Tessem et al. 2008). The increase in alanine concentration of cancer cells could be a consequence of an increase in the catabolism of cellular proteins and/or of the increased protein synthesis required.

Rapidly proliferating cells also rely on an altered lipid metabolism (Fig. 11.2). The bulk of cell membrane lipids are phospholipids, sterols, and sphingolipids. The production of these lipids requires fatty acids as building blocks that come from either exogenous sources or from de novo fatty acid synthesis. While most normal human cells prefer exogenous sources, tumor cells have developed the capability to easily synthesize fatty acids de novo (Ookhtens et al. 1984; Medes et al. 1956). Consistent with these findings, the expression of CD36 which is a widely expressed transmembrane protein involved in the uptake of fatty acids is implicated in breast cancer and significantly decreases its expression in malignant and stromal tissue as an early step in tumorigenesis (DeFilippis et al. 2012). As we have discussed, de novo synthesis of fatty acids for the formation of membranes often derives from glucose and glutamine catabolism (Currie et al. 2013). However, some tumors also consume lipids from their environment rendering fatty acid uptake as a potential therapeutic target. For example, fatty acid binding protein 4 (FABP4), a lipid chaperone, is involved in providing fatty acids from surrounding adipocytes to ovarian tumors increasing metastasis and tumor growth (Nieman et al. 2011). The treatment of prostate cancer cells with fatty acid synthase (C75) or ATP citrate lyase (SB-204990) inhibitors showed reduced viability of the cells only when cultured in the absence of lipoproteins, an exogenous lipid source (Ros et al. 2012). Figure 11.2 provides a summary of the changes and relative activities in metabolic pathways observed in proliferating cells.

11.3 The Bioenergetic Function of Mitochondria in Cancer Cells

If the induction of glycolysis in cancer cells is nowadays no longer questioned, the impairment of mitochondrial function is still a subject of debate (Koppenol et al. 2011; Krebs 1981; Warburg 1966; Weinhouse 1976; Dang 2010; Schulze and Harris 2012). A key player in regulating the bioenergetic function of mitochondria is the H^+-ATP synthase (Fig. 11.1), a reversible engine located in the inner mitochondrial membrane that synthesizes or hydrolyzes ATP upon changes in cellular conditions. The mammalian H^+-ATP synthase is a multiprotein complex of 650 kDa that consists of two main domains: F0, which is a hydrophobic subcomplex embedded in the inner membrane and contains the proton channel, and the hydrophilic F1 subcomplex exposed to the matrix that is responsible for the catalytic function (Boyer 1997). The F1 portion consists of three catalytic subunits (β-F1-ATPase) and three regulatory subunits (α-F1-ATPase) that are arranged around a central stalk (the γ subunit) as segments of an orange (Abrahams et al. 1994). The F0 domain is a rotary motor that uses the proton flux to generate the conformational changes in the F1 domain needed for the synthesis of ATP (Boyer 1993; Capaldi and Aggeler 2002; Yoshida et al. 2001). The ATPase inhibitory factor 1 (IF1) is a physiological inhibitor of the H^+-ATP synthase that exerts a very important regulation of its activity in cancer (Sanchez-Cenizo et al. 2010; Sanchez-Arago et al. 2012).

Despite recent claims that cancer cells retain a functional bioenergetic activity of mitochondria (Koppenol et al. 2011; Dang 2010; Schulze and Harris 2012), a large number of studies established the impaired bioenergetic function of the organelle in the cancer cell [for a detailed review, see Pedersen (1978)]. To list some, it was shown that pyruvate dehydrogenase of tumor mitochondria kinetically resembles that of embryonic tissues (Lazo and Sols 1980). As indicated for proliferating cells (Fig. 11.2), the Krebs cycle in tumor mitochondria was shown to be truncated at the level of the conversion to citrate (Parlo and Coleman 1984), later on confirmed by a proteomic approach (Bi et al. 2006). In this situation, glutamine primes the TCA cycle with carbon skeletons (DeBerardinis et al. 2008; Baggetto 1992). Deficiencies in enzyme complexes of the respiratory chain and oxidative phosphorylation have also been described in cancer (Krieg et al. 2004). Consistent with some of the molecular and functional alterations described in cancer mitochondria, the organelles have less *cristae* than in normal cells (Pedersen 1978; Cuezva et al. 2002). Likewise, carcinomas have a diminished mitochondrial content when compared to noncancerous cells (Pedersen 1978; Cuezva et al. 2002; de Heredia et al. 2000). Mutations in mitochondrial DNA of human carcinomas (Carew and Huang 2002) and in nuclear genes involved in energy transduction are known to predispose to some types of inherited neoplasia syndromes (Eng et al. 2003). Conversely, a recent study has demonstrated that the introduction of noncancerous mitochondria into highly metastatic cells (cybrid) reverses the oncogenic characteristics by inhibiting tumor promoting pathways of the cells (Kaipparettu et al. 2013), thus supporting

that rectifying mitochondrial function is a promising target in cancer therapy. All these findings ultimately suggest that by one mechanism or another mitochondrial activity of the cancer cell is compromised.

Transcriptomic, proteomic, functional, and structural studies of human carcinomas strongly emphasize that a repressed bioenergetic activity of mitochondria is concomitant with the enhanced glycolytic flux required for tumor progression (Cuezva et al. 2009; Martinez-Reyes et al. 2012). To list few examples, it has been documented that the transformed state is accompanied by an increased flux of glycolysis due to aberrant mitochondrial respiration (Ramanathan et al. 2005). In H-RASV12/E1A-transformed cells, the tumorigenic potential of the cells is directly proportional to the enhancement of glycolysis and the suppression of mitochondrial respiration (de Groof et al. 2009). Other findings demonstrate that the previous selection of cancer cells with a diminished bioenergetic activity of mitochondria is needed for in vivo tumor progression (Sanchez-Arago et al. 2010). Importantly, the acquisition of the metabolic phenotype that is compatible with tumor progression is a reversible trait acquired by adaptation of the cells to the milieu where tumors develop in vivo (Sanchez-Arago et al. 2010). In gliomas, it has been documented that the enhanced infiltration in the brain parenchyma parallels the repression of mitochondrial biogenesis and an increased glycolysis (Keunen et al. 2011). A recent study has reported that inhibition of complex I of the mitochondrial electron transport chain promotes breast cancer cell migration and invasion by the upregulation of HIF1α and VEGF as a consequence of an increased production of ROS (Ma et al. 2013).

Hence, to understand the role of mitochondria in cancer biology, it is necessary to take into account other aspects different from the impact of cancer genes in energy metabolism. Specifically, we have to consider the inverse correlation that exists between the activity of OXPHOS and glycolysis (Sanchez-Arago et al. 2010; Isidoro et al. 2005; Aldea et al. 2011). As it has been extensively discussed, the activation of several oncogenes and the repression of tumor suppressors do have an impact on the metabolic switch of the cells. However, the overwhelming list of different mutations described so far in cancers cannot explain why most prevalent carcinomas, independently of the tissue of origin, histological type, and the diversity of the genetic alterations that they contain, converge on the same protein signature of energy metabolism (Acebo et al. 2009). The simplest explanation is that cancer cells just reproduce the bioenergetic phenotype of the metabolism of proliferating cells (Fig. 11.2). In fact, the rewiring of energy metabolism to an enhanced glycolysis or to an increased dependence on OXPHOS is a two-way path through which cells can transit in any direction without the need of genetic alterations. In other words, it is a reversible trait that can be taken in either of the two directions depending upon environmental, developmental, and/or pathological cues. Some relevant examples to understand the bioenergetic phenotype of carcinomas are the metabolic changes experienced by cells during adaptation to hypoxic milieus (Semenza 2008, 2011) and the dedifferentiation of adult somatic cells into iPS cells (Folmes et al. 2011), where the activation of glycolysis is concurrent with the repression of OXPHOS. Two other examples walking down the opposite

direction of the trail are the metabolic rewiring experienced by hepatocytes from the fetal to the neonatal stage (Cuezva et al. 2007) and the differentiation of stem cells into different cellular lineages (Sanchez-Arago et al. 2013b; Rehman 2010; Chen et al. 2008). In these latter cases, it involves the repression of glycolysis with the further concurrent development of the bioenergetic function of mitochondria. Development further affords the example of the switch in the expression of protein isoforms typical of cancer cells and embryonic tissues to adult-type proteins (Cuezva et al. 1997, 2007; Mazurek 2011). Moreover, the parallelism between cancer and embryonic cells not only impinges on the content and dedifferentiated state of mitochondria (de Heredia et al. 2000; Cuezva et al. 2007) but also in the structure of the organelle since they have less *cristae* when compared to mitochondria in normal cells (Cuezva et al. 2002; Arismendi-Morillo 2011). It is well established that dimers of the H^+-ATP synthase promote the high local curvature of the inner mitochondrial membrane at *cristae* ridges (Paumard et al. 2002; Strauss et al. 2008; Davies et al. 2011, 2012). Apparently, aging seems to meltdown the inner membrane *cristae* of mitochondria by age-dependent dissociation of ATP synthase dimers (Daum et al. 2013). We suggest that the lower relative expression of the H^+-ATP synthase in cancer cells as we will see in the next section explains the aberrant structure of mitochondria observed in carcinomas.

11.4 The Clinical Relevance of the Bioenergetic Signature of Cancer

As mentioned above, studies in development highlighted the inverse correlation that exists between the expression of a key component of OXPHOS such as the catalytic subunit of the H^+-ATP synthase (β-F1-ATPase) with the expression and activity of the enzymes of glycolysis (Cuezva et al. 1997, 2007). These observations prompted us to design a simple test to determine the protein signature of energy metabolism (Cuezva et al. 2002) that could be translated into the cancer field with the purpose of contrasting the feasibility of Warburg's hypothesis (Warburg 1956a, b). With this aim, we studied in normal and tumor biopsies derived from the same cancer patients the expression level of two mitochondrial proteins: β-F1-ATPase and Hsp60, respectively, representing the bioenergetic and structural function of mitochondria. We also determine the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), used as representative of the glycolytic pathway (Cuezva et al. 2002; Isidoro et al. 2004). Whereas the β-F1-ATPase/Hsp60 ratio provides an index of the bioenergetic competence of the organelle, the β-F1-ATPase/Hsp60/GAPDH or the β-F1-ATPase/GAPDH ratio provides indexes of the overall mitochondrial potential of the cell, which are two levels that could compromise the bioenergetic activity of mitochondria in carcinomas (Cuezva et al. 2002). The ratio that estimated the overall mitochondrial potential of the

cell was defined as the bioenergetic cellular (BEC) index (Cuezva et al. 2002), nowadays simplified to the β-F1-ATPase/GAPDH ratio (Lopez-Rios et al. 2007).

Consistent with Warburg's postulates, a significant reduction of the β-F1-ATPase/Hsp60 ratio was observed in colon, lung, breast, esophageal, gastric, and renal carcinomas when compared to paired normal tissues (Cuezva et al. 2002; Isidoro et al. 2004), suggesting a deficit in the bioenergetic competence of the mitochondrion. Remarkably, these changes in the mitochondrial proteome of the tumor were accompanied by the concurrent sharp increase of the glycolytic GAPDH marker (Cuezva et al. 2002; Isidoro et al. 2004) as well as by several other glycolytic proteins such as lactate dehydrogenase (Isidoro et al. 2005) and pyruvate kinase M2 (Isidoro et al. 2004, 2005; Aldea et al. 2011). 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 tissues, thus supporting a bioenergetic deficit of mitochondria in the carcinomas (Cuezva et al. 2002). This proteomic feature was defined as the "bioenergetic signature of cancer" (Cuezva et al. 2002, 2009) and its downregulation has been confirmed and extended to different carcinomas (He et al. 2004; Hervouet et al. 2005; Meierhofer et al. 2004; Unwin et al. 2003; Yin et al. 2004; Lin et al. 2008) (see (Cuezva et al. 2009) for a recent review). More recently, we have functionally supported the relevance of Warburg's postulates after the demonstration that the rates of glucose capture assessed by FDG-PET imaging inversely correlate with the bioenergetic signature in lung carcinomas (Lopez-Rios et al. 2007). Moreover, the generation of isogenic HCT116 colon cancer cell lines expressing different levels of β-F1-ATPase, to assess the contribution of mitochondrial bioenergetics in cancer progression (Sanchez-Arago et al. 2010), allowed us to demonstrate that the activity of glycolysis and OXPHOS is inversely correlated, being the overall activity of OXPHOS the one that defines the rate of glucose utilization by aerobic glycolysis (Sanchez-Arago et al. 2010). Hence, interference with OXPHOS is part of the mechanism that triggers the increased glucose avidity of the cancer cell. Quantitative assays, using high-affinity monoclonal antibodies against proteins of the "bioenergetic signature" (Acebo et al. 2009), have revealed an unanticipated finding: tumors from different tissues and/or histological types have the same proteomic signature of energy metabolism, indicating that cancer abolishes the tissue-specific differences in the bioenergetic phenotype of the cell (Acebo et al. 2009). In other words, energy metabolism represents an additional hallmark of the cancer phenotype (Ortega et al. 2009; Hanahan and Weinberg 2000) and a promising target for the treatment of diverse neoplasias (Cuezva et al. 2009; Acebo et al. 2009).

Many of the potential cancer biomarkers that are being discovered at the bench-side are rarely translated in benefit of the management of the patients because they lack support from data obtained in clinically oriented studies. To bridge this gap, we have studied the bioenergetic signature of tumors in large cohorts of different cancer patients (Cuezva et al. 2002, 2004; Isidoro et al. 2005; Aldea et al. 2011; Lopez-Rios et al. 2007; Ortega et al. 2008) to explore its relevance as predictor of overall survival (OS) and/or of disease recurrence (DFS). Figure 11.3 provides

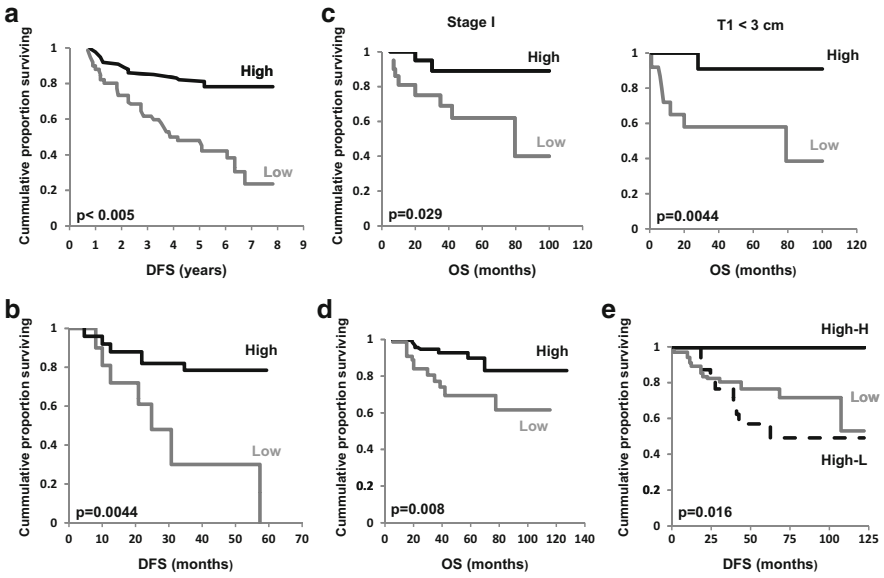

Fig. 11.3 Bioenergetic signature is a biomarker of cancer prognosis. Kaplan–Meier survival analysis show the association of markers of the bioenergetic signature with overall (OS) and/or disease-free (DFS) survival in different cohorts of cancer patients. (**a**) and (**b**). Significant association of the high expression level of β-F1-ATPase (**a**) and of the β-F1-ATPase/GAPDH ratio (**b**) (*black curves*) with better prognosis in two independent cohorts of colon cancer patients (Cuezva et al. 2002; Aldea et al. 2011). (**c**) A high BEC index (*black curve*) in early stage disease of lung cancer patients predicts better outcome (Cuezva et al. 2004). (**d**) A high β-F1-ATPase/Hsp60 ratio in breast carcinomas is a significant marker of better prognosis (Isidoro et al. 2005). (**e**) The combination of markers of the bioenergetic signature (high and low, *black and gray curves*, respectively) with the expression level of HuR [high (-H) and low (-L)] allows the discrimination of breast cancer patients that within the group of higher bioenergetic signature have higher risk of disease recurrence (*dotted curve*) because they have low expression of HuR (high-L) (Ortega et al. 2008). High and low expression levels are represented in *black and gray curves*, respectively. The log-rank test *p* value is shown. The curves have been redrawn from the indicated references

representative examples of this sort of studies in large cohorts of colon (Fig. 11.3a, b), lung (Fig. 11.3c), and breast (Fig. 11.3d, e) cancer patients. Immunohistochemical determination of β-F1-ATPase in a large cohort of stage II colon cancer patients using colon-tissue microarrays (Fig. 11.3a) indicated that a low tumor expression of β-F1-ATPase afforded an excellent marker of both the overall (not shown) and disease-free survival (Fig. 11.3a) of colon cancer patients (Cuezva et al. 2002). The clinical utility of β-F1-ATPase as a marker of colon cancer prognosis has been independently confirmed in a different large cohort of colon cancer patients (Lin et al. 2008). A more recent study in a more heterogeneous cohort of colon cancer patients using reverse phase protein microarrays, a technique that affords a quantitative approach to assess the bioenergetic signature (β-F1-ATPase/GAPDH ratio), provided essentially the same findings (Fig. 11.3b) (Aldea et al. 2011). It is worth mentioning here that a study in a large cohort of colon cancer patients investigating

the genetic alterations that impact on metabolic genes has concluded that the impairment of OXPHOS is the only metabolic pathway that correlates with patient prognosis (Sheffer et al. 2009). The bioenergetic signature also predicts the overall survival of lung cancer patients at early stages of the disease (stage I or tumor size less than 3 cm) (Fig. 11.3c), and the lower the bioenergetic signature (β-F1-ATPase/Hsp60/GAPDH ratio) of the tumor, as assessed by protein expression on 2D gels (Cuezva et al. 2004), the worse the prognosis for lung cancer patients (Fig. 11.3c). Similar results have been obtained in a different cohort of lung cancer patients using immunohistochemistry to assess β-F1-ATPase expression in the tumors (Lopez-Rios et al. 2007). In that study (Lopez-Rios et al. 2007), a low expression of the protein also correlated with a worse prognosis for lung cancer patients. Interestingly, multivariate Cox regression analysis indicated that tumor β-F1-ATPase expression in lung and colon carcinomas is an independent marker of survival (Aldea et al. 2011; Lopez-Rios et al. 2007). Similarly, the bioenergetic signature has also been shown to provide relevant markers of disease progression in breast cancer patients (Fig. 11.3d) (Isidoro et al. 2005). The robustness of markers of the bioenergetic signature for cancer diagnosis has been illustrated by Fisher linear discriminant analysis (Isidoro et al. 2005; Aldea et al. 2011; Cuezva et al. 2004). Using cross-validation, a classification sensitivity of lung, colon, and breast tumor biopsies >95 % has been reported, strongly supporting that alteration of the bioenergetic function of mitochondria is a hallmark of cancer. Overall, these studies emphasized that an impaired bioenergetic function of mitochondria compromises survival by favoring recurrence of the disease and cancer progression, strongly supporting the original Warburg hypothesis.

A translation repression mechanism regulates the expression of β-F1-ATPase in fetal rat liver (Izquierdo and Cuezva 1997) and in rat hepatomas (de Heredia et al. 2000). Consistently, the downregulation of β-F1-ATPase expression in prevalent human carcinomas (lung, breast, colon) is also exerted at posttranscriptional levels by a specific translation masking event of the β-F1-ATPase mRNA (Willers et al. 2010). Mechanistically, translational regulation could be explained by differences in the affinity of the mRNA to the translational machinery as well as by the action of regulatory proteins and miRNAs that bind sequence elements within the mRNA for controlling its translation (Willers and Cuezva 2011). In this regard, we have described that the 3′UTR of rat β-F1-ATPase mRNA is essential for translation of the transcript due to its ability to interact with components of the translational machinery (Izquierdo and Cuezva 1997). That is, the 3′UTR behaves as a translational enhancer both in vitro (Izquierdo and Cuezva 1997, 2000) and in transfected cells (Di Liegro et al. 2000). This activity of the 3′UTR is essential for conferring the appropriate bioenergetic phenotype to daughter cells during cellular proliferation because it drives the synthesis of β-F1-ATPase at the G2/M phase of the cycle (Martinez-Diez et al. 2006) when *cap*-dependant translation is partially inhibited (Pyronnet and Sonenberg 2001). More recently, we have demonstrated that the 3′UTR of human β-F1-ATPase mRNA is also required for efficient translation of the transcript (Willers et al. 2010).

The control of the translation of β-F1-ATPase mRNA during development (Izquierdo and Cuezva 1997) and in rat hepatomas (de Heredia et al. 2000) involves specific proteins that bind the rat transcript. The binding of these proteins is regulated by the energy and *redox* state of the cell (Izquierdo and Cuezva 2005). It is assumed that the binding of proteins to the 3′UTR of β-F1-ATPase mRNA sterically hinders the initiation of translation (Cuezva et al. 2007; Izquierdo and Cuezva 1997). With these findings in mind, we have pursued the molecular and functional characterization of the human β-F1-ATPase mRNA interacting proteins (Ortega et al. 2008, 2010). We have identified that the AU-rich element-binding protein HuR, which is a central regulator of posttranscriptional gene expression (Levy et al. 1998), interacts with the human 3′UTR of β-F1-ATPase mRNA (Ortega et al. 2008). However, functional studies demonstrated that HuR plays an ancillary role in β-F1-ATPase expression in human cells (Ortega et al. 2008). 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 (Ortega et al. 2008). 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 (Fig. 11.3e) (Ortega et al. 2008). 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.

In addition, we have identified nine RNA binding proteins that interact in vitro with β-F1-ATPase mRNA (Ortega et al. 2010) and found the in vivo association of G3BP1 (Ras-GAP SH3 binding protein 1) with the 3′UTR of human β-F1-ATPase mRNA (Ortega et al. 2010). This interaction is functionally relevant because it represses β-F1-ATPase mRNA translation by preventing its recruitment into active polysomes (Ortega et al. 2010). Since G3BP1 is overexpressed in several tumors and cancer cell lines (Barnes et al. 2002; Guitard et al. 2001; Zhang et al. 2007) and β-F1-ATPase expression in human cancer is exerted at the level of translation (Willers et al. 2010), the findings suggest that G3BP1 could play an essential role in the glycolytic switch that occurs in cellular transformation, contributing to define the bioenergetic phenotype of cancer (Ortega et al. 2010). Ongoing studies are aimed at establishing the potential role of G3BP1 as biomarker in cancer prognosis.

In contrast to these findings, the downregulation of β-F1-ATPase expression in chronic myeloid leukemia is mediated by hypermethylation of the promoter of the gene (Li et al. 2010). In addition, in colorectal cancer, the deregulation of the activity of the H^+-ATP synthase due to chromosomal instability of the ATP5A1 gene has been described (Bacolod and Barany 2010). Moreover, the decreased expression of this gene also affords a marker of unfavorable clinical outcome (Bacolod and Barany 2010). It has been recently described that miRNAs that are deregulated in various cancers have an important impact on signaling pathways in mitochondria (Bienertova-Vasku et al. 2013). Interestingly, translation silencing of β-F1-ATPase mRNA during development of the human fetal liver seems to be exerted by the action of miRNA-127-5p (Willers et al. 2012), a mechanism that is not operative in prevalent human carcinomas because this miRNA is not expressed in carcinomas.

11.5 The H^+-ATP Synthase and Tumor Suppression

A dysfunctional OXPHOS promotes cellular proliferation and invasion (Sanchez-Arago et al. 2013a; Amuthan et al. 2001), whereas an increase in oxidative metabolism halts cellular proliferation and tumor progression (Sanchez-Arago et al. 2013a; Cuezva et al. 2009; McFate et al. 2008; Bonnet et al. 2007). In this regard, the activity of OXPHOS has been demonstrated to be specifically required for the execution of cell death (Sanchez-Arago et al. 2013a; Dey and Moraes 2000; Kim et al. 2002; Tomiyama et al. 2006). In particular, molecular components that participate in OXPHOS including subunits of the H^+-ATP synthase are needed for the execution of cell death (Wang 2001; Matsuyama et al. 1998; Plas and Thompson 2002; Santamaria et al. 2006; Vahsen et al. 2004). Hence, bioenergetics and cell death are two master tasks of mitochondria that are molecularly and functionally integrated (Sanchez-Arago et al. 2013a). The inhibition of the activity of the H^+-ATP synthase with oligomycin blunts mitochondrial hyperpolarization and ROS production, prevents the oxidation and modification of mitochondrial proteins, and delays the release of *cyt c* and the execution of cell death (Santamaria et al. 2006). Consistent with these findings, the cell death response to different chemotherapeutic agents varies largely depending upon the relative activity of the two pathways that sustain energy metabolism (Santamaria et al. 2006; Sanchez-Arago and Cuezva 2011). Indeed, highly glycolytic cells with negligible contribution of OXPHOS for ATP provision have a cell death-resistant phenotype because mitochondrial ROS signaling after chemotherapeutic targeting is blunted (Santamaria et al. 2006; Sanchez-Arago and Cuezva 2011). Consistent with the tumor suppressor function of mitochondrial activity, there is a large body of data supporting that OXPHOS, both under basal conditions and in response to chemotherapeutic agents, abolishes tumorigenicity [see Sanchez-Arago et al. (2013a) for updated review]. A likely mechanism that explains the preferential death of cancer cells when forced to oxidize mitochondrial substrates is the overproduction of superoxide radical as a result of the stimulation of mitochondrial metabolism (Sanchez-Arago et al. 2013a; Santamaria et al. 2006; Michelakis et al. 2010). Both genetic (D'Errico et al. 2011) and pharmacological (Wang and Moraes 2011) studies have shown that the PGC1-α-mediated improvement of mitochondrial activity and metabolism restrains cancer progression by increasing ROS-mediated apoptosis in cancer cells (D'Errico et al. 2011).

Permeabilization of the inner mitochondrial membrane to low molecular weight solutes, the so-called permeability transition pore (PTP) opening, is the point of no return in cell death (Galluzzi et al. 2009; Bernardi 2013). Downregulation of the bioenergetic signature is also functionally linked to the resistance to chemotherapy in many different cancer cells (Sanchez-Arago et al. 2013a; Li et al. 2010; Shin et al. 2005; Hernlund et al. 2009) and in colon cancer patients (Lin et al. 2008), emphasizing that a low bioenergetic activity of mitochondria predisposes to cancer onset and progression, highlighting the emerging role that the H^+-ATP synthase plays in cell death (Matsuyama et al. 1998; Santamaria et al. 2006; Alavian

et al. 2011; Chivasa et al. 2011; Giorgio et al. 2013). In this regard, and although the molecular composition of the PTP remains unknown, recent findings support that a critical component of the PTP is subunit c of the H^+-ATP synthase (Bonora et al. 2013). Moreover, it has been shown that dimers of the H^+-ATP synthase form a channel with electrophysiological properties identical to those of the PTP (Giorgio et al. 2013). Overall, the downregulation of the H^+-ATP synthase, and thus of OXPHOS, is part of the molecular strategy adapted by cancer cells to prevent cell death. Consistently, cancer progression requires the silencing of the bioenergetic activity of mitochondria not only by downregulating the content of the H^+-ATP synthase as above discussed but also by overexpressing the natural physiological inhibitor of the enzyme, the so-called ATPase inhibitory factor 1 (IF1) (Sanchez-Cenizo et al. 2010; Sanchez-Arago et al. 2013c; Formentini et al. 2012).

Despite the relevance of OXPHOS as a tumor suppression pathway in different cancer cells, we have to take into consideration the diversity of mitochondrial functions in different cellular lineages. In this regard, it should be noted the case of melanomas. The common activating mutations in the BRAF oncogene drive dysfunctional OXPHOS that increases the tumorigenic properties of malignant melanomas making cells addicted to the glycolytic and the pentose phosphate pathways (Hall et al. 2013). The regulation of oxidative metabolism by BRAF is mediated by PGC1α and by the melanocyte lineage factor (MITF) (Haq et al. 2013). The use of inhibitors of the BRAF oncogene to treat melanomas seems to have a limited efficacy because cells put in place an adaptive metabolic program to induce OXPHOS (Haq et al. 2013). Apparently, the mechanism that mediates drug resistance is the increase of the expression of enzymes of OXPHOS in a slow-cycling subpopulation of cells that survive upon multidrug treatment (Roesch et al. 2013). The increased mitochondrial capacity and resistance to oxidative stress of these survivor cells is mediated by the action of PGC1α (Vazquez et al. 2013). Interestingly, the inhibition of mitochondrial respiration eliminates the emergence of these survivor cells sensitizing melanoma cells to therapy (Roesch et al. 2013). This is an example of how important is to understand mitochondrial function in particular cell types to prevent cancer onset and progression and its treatment.

11.6 IF1-Mediated Inhibition of the H^+-ATP Synthase Also Propitiates Cancer Progression

In addition to the down regulation of the H^+-ATP synthase, most prevalent human carcinomas also inhibit the activity of the complex by the overexpression of the ATPase inhibitory factor 1 (IF1). This small protein is overexpressed in lung, colon, breast, and ovarian carcinomas being its expression negligible in normal tissues (Sanchez-Cenizo et al. 2010; Sanchez-Arago et al. 2013c; Formentini et al. 2012). The overexpression of IF1 triggers the inhibition of the H^+-ATP synthase, the metabolic switch to an enhanced aerobic glycolysis, and the concurrent increase

in the mitochondrial membrane potential due to the prevention of the backflow of protons into the mitochondrial matrix (Sanchez-Cenizo et al. 2010; Formentini et al. 2012). Conversely, the silencing of IF1 has the opposite metabolic effects (Sanchez-Cenizo et al. 2010). These findings emphasize the relevance that the inhibition of the activity of the H^+-ATP synthase has for metabolic adaptation of cancer cells and tumor development. In fact, and in addition to promoting the metabolic switch observed in cancer cells, IF1 simultaneously triggers a ROS signaling cascade that mediates a nuclear response via NFkB to promote cell proliferation, invasion, and cell survival (Formentini et al. 2012). Remarkably, the regulation of the expression of this short-lived protein is exerted at posttranscriptional levels in colon, lung, breast, and ovarian carcinomas, further emphasizing the importance of translation and/or protein stabilization in favoring the metabolic reprogramming of cancer and stem cells (Sanchez-Arago et al. 2013b, c). In fact, the degradation of IF1 is essential in promoting the metabolic changes that support stem cell differentiation (Sanchez-Arago et al. 2013b). Moreover, IF1 is also a relevant predictive marker for clinical outcome of breast and colon cancer patients, suggesting the high potential of IF1 as a therapeutic target (Sanchez-Arago et al. 2013c).

Concluding Remarks

The study of metabolism in cancer has experienced a renaissance in the last decade. Moreover, the focus of cancer research in the twenty-first century has moved from the exclusive genetic interest to the broad study of the signaling pathways that regulate cell metabolism. The knowledge that cancer cells become addicted to certain metabolic pathways has opened up new and promising therapeutic approaches. The identification of the mechanisms that control the cell type-specific bioenergetic phenotype of tumor cells is a needed step to combat the disease. In this regard, basic studies are required to better characterize the alterations of the mitochondrial proteome especially of those that affect the content/activity of the H^+-ATP synthase and that are responsible for mediating cancer progression. Unveiling these mechanisms would make possible the development of effective therapies against cancer.

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