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Review

Oxidative phosphorylation in cancer cells ☆

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ABSTRACT

Evidence suggests that mitochondrial metabolism may play a key role in controlling cancer cells life and proliferation. Recent evidence also indicates how the altered contribution of these organelles to metabolism and the resistance of cancer mitochondria against apoptosis-associated permeabilization are closely related. The hallmarks of cancer growth, increased glycolysis and lactate production in tumours, have raised attention due to recent observations suggesting a wide spectrum of oxidative phosphorylation *deficit* and decreased availability of ATP associated with malignancies and tumour cell expansion. More specifically, alteration in signal transduction pathways directly affects mitochondrial proteins playing critical roles in controlling the membrane potential as UCP2 and components of both MPTP and oxphos complexes, or in controlling cells life and death as the Bcl-2 proteins family. Moreover, since mitochondrial bioenergetics and dynamics, are also involved in processes of cells life and death, proper regulation of these mitochondrial functions is crucial for tumours to grow. Therefore a better understanding of the key pathophysiological differences between mitochondria in cancer cells and in their non-cancer surrounding tissue is crucial to the finding of tools interfering with these peculiar tumour mitochondrial functions and will disclose novel approaches for the prevention and treatment of malignant diseases. Here, we review the peculiarity of tumour mitochondrial bioenergetics and the mode it is linked to the cell metabolism, providing a short overview of the evidence accumulated so far, but highlighting the more recent advances. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. Introduction

Mitochondria are essential organelles and key integrators of metabolism, but they also play vital roles in cell death and cell signaling pathways critically influencing cell fate decisions [1–3]. Mammalian mitochondria contain their own DNA (mtDNA), which encodes 13 polypeptides of oxidative phosphorylation complexes, 12S and 16S rRNAs, and 22 tRNAs required for mitochondrial function [4]. In order to synthesize ATP through oxidative phosphorylation (oxphos), mitochondria consume most of the cellular oxygen and produce the majority of reactive oxygen species (ROS) as by-products [5]. ROS have been implicated in the etiology of carcinogenesis via oxidative damage to cell macromolecules and through modulation of mitogenic signaling pathways [6–8]. In addition, a number of mitochondrial dysfunctions of genetic origin are implicated in a range of age-related diseases, including tumours [9]. How mitochondrial functions are associated with cancer is a crucial and complex issue in biomedicine that is still unravelled [10,11], but it warrants an extraordinary importance since mitochondria play a major role not only as energy suppliers and ROS "regulators", but also because of their control on cellular life and death. This is of particular relevance since tumour cells can acquire resistance to apoptosis by a number of mechanisms, including mitochondrial dysfunction, the expression of anti-apoptotic proteins or by the down-regulation or mutation of pro-apoptotic proteins [12].

Cancer cells must adapt their metabolism to produce all molecules and energy required to promote tumour growth and to possibly modify their environment to survive. These metabolic peculiarities of cancer cells are recognized to be the outcome of mutations in oncogenes and tumour suppressor genes which regulate cellular metabolism. Mutations in genes including P53, RAS, c-MYC, phosphoinosine 3-phosphate kinase (PI3K), and mTOR can directly or through signaling pathways affect metabolic pathways in cancer cells as discussed in several recent reviews [13–17]. Cancer cells harboring

Abbreviations: HIF-1, hypoxia-inducible factor 1; HIFs, hypoxia-inducible factors; IF_1, ATP synthase natural inhibitor protein; F_1F_0-ATPase, ATP synthase or Complex V; COX, cytochrome c oxidase or Complex IV; Complex III, ubiquinone cytochrome c oxidoreductase; $\Delta\Psi m$, electrical membrane potential of mitochondria; mtDNA, mitochondrial DNA; AMPK, AMP-activated protein kinase; oxphos, oxidative phosphorylation; ROS, reactive oxygen species; PDH, pyruvate dehydrogenase; LDH, lactate dehydrogenase; HK-II, hexokinase II; ANT, adenine nucleotide translocator; VDAC, voltage-dependent anion channel; MPTP, mitochondrial permeability transition pore; CyP-D, cyclophilin D; UCP, uncoupling protein; SDH, succinate dehydrogenase; FH, fumarate hydratase; Bcl2, B cell lymphoma gene-2; P53, tumour protein 53; c-MYC, MYC protein gene; ras, gene coding a family of ras proteins; PI3K, phosphatidylinositol 3-kinases; AKT, protein kinases B; mTOR, the mammalian target of rapamycin, serine/threonine protein kinase; ERK, extracellular-signal-regulated kinases; STAT3, signal transducer and activator of transcription 3, is a transcription factor which in humans is encoded by the *STAT3* gene

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the genetic mutations are also able to thrive in adverse environments such as hypoxia inducing adaptive metabolic alterations which include glycolysis up-regulation and angiogenesis factor release [18,19]. In response to hypoxia, hypoxia-induced factor 1 (HIF-1) [20], a transcription factor, is up-regulated, which enhances expression of glycolytic enzymes and concurrently it down regulates mitochondrial respiration through up-regulation of pyruvate dehydrogenase kinase 1 (PDK1) (see recent reviews [21,22]). However, several tumours have been reported to display high HIF-1 activity even in normoxic condition, now referred to as pseudohypoxia [23–25]. In addition, not only solid tumours present a changed metabolism with respect to matched normal tissues, hematological cell malignancies also are characterized by peculiar metabolisms, in which changes of mitochondrial functions are significant [26–28], therefore indicating a pivotal role of mitochondria in tumours independently from oxygen availability.

Collectively, actual data show a great heterogeneity of metabolism changes in cancer cells, therefore comprehensive cellular and molecular basis for the association of mitochondrial bioenergetics with tumours is still undefined, despite the numerous studies carried out. This review briefly revisits the data which are accumulating to account for this association and highlights the more recent advances, particularly focusing on the metabolic and structural changes of mitochondria.

2. Mitochondria-related metabolic changes of cancer cells

Accumulating evidence indicate that many cancer cells have an higher glucose consumption under normoxic conditions with respect to normal differentiated cells, the so-called “aerobic glycolysis” (Warburg effect), a phenomenon that is currently exploited to detect and diagnose staging of solid and even hematological malignancies [27]. Since the initial publication by Otto Warburg over half a century ago [29], an enormous amount of studies on many different tumours have been carried out to explain the molecular basis of the Warburg effect. Although the regulatory mechanisms underlying aerobic and glycolytic pathways of energy production are complex, making the prediction of specific cellular responses rather difficult, the actual data seem to support the view that in order to favour the production of biomass, proliferating cells are commonly prone to satisfy the energy requirement utilizing substrates other than the complete oxidation of glucose (to CO_2 and H_2O). More precisely, only part (40 to 75%, according to [30]) of the cells need of ATP is obtained through the scarcely efficient catabolism of glucose to pyruvate/lactate in the cytoplasm and the rest of the ATP need is synthesized in the mitochondria through both the tricarboxylic acid (TCA) cycle (one ATP produced each acetyl moiety oxidized) and the associated oxidative phosphorylation that regenerates nicotinamide- and flavin-dinucleotides in their oxidized state(NAD^+ and FAD). This might be due to the substrate availability as it was shown in HeLa cells, where replacing glucose with galactose/glutamine in the culture medium induced increased expression of oxphos proteins, suggesting an enhanced energy production from glutamine [31]. As a conclusion the authors proposed that energy substrate can modulate mitochondrial oxidative capacity in cancer cells. A direct evidence of this phenomenon was provided a few years later in glioblastoma cells, in which it was demonstrated that the TCA cycle flux is significantly sustained by anaplerotic alfa-ketoglutarate produced from glutamine and by acetyl moieties derived from the pyruvate dehydrogenase reaction where pyruvate may have an origin other than glucose [32]. The above changes are the result of genetic alteration and environmental conditions that induce many cancer cells to change their metabolism in order to synthesize molecules necessary to survive, grow and proliferate, including ribose and NADPH to synthesize nucleotides, and glycerol-3 phosphate to produce phospholipids. The synthesis of the latter molecules requires major amount of acetyl moieties that are derived from beta-oxidation of fatty acids and/or from cytosolic citrate (citrate lyase reaction) and/or from the pyruvate dehydrogenase reaction. Given the important requirement for NADPH in macromolecular synthesis and redox control, NADPH production in cancer cells besides being produced through the phosphate pentose shunt, may be significantly sustained by cytosolic isocitrate dehydrogenases and by the malic enzyme (see Ref. [33] for a recent review). Therefore, many cancer cells tend to have reduced oxphos in the mitochondria due to either or both reduced flux within the tricarboxylic acid cycle and/or respiration (Fig. 1). The latter being also caused by reduced oxygen availability, a typical condition of solid tumours, that will be discussed below.

Of particular relevance in the study of the metabolic changes occurring in cancer cells, is the role of hexokinase II. This enzyme is greatly up-regulated in many tumours being its gene promoter sensitive to typical tumour markers such as HIF-1 and P53 [30]. It plays a pivotal role in both the bioenergetic metabolism and the biosynthesis of required molecules for cancer cells proliferation. Hexokinase II phosphorylates glucose using ATP synthesized by the mitochondrial oxphos and it releases the product ADP in close proximity of the adenine nucleotide translocator (ANT) to favour ATP re-synthesis within the matrix (Fig. 1). Obviously, the expression level, the location, the substrate affinity, and the kinetics of the enzyme are crucial to the balancing of the glucose fate, to either allowing intermediates of the glucose oxidation pathway towards required metabolites for tumour growth or coupling cytoplasmic glycolysis with further oxidation of pyruvate through the TCA cycle, that is strictly linked to oxphos. This might be possible if the mitochondrial-bound hexokinase activity is reduced and/or if it limits ADP availability to the mitochondrial matrix, to inhibit the TCA cycle and oxphos. However, the mechanism is still elusive, although it has been shown that elevated oncogene kinase signaling favours the binding of the enzyme to the voltage-dependent anion channel (VDAC) by AKT-dependent phosphorylation [34] (Fig. 2). VDAC is a protein complex of the outer mitochondrial membrane which is in close proximity of ANT that exchanges ADP for ATP through the inner mitochondrial membrane [35]. However, the enzyme may also be detached from the mitochondrial membrane, to be redistributed to the cytosol, through the catalytic action of sirtuin-3 that deacylates cyclophilin D, a protein of the inner mitochondrial membrane required for binding hexokinase II to VDAC (Fig. 2) [36]. Removing hexokinase from the mitochondrial membrane has also another important consequence in cancer cells: whatever mechanism its removal activates, apoptosis is induced [37,38]. These observations indicate hexokinase II as an important tool used by cancer cells to survive and proliferate under even adverse conditions, including hypoxia, but it may result an interesting target to hit in order to induce cells cytotoxicity. Indeed, a stable RNA interference of hexokinase II gene showed enhanced apoptosis indices and inhibited growth of human colon cancer cells; in accordance *in vivo* experiments indicated a decreased tumour growth [39].

In addition to having to adopt the aerobic glycolysis, many cancer cells present a number of other metabolic changes that in the mitochondria include: decreased oxidation of substrates (mostly NADH-linked), altered expression and activity of respiratory chain subunits, overproduction of ROS, mitochondrial DNA (mtDNA) mutations, impaired both respiratory chain complexes and ATP synthase organization within the inner mitochondrial membrane, and altered control of apoptosis.

3. Oxidative phosphorylation peculiarities in cancer cells

3.1. Respiratory chain complexes and ATP synthase

Beyond transcriptional control of metabolic enzyme expression by oncogenes and tumour suppressors, it is becoming evident that environmental conditions affect the mitochondrial energy

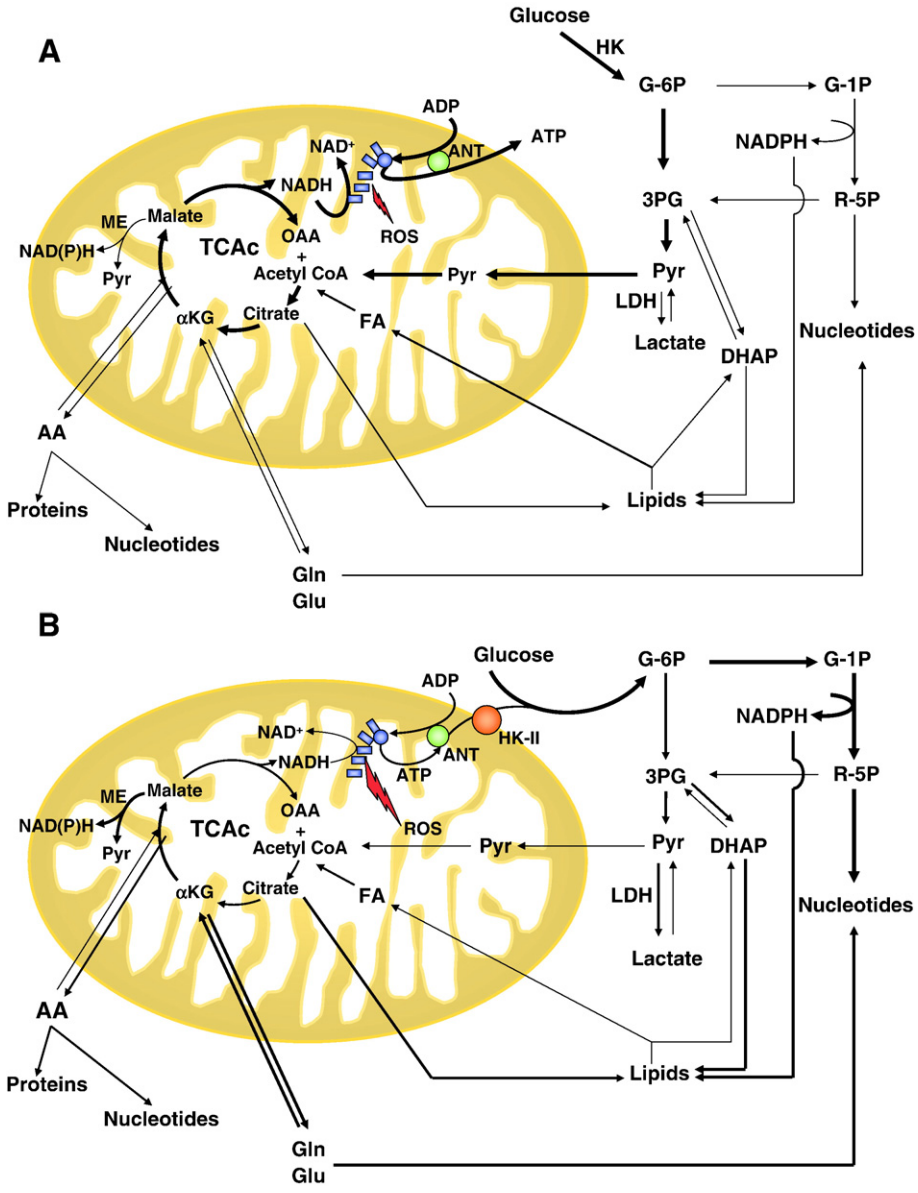

Fig. 1. Schematic illustration of mitochondrial metabolism and metabolic reprogramming in tumours. In normal cells (A), glucose is phosphorylated by HK-I, then the major part is degraded via glycolysis to pyruvate, which prevalently enters the mitochondria, it is decarboxylated and oxidized by PDH to acetyl-coenzyme A, which enters the TCA cycle where the two carbons are completely oxidized to CO_2 whereas hydrogen atoms reduce NAD^+ and FAD, which feed the respiratory chain (turquoise). Minor part of glycolytic G-6P is diverted to produce ribose 5-phosphate (R-5P) and NADPH, that will be used to synthesize nucleotides, whereas triose phosphates in minimal part will be used to synthesize lipids and phospholipids with the contribution of NADPH and acetyl-coenzyme A. Amino acids, including glutamine (Gln) will follow the physiological turnover of the proteins, in minimal part will be used to synthesize the nucleotides bases, and the excess after deamination will be used to produce energy. In the mitochondria inner membranes are located the respiratory chain complexes and the ATP synthase (turquoise), which phosphorylates ADP releasing ATP, that in turn is carried to the cytosol by ANT (green) in exchange for ADP. About 1–2% O_2 uptaken by the mitochondria is reduced to superoxide anion radical and ROS. In cancer cells (B), where anabolism is enhanced, glucose is mostly phosphorylated by HK-II (red), which is up-regulated and has an easy access to ATP being more strictly bound to the mitochondria. Its product, G-6P, is only in part oxidized to pyruvate. This, in turn, is mostly reduced to lactate being both LDH and PDH kinase up-regulated. A significant part of G-6P is used to synthesize nucleotides that also require amino acids and glutamine. Citrate in part is diverted from the TCA cycle to the cytosol, where it is a substrate of citrate lyase, which supplies acetyl-coenzyme A for lipid and phospholipid synthesis that also requires NADPH. As indicated, ROS levels in many cancer cells increase.

metabolism, and many studies in the last decade indicate that mitochondrial dysfunction is one of the more recurrent features of cancer cells, as reported at microscopic, molecular, biochemical, and genetic level [7,40,41]. Although cancer cells under several conditions, including hypoxia, oncogene activation, and mDNA mutation, may substantially differ in their ability to use oxygen, only few reports have been able to identify a strict association between metabolic changes and mitochondrial complexes composition and activity. In renal oncocytomas [42] and in lung epidermoid carcinoma [43], the NADH dehydrogenase activity and protein content of Complex I were found to be strongly depressed; subsequently, in a thyroid oncocytoma cell line [44] a similar decrease of Complex I activity was ascribed to a specific mutation in the ND1 gene of mitochondrial DNA. However, among the respiratory chain complexes, significant decrease of the only Complex I content and activity was found in K-ras transformed cells in our laboratory [45], and could not be ascribed to mtDNA mutations, but rather, based on microarray analysis of oxphos genes, we proposed that a combination of genetic (low transcription of some genes) and biochemical events (assembly factors deficiency, disorganization of structured supercomplexes, and ROS-induced structural damage) might cause the Complex I defects.

In some hereditary tumours (renal cell carcinomas) a correlation has been identified between mitochondrial dysfunctions and content of oxphos complexes [46]. For instance, the low content of ATP

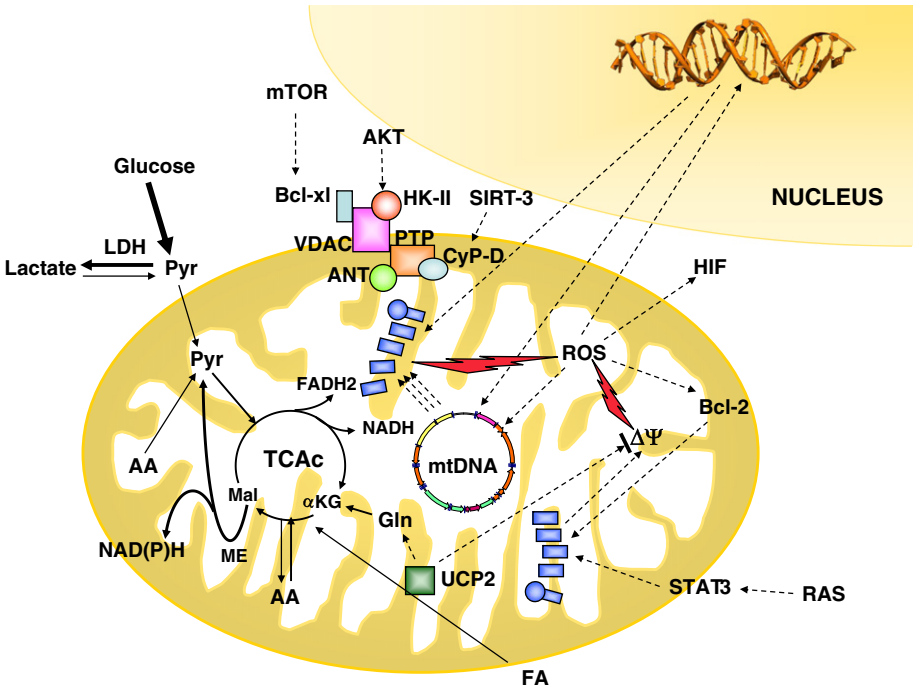

Fig. 2. Schematic illustration of the main mitochondrial changes frequently occurring in cancer cells. The reprogramming of mitochondrial metabolism in many cancer cells comprises reduced pyruvate oxidation by PDH followed by the TCA cycle, increased anaplerotic feeding of the same cycle, mostly from Gln, whose entry in the mitochondrial matrix is facilitated by UCP2 up-regulation. This increases also the free fatty acids uptake by mitochondria, therefore β-oxidation is pushed to produce acetyl-coenzyme A, whose oxidation contributes to ATP production. In cancer cells many signals can converge on the mitochondrion to regulate the mitochondrial membrane permeability, which may respond by elevating the MPTP (PTP) threshold, with consequent enhancement of apoptosis resistance. ROS belong to this class of molecules since it can enhance Bcl2 and may induce DNA mutations. Dotted lines indicate regulation; solid lines indicate reaction(s).

synthase, often observed in clear cell type renal cell carcinomas and in chromophilic tumours, seems to indicate that the mitochondria are in an inefficient structural and functional state [46]. However, it cannot be excluded that, in some cases, the structural alteration of ATP synthase may offer a functional advantage to cells exhibiting a deficient respiratory chain for instance to preserve the transmembrane electrical potential ($\Delta\psi_m$) [47]. It is likely that low levels of ATP synthases may play a significant role in cancer cell metabolism since it has been reported that in tumours from many different tissues, carcinogenesis specifically affects the expression of F_1-ATPase β subunit, suggesting alterations in the mechanisms that control mitochondrial differentiation (see for a detailed review [48]). What it seems intriguing is the overexpression of the inhibitor protein, IF_1, reported in hepatocellular carcinomas [49,50] and in Yoshida sarcoma [51]. Normally, this protein binds to the F_1 domain of the ATP synthase inhibiting its activity [52], and it is believed to limit the ATP hydrolysis occurring in the mitochondria of hypoxic cells, avoiding ATP depletion and maintaining $\Delta\psi_m$ to a level capable to avoid the induction of cell death [5]. But why is its expression in cancer cells enhanced in front of a reduced F_1-ATPase β subunit?

The first possibility is that IF_1 has a function similar to that in normal cells, simply avoiding excessive ATP hydrolysis therefore limiting $\Delta\psi_m$ enhancement, but in cancer cells this is unlikely due to both the reduced level of ATP synthase [46] and the high affinity of IF_1 for the enzyme. A second possibility might be that cancer cells need strongly reduced oxphos to adapt their metabolism and acquire a selective growth advantage under adverse environmental conditions such as hypoxia, as it has been experimentally shown [53]. Finally, IF_1 might contribute to the saving of the inner mitochondrial membrane structure since it has been reported its capability to stabilize oligomers of ATP synthase, which in turn can determine cristae shapes [54]. In this regard, recent experimental evidence has shed some light on a critical role of mitochondrial morphology in the control of important mitochondrial functions including apoptosis [55] and oxidative phosphorylation [56]. In particular, dysregulated mitochondrial fusion and fission events can now be regarded as playing a role in cancer onset and progression [57]. Accordingly, mitochondria-shaping proteins seem to be an appealing target to modulate the mitochondrial phase of apoptosis in cancer cells. In fact, several cancer tissues: breast, head-and-neck, liver, ovarian, pancreatic, prostate, renal, skin, and testis, showed a pattern suggestive of enlarged mitochondria resulting from atypical fusion [58].

As already mentioned in the above paragraphs, mitochondrial metabolism is reprogrammed in many tumours with a high variability. However, relatively few reports focus on the main functional parameters of mitochondria, including the membrane potential and intrinsic proteins controlling it, the coupling of respiration to ATP synthesis, and the ATP synthesis rate itself. Since both mtDNA mutations and oncogene products modify cells bioenergetics, which is strictly associated with ROS generation and apoptosis, analysis of the mitochondrial main functional parameters might provide useful information for both cancer diagnosis and therapeutical approaches.

3.2. Mitochondrial membrane potential in cancer cells

Critical mitochondrial functions, including ATP synthesis, ion homeostasis, metabolites transport, ROS production, and cell death are highly dependent on the electrochemical transmembrane potential, a physico-chemical parameter consisting of two components, the major of which being the transmembrane electrical potential ($\Delta\psi_m$) (see for a recent review [59]). In normal cells, under normoxic conditions, $\Delta\psi_m$ is build up by the respiratory chain and is mainly used to drive ATP synthesis, whereas in anoxia or severe hypoxia it is generated by the hydrolytic activity of the ATP synthase complex and by the electrogenic transport of ATP in exchange for ADP from the cytosol to the matrix, operated by the adenine nucleotide translocator [17]. Dissipation of the mitochondrial membrane potential (proton

leak) causes uncoupling of the respiratory chain electron transport from ADP phosphorylation by the ATP synthase complex. Proton leak functions as a regulator of mitochondrial ROS production and its modulation by uncoupling proteins may be involved in pathophysiology, including tumours. In addition, $\Delta\psi_m$ plays a role in the control of the mitochondrial permeability transition pore (MPTP), that might be critical in determining reduced sensitivity to stress *stimuli* that were described in neoplastic transformation [60], implying that dysregulation of pore opening might be a strategy used by tumour cells to escape death. Indeed, it has recently been reported that ERK is constitutively activated in the mitochondria of several cancer cell types, where it inhibits glycogen synthase kinase-3-dependent phosphorylation of CyP-D and renders these cells more refractory to pore opening and to the ensuing cell death [61].

It is worth mentioning a second protein of the inner mitochondrial membrane, the uncoupling protein, UCP2 (Fig. 2), which contributes to regulate $\Delta\psi_m$. Indeed, recent observations evidenced its overexpression in various chemoresistent cancer cell lines and in primary human colon cancer. This overexpression was associated with an increased apoptotic threshold [62]. Moreover, UCP2 has been reported to be involved in metabolic reprogramming of cells, and appeared necessary for efficient oxidation of glutamine [63]. On the whole, these results led to hypothesize an important role of the uncoupling protein in the molecular mechanism at the basis of the Warburg effect, that suppose a reduced $\Delta\psi_m$-dependent entry of pyruvate into the mitochondria accompanied by enhanced fatty acid oxidation and high oxygen consumption (see for a review [64]). However, in breast cancer Sastre-Serra et al. [65] suggested that estrogens by down-regulating UCPs, increase mitochondrial $\Delta\psi_m$, that in turn enhances ROS production, therefore increasing tumorigenicity. While the two above points of view concur to support increased tumorigenicity, the mechanisms at the basis of the phenomenon appear on the opposite of the other. Therefore, although promising for the multiplicity of metabolic effects in which UCPs play a role (see for a recent review [66]), at present it seems that much more work is needed to clarify how UCPs are related to cancer.

A novel intriguing hypothesis has recently been put forward regarding effectors of mitochondrial function in tumours. Wegrzyn J et al. [67] demonstrated the location of the transcription factor STAT3 within the mitochondria and its capability to modulate respiration by regulating the activity of Complexes I and II, and Gough et al. [68] reported that human ras oncoproteins depend on mitochondrial STAT3 for full transforming potential, and that cancer cells expressing STAT3 have increased both $\Delta\psi_m$ and lactate dehydrogenase level, typical hallmarks of malignant transformation (Fig. 2). A similar increase of $\Delta\psi_m$ was recently demonstrated in K-ras transformed fibroblasts [45]. In this study, the increased $\Delta\psi_m$ was somehow unexpected since the cells had shown a substantial decrease of NADH-linked substrate respiration rate due to a compatible reduced Complex I activity with respect to normal fibroblasts. The authors associated the reduced activity of the enzyme to its peculiar low level in the extract of the cells that was confirmed by oxphos nuclear gene expression analysis. This significant and peculiar reduction of Complex I activity relative to other respiratory chain complexes, is recurrent in a number of cancer cells of different origin [42,44,45,69]. Significantly, all those studies evidenced an overproduction of ROS in cancer cells, which was consistent with the mechanisms proposed by Lenaz et al. [70] who suggested that whatever factor (i.e. genetic or environmental) initiate the pathway, if Complex I is altered, it does not associate with Complex III in supercomplexes, consequently it does not channel correctly electrons from NADH through coenzyme Q to Complex III redox centres, determining ROS overproduction. This, in turn, enhances respiratory chain complexes alteration resulting in further ROS production, thus establishing a vicious cycle of oxidative stress and energy depletion, which can contribute to further damaging cells pathways and structures with consequent tumour progression and metastasis [69].

Concerning association of oxphos complexes in supramolecular complexes within the mitochondrial membrane, of notice is the organization of ATP synthase complexes in K-ras transformed cells. Fig. 3 exhibits a typical pattern obtained from 2D electrophoresis analysis of normal and transformed fibroblasts, showing a strong reduction of ATP synthase oligomers in the transformed cells, which suggests a changed organization of the mitochondrial cristae. Further studies are in due course in our laboratory to verify this hypothesis and to evaluate whether the natural inhibitor protein of the ATP synthase complex [52] plays a role in the induction of this phenomenon, as hypothesized by Campanella et al. [71]. If this is the case, a novel possible target of interest in developing therapies for treatment of certain tumours might be considered.

3.3. Mutation of nuclear genes encoding mitochondrial proteins

Mutations of nuclear encoded mitochondrial proteins have been linked to cancer. Here we just mention mutations in two enzymes of the TCA cycle: succinate dehydrogenase (SDH) and fumarate hydratase (FH), that were associated with phaeochromocytomas and renal cancer, respectively (see for an extensive review [72]). In both conditions an accumulation of TCA cycle intermediates succinate and fumarate, respectively, was observed, and this accumulation was shown capable to stabilize HIF-1α, supporting the conclusions of Selak et al. [73] who demonstrated the inhibiting effect of succinate on the HIF-1α prolyl hydroxylase, a crucial enzyme for HIF-1α removal, that resulted in the stabilization of HIF-1. A mutation in a third TCA cycle enzyme, isocitrate dehydrogenase, has recently been described in the majority of grade II and grade III gliomas and secondary glioblastomas [74]. The single amino acid change (Arg-132 to His) in the enzyme results in loss of the enzyme's ability to catalyze conversion of isocitrate to α-ketoglutarate, and it determines the formation and accumulation of 2-hydroxyglutarate, which has been shown to be an onco-metabolite. Other mutations have been reported in nuclear genes encoding proteins being related with both replication of mtDNA and assembly of respiratory chain complexes. Indeed, 63% of the breast tumours examined by Singh et al. [75] harbored mutations in the polymerase γ gene, resulting in severe mtDNA depletion and oxphos impairment.

3.4. Mitochondrial DNA mutation and cancer

In the last decade, there has been considerable interest in the possibility that mtDNA mutations might predispose or at least play a role in common diseases, including human cancer. Accordingly, many reports are being focused on mitochondrial DNA mutation and cancer. Nonetheless the mechanisms responsible for the initiation and evolution of mtDNA mutations, and their roles in the development of cancer and disease progression still remain to be fully elucidated. It is intriguing that, as recently reported, the high heterogeneity of human mtDNA was found to be significantly amplified in tumours [76]. The first paper consistently describing the presence of somatic mtDNA mutations in human tumours was reported by Polyak et al. [77]. In 7 out of 10 cell lines from patients with colorectal tumours, the authors evidenced the occurring of homoplasmic mtDNA mutations, which were neither found in normal colon nor in other tissues from the same patients. Of notice is the almost absence of the mutations effects on the mitochondrial function, a situation reported also in another study, in which the entire mitochondrial genome sequence from normal and leukaemic cells obtained from 24 patients with both chronic and acute presentations were compared [78]. Incidentally, in any of the above cases, there was no evidence of whether mtDNA mutations themselves contributed to the development of the tumour. However, some years later, in a very interesting study, Petros et al. [79] found that 11–12% of all prostate cancer patients treated over the previous 7 years at their institutional tissue resources harbored

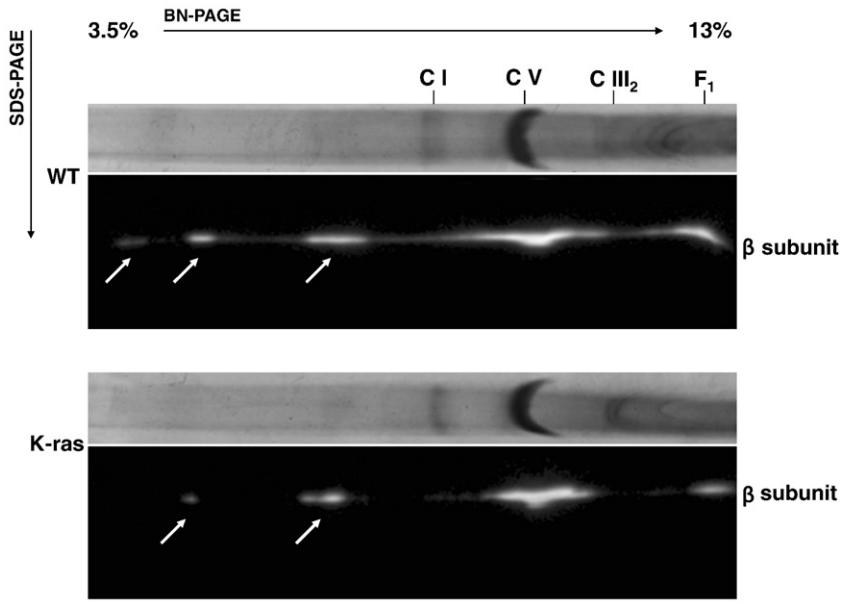

Fig. 3. Two-dimensional analysis of mitochondrial ATP synthase (F_1F_0-ATPase) in wild-type (Top) and K-ras transformed (Bottom) fibroblasts. Monomer and oligomers separation was achieved by 1D Blue-Native electrophoresis followed by 2D SDS-PAGE. 2D gels were blotted onto the nitrocellulose membrane and then exposed to a monoclonal antibody specific for subunit β of the F_1F_0-ATPase complex. In 1D, monomeric Complex I and Complex V (molecular mass about 1000 and 600 kDa, respecively), dimeric Complex III (molecular mass about 500 kDa), and F_1 (molecular mass about 400 kDa), the catalytic domain of the ATP synthase complex are indicated. Arrows indicate dimeric and oligomeric F_1F_0-ATPases. The amount of the oligomers of the ATP synthase is higher in wild-type than in transformed cells. Moreover, the highest molecular mass oligomer is completely absent in K-ras transformed fibroblasts. Details of electrophoretic and blotting methods are reported in Ref. [45].

mutations on the cytochrome c oxidase subunit I gene. This observation induced the authors to evaluate whether mutant tumours had increased tumour growth rate. Therefore, the pathogenic mtDNA nt8993T>G mutation in the ATP6 gene [80] was introduced into PC3 prostate cancer cells through cybrids (trans-mitochondrial hybrids) transfer. After injection in nude mice tumour growth was tested. These experiments revealed that the average tumour volume of the mutant PC3 cybrids was significantly higher (7-fold) than that of controls, and induced increased ROS generation. Therefore it could be shown that mtDNA mutations increase tumorigenicity in animal models of prostate cancer. Similarly, Shidara et al. [81] showed the positive contribution of pathogenic mutations in mtDNA to the promotion of cancer, and in addition, they demonstrated that these mutations can effectively promote cancer growth by preventing apoptosis. In accordance, it was recently shown that the presence of heteroplasmic (only a fraction of the mtDNA molecules is mutated) mutations in two genes encoding polypeptides of the respiratory chain Complex I (ND1 subunit) and III (cytochrome b), respectively, could result in thyroid oncocytic carcinoma [44]. Again, the authors found a dramatic increase in ROS production, which was associated with a concurrent dramatic activity decrease of Complex I and to a lesser extent of Complex III, the main mitochondrial sources of ROS [82]. Similar results have been reported by Ishikawa et al. [69], who also showed an increase of tumorigenicity and development of metastasis in transformed cells transfected with pathogenic mtDNA mutations. Very recent papers report somatic mutations in the mitochondrial genome in nearly one out of four gastric cancer specimen and stress the potential role of those mutations in the progression of the disease [83], whereas Kulawiec et al. [84] showed that in some samples of breast cancer cells, mtDNA mutations were not associated with ROS production, but constitutively activate the PI3K/AKT pathway contributing to increased metastasis. In addition, this pathway is strictly linked and activated in association with the serine/threonine kinase target of rapamycin (TOR) that controls key cellular processes such as cell survival, growth and proliferation. Consistent with its role in cell proliferation, the mTOR pathway is frequently hyperactivated in a number of human malignancies [85] and its TORC1 protein complex exerts a direct control of mitochondrial function via a complex comprising Bcl-xl and VDAC1 at the mitochondrial outer membrane (Fig. 2) [86]. For this reason, several mTOR inhibitors have been approved for cancer therapy, and late-stage clinical trials are underway [87].

3.5. Hypoxia and oxidative phosphorylation in cancer cells

Tumour cells experience an extensive heterogeneity of oxygen levels, from normoxia (around 2–4% oxygen tension), through hypoxia, to anoxia (<0.1% oxygen tension). The growth of tumours beyond a critical mass >1–2 mm^3 is dependent on adequate blood supply to receive nutrients and oxygen by diffusion [88]. Cells adjacent to capillaries were found to exhibit a mean oxygen concentration of 2%, therefore, beyond this distance, hypoxia occurs: indeed, cells located at 200 μm displayed a mean oxygen concentration of 0.2%, which is a condition of severe hypoxia [89]. Oxygen shortage results in hypoxia-dependent inhibition of mitochondrial activity, mostly mediated by the hypoxia-inducible factor 1 (HIF-1) [90,91]. More precisely, hypoxia affects structure, dynamics, and function of the mitochondria, and in particular it has a significant inhibitory effect on the oxidative phosphorylation machinery, which is the main energy supplier of cells (see Ref. [22] for a recent review). The activation of HIF-1 occurs in the cytoplasmic region of the cell, but the contribution of mitochondria is critical being both cells oxygen sensors and suppliers of effectors of HIF-1α prolyl hydroxylase like α-ketoglutarate and probably ROS, that inhibit HIF-1α removal [92]. As reported above, mitochondria can also promote HIF-1α stabilization if the TCA flux is severely inhibited with release of intermediate molecules like succinate and fumarate into the cytosol. On the other hand, HIF-1 can modulate mitochondrial functions through different mechanisms, that besides metabolic reprogramming [7,22,93,94], include alteration of mitochondrial structure and dynamics [58], induction of microRNA-210 that decreases the cytochrome c oxidase (COX) activity by inhibiting the gene expression of the assembly protein COX10 [95], that also increases ROS generation. Moreover, these stress conditions could induce the anti-apoptotic protein Bcl-2,

which has also been reported to regulate COX activity and mitochondrial respiration [96] conferring resistance to cells death in tumours (Fig. 2). This effect might be further enhanced upon severe hypoxia conditions, since COX is also inhibited by NO, the product of activated nitric oxide synthases [97].

The reduced respiration rate occurring in hypoxia favours the release of ROS also by Complex III, which contribute to HIF stabilization and induction of Bcl-2 [98]. In addition, hypoxia reduces oxphos by inhibiting the ATP synthase complex through its natural protein inhibitor IF_1 (discussed in a previous section), which contributes to the enhancement of the "aerobic glycolysis", all signatures of cancer transformation.

Interestingly, a recent study based on metabolome analysis of colon and stomach cancer cells suggests a significant energy generation by the so-called fumarate respiration (i.e. fumarate rather than molecular oxygen is used as electron acceptor) under conditions of glucose deprivation and severe hypoxia [99].

Considering the pro-tumoral effect of hypoxia, some research groups have investigated whether hyperoxia may be useful in cancer therapy. For instance, Cannizzaro et al. [100] studied the effect of exposition at high oxygen tension of two human neuroblastoma cell lines (SK-N-SH and SK-N-DZ) and found that the treatment was able to induce cell growth inhibition and cell cycle perturbation. In particular, it was observed an arrest at G(2) phase, accompanied by an alteration in the expression and localization of cyclin B1/cdk1 complex and a reduction in its activity in SK-N-SH cells. Based on a different mechanism, hyperoxia induced apoptosis in SK-N-DZ cells via caspase 3 activation and Poly ADP-ribose polymerase-1 (PARP) cleavage, associated with increased pro-apoptotic Bax protein. In addition, preliminary observations demonstrated increased ROS and membrane lipid peroxidation in cultured U87 human glioma cells exposed to either normobaric hyperoxia or hyperbaric hyperoxia. On the same study, it was also shown that membrane blebbing enhanced with increasing O_2 tension, therefore suggesting a possible use of hyperoxia to induce cells death [101]. These very preliminary investigations seem interesting, but much more has to be known in order to attempt therapeutic treatments of tumours by this approach.

4. Conclusions

The observations reported to date indicate that cancer cells exhibit large varieties of metabolic changes which are associated with alterations in the mitochondrial structure, dynamics and function, and with tumour growth and survival. On one hand, mitochondria can regulate tumour growth through modulation of the TCA cycle and oxidative phosphorylation. The altered TCA cycle provides intermediates for both macromolecular biosynthesis and regulation of transcription factors such as HIF, and it allows cytosolic reductive power enhancement. Oxphos provides significant amounts of ATP which varies among tumour types. On the other hand, mitochondria are crucial in controlling redox homeostasis in the cell, inducing them to be either resistant or sensitive to apoptosis. All these reasons locate mitochondria at central stage to understanding the molecular basis of tumour growth and to seeking for novel therapeutical approaches.

Due to the complexity and variability of mitochondrial roles in cancer, careful evaluation of mitochondrial function in each cancer type is crucial. Deeper and more integrated knowledge of mitochondrial mechanisms and cancer-specific mitochondrial modulating means are expected for reducing tumorigenicity and/or improving anticancer drugs efficacy at the mitochondrial level. Although the great variability of biochemical changes found in tumour mitochondria, some highlighted peculiarities such as reduced TCA cycle flux, reduced oxphos rate, and reduced Complex I activity with respect to tissue specific normal counterparts are more frequent. In addition, deeper examination of supramolecular organization of the complexes in the inner mitochondrial membrane has to be considered in relation to oxphos dysfunction. Indeed, investigations on this topic in a set of tumour cells of different origins are currently carried out in our laboratory. Preliminary results here reported (Fig. 3) suggest a significant reorganization of the mitochondrial inner membrane at least in K-ras transformed cells.

Moreover, investigations into mechanisms of mitochondrial metabolic changes and how critical signaling pathways interact will uncover new therapeutic approaches in a diverse range of tumours. In this context, developing therapies based on RNA interference: post-transcriptional gene silencing mediated by small RNA duplexes, which has the advantage of high specificity and potent gene silencing, will disclose powerful weapons against tumours. The specificity of the treatment at present seems crucial due to the interdependence of metabolic pathways that makes very difficult to have benefits without altering any other important process within the cells. However, in the early and mid future, we might expect the developing of therapeutic interventions based on controlling the mitochondrial pathway for apoptosis that seem very promising. In addition, mitochondrial targeting of ROS scavengers and compounds that interfere with the unique biochemistry in the mitochondria are under investigation as promising therapeutic attempts.

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