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Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis

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1. ABSTRACT

In this review, we discuss the concept of metabolic remodeling and signaling in tumors, specifically the various metabolites that participate in the regulation of gene expression in cancer cells. In particular, pyruvate, oxaloacetate, succinate and fumarate, four mitochondrial metabolites, activate genes relevant for tumor progression. When the balance between glycolysis and oxidative phosphorylation is altered, these metabolites accumulate in the cytoplasm and regulate the activity of the Hypoxia Inducible Factor 1alpha (HIF-1alpha). HIF is one of the main factors that orchestrate the metabolic switch observed during oncogenesis. There is also an important role for lactate, fructose 1-6 bisphosphate or citrate that leads to the diversion of glucose metabolites to anabolism. In addition reactive oxygen species, which are produced by the respiratory chain, could serve as an endogenous source of DNA-damaging agents to promote genetic instability. Accordingly, several mitochondrial DNA mutations were reported in tumors, and the construction of cybrids recently demonstrated their role in the control of tumor progression.

2. MITOCHONDRIA AND ENERGY METABOLISM

Mitochondria were first described by cytologists around 1840. The first experiments on respiration and the characterization of cytochrome c oxidase were reported in 1930 by Otto Warburg (1). In 1949, Lehninger and Kennedy demonstrated that mitochondria are the organelles where the Krebs cycle, fatty acid oxidation and oxidative phosphorylation (OXPHOS) occur. Using subcellular fractionation, de Duve and Palade showed with Hogeboom and Hotchkiss that respiration takes place within mitochondria. Later, B. Ephrussi provided evidence that their genetic information was not only nuclear but also cytoplasmic, as the non-respiring yeast “Petite” mutants were shown to randomly segregate, rather than following Mendel’s laws. In 1961, P. Mitchell proposed the chemiosmotic theory, which considered that the transformation of energy from NADH, H^+ and $FADH_2$ to ATP occurs through a succession of redox reactions in a respiratory chain coupled to the extrusion of protons across the inner membrane and the ultimate phosphorylation of ADP by the F_1F_0-ATP synthase (complex V). In 1963,

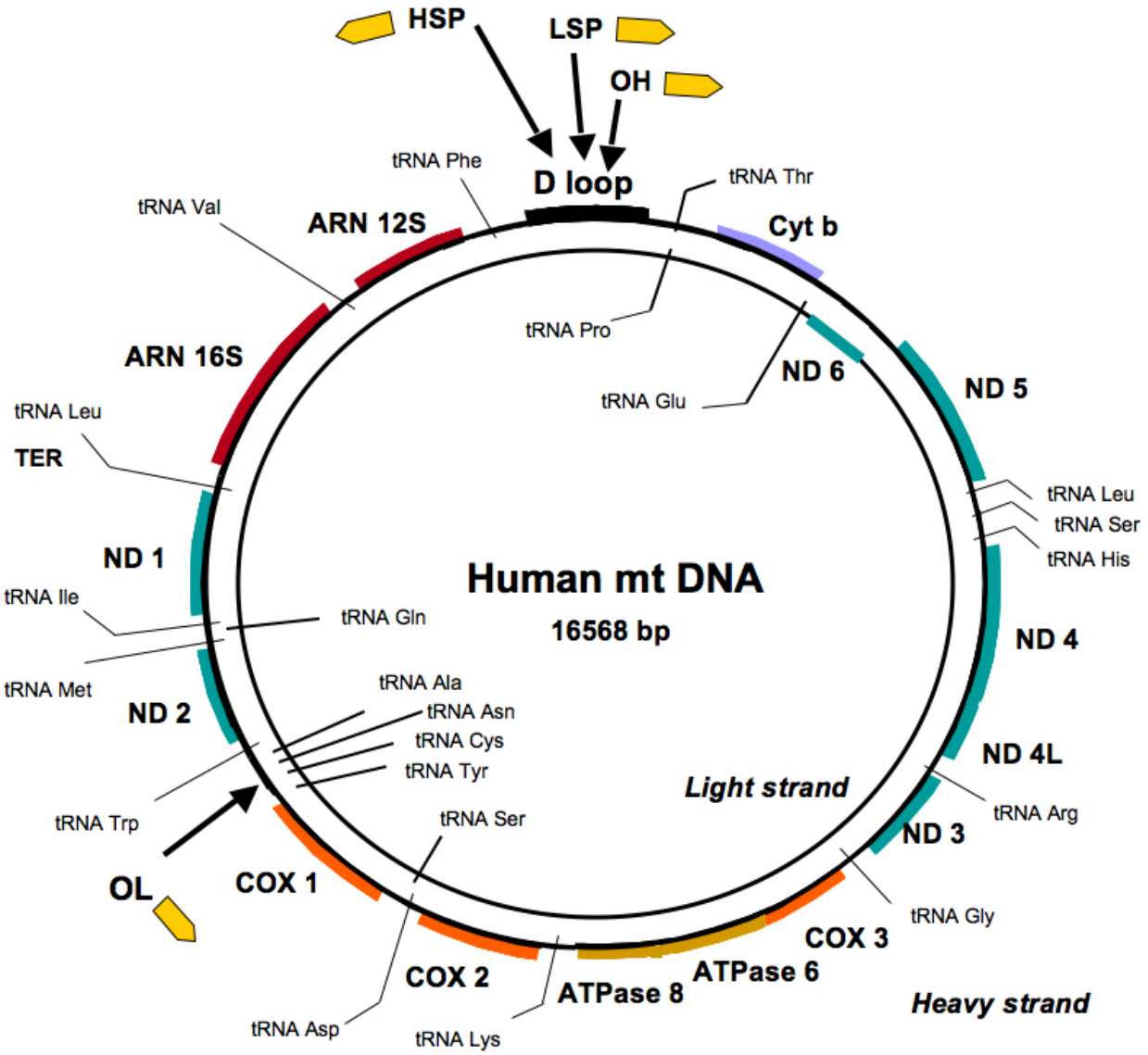

Figure 1. Human mtDNA. The human mitochondrial DNA consists of 16,568 base pairs and was the first entire genome sequenced in 1981 (92). It encodes 22 tRNA, the 12 S and the 16 S rRNA, and 13 peptides forming with the 74 nuclear-encoded polypeptides the five respiratory chain complexes. The regulatory region is a non coding sequence, or three stranded D-loop of nearly 600 base pairs. It contains the promoters of the heavy and light strands (HSP and LSP respectively), and the origin of replication of Purine-rich Heavy strand (OH). Transcription is polycistronic, and translation uses a specific code different from the universal. Replication is asymmetric, starting with the synthesis of the Heavy strand until the origin of the Light strand (OL) located about 2/3 from OH from which synthesis proceeds counter-clockwise. Thus, the Heavy strand is single stranded during about half of the replication cycle which lasts about 2 hours. ND1-6: NADH dehydrogenase subunits, COX1-3: Cytochrome c oxidase subunits, ATP6 and ATP8: subunits of ATP synthase, Cyt b: Cytochrome b.

mtDNA (Figure 1) was characterized by Nass and Schatz and then entirely sequenced by Sanger and co-workers in 1981. By that time, L. Margulis had proposed the endosymbiotic theory of mitochondrial evolution, which states that aerobic bacteria, upon symbiosis, transferred most of their DNA to the nucleus of the protocells and gained the selective advantage of being able to consume oxygen while producing energy in the form of ATP. By examining the polymorphisms in mtDNA, H. Blanc discovered its maternal inheritance; others discovered that it had a mutation rate 10 times higher than that of nuclear DNA. Furthermore, some mitochondrial DNA genes were found to be localized in the chromosomes, but are silent. In 1979, Barell found differences in the genetic code as compared with the universal one. Two years later, the entire sequence of the human mitochondrial DNA was established by F. Sanger and collaborators. The first diseases associated with mtDNA deletions were reported in early 1988; these disorders are associated with several clinical symptoms, such as myopathy, ptosis, external

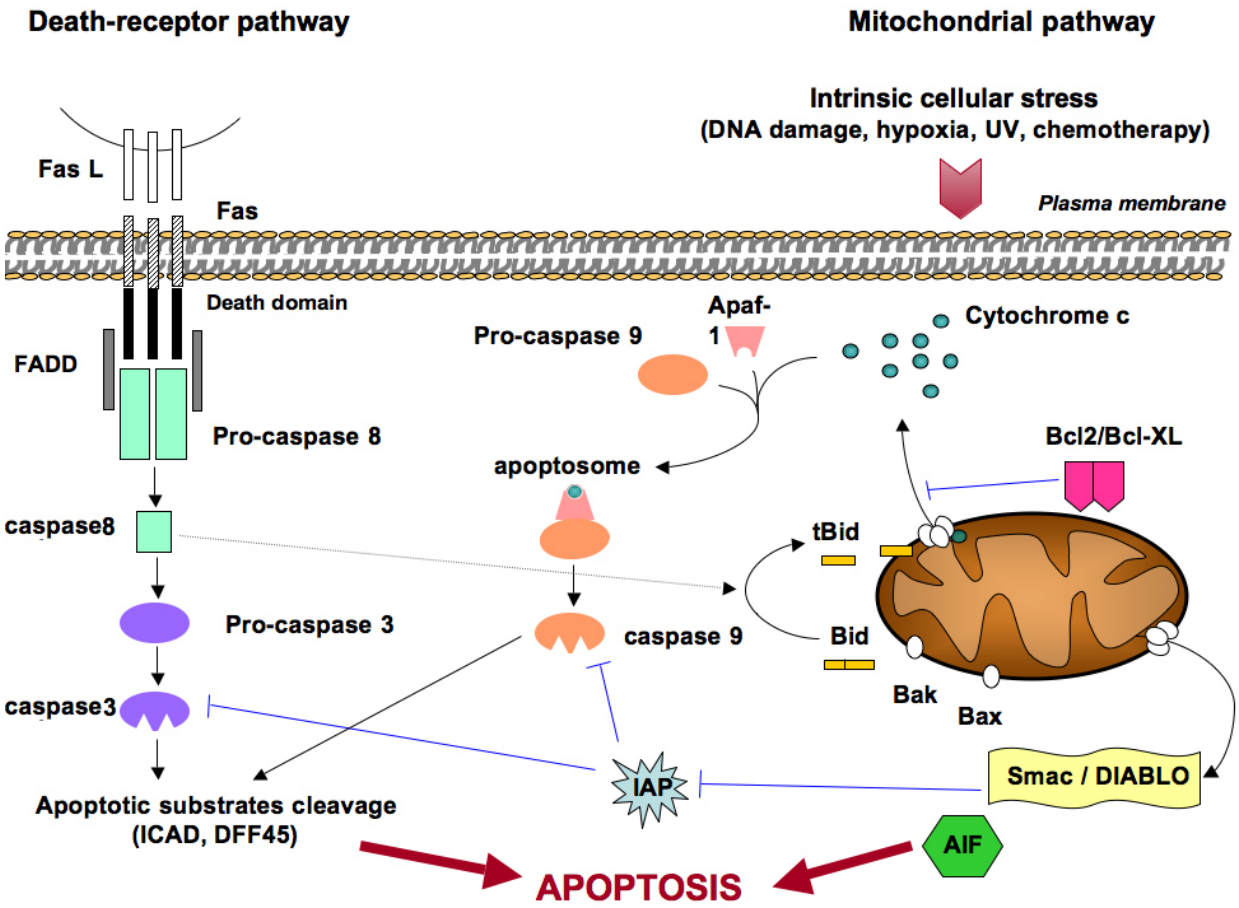

Figure 2. Mitochondrial apoptotic pathway. Death can be induced by the binding of ligands (such as FasL) to specific receptors (such as FAS) located at the cell surface. FAS contains a cytoplasmic death domain where FADD (Fas-associated death domain) can bind in presence of FasL, and recruit Pro-caspase 8 for subsequent activation in caspase 8. This induces caspase 3 activation. Caspase 3 cleaves I-CAD, the inhibitor of CAD (Caspase-activated DNase), which is released to enter the nucleus and cleaves DNA. In addition caspase 8 cleaves Bid protein, resulting in a truncated Bid (tBid) that, upon dimerisation of Bax or Bad, causes the release of cytochrome c from mitochondria. The mechanisms by which Bax leads to mitochondrial membrane permeabilisation and subsequent release of pro-apoptotic factors still remain unclear. It is proposed that Bax could interact with the permeability transition pore, or form channels by self-oligomerization. This leads to the mitochondrial release of cytochrome c and Smac/Diablo (Smac: second mitochondrial- derived activator of caspase; Diablo: direct IAP-binding protein with low pI), AIF (apoptosis inducing factor) and various procaspases. Bcl_2 inhibits the release of cytochrome C and AIF in the cytoplasm and prevents the variation of the permeability transition pore. In the cytosol, cytochrome c binds to Apaf-1 (apoptosis- protease-activating factor). Both proteins form the apoptosome, which converts procaspase 9 in caspase 9. This results in activation of downstream effector caspases. Smac/DIABLO binds to IAP (Inhibitors of apoptosis) and prevent them from inhibition of the caspase 9 and caspase 3 activation. AIF has an indirect role in chromosome degradation as it activates endonuclease G, a DNase that moves from the mitochondria to the nucleus during apoptosis. Interestingly to note, the mtDNA is not fragmented during apoptosis (93).

ophthalmoplegia and cardiac block branch (for an extensive review, see (2)). The first point mutation identified was associated with Leber's disease, which is mainly characterized by visual loss. During the 1990s, the three-dimensional structures of the cytochrome c oxidase (complex IV) and of the ATP synthase were determined by the groups of S. Yoshikawa and J. Walker, respectively. The identification of proapoptotic factors that are released by this organelle indicated that mitochondria play a pivotal role in deciding cell survival or death through intracellular signaling. Apoptosis can be divided in three phases: induction, execution and degradation (Figure 2). In this review, we will not cover the downregulation of mitochondrial apoptotic mechanisms in cancer cells. We will focus more on the particularities of their energy metabolism in relationship with metabolic signaling and tumor biology.

In the last decade, much progress has been made in deciphering the link between mitochondrial structure and function with the discovery (consideration) of a networked and dynamic organization of the mitochondrion in living

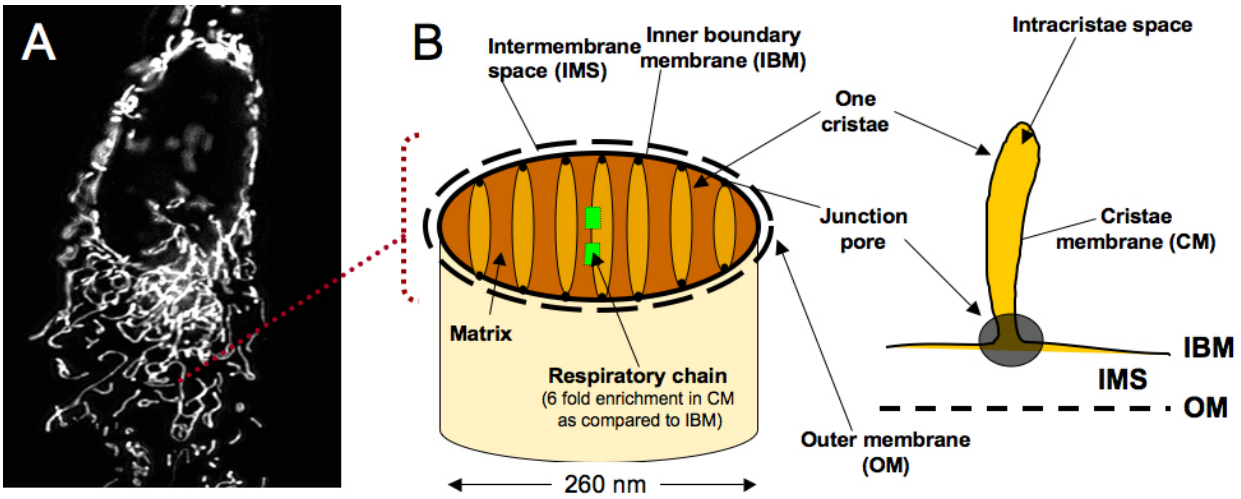

Figure 3. Mitochondrial structure. (A) Overview of the mitochondrial network in living human HeLa cells. The mitochondrial network was imaged by fluorescence microscopy (bi-photonic), using a matrix-targeted GFP. (B) Internal organization of the mitochondrial network; section of a tubule. This scheme illustrates the "cristae junction model" of mitochondrial interior. In this view, the pores could serve to regulate the release of cytochrome c during apoptosis.

human cells of diverse origin (Figure 3A). Studies examining the mitochondrial interior by electron tomography helped to define the "cristae-junction model" (3), which separates the inner boundary membrane (IBM) closely opposed to the outer membrane from the cristae membrane that projects into the matrix compartment (Figure 3B). These two components may be connected by tubular structures called cristae junctions of relatively uniform size. Recent advances in mitochondrial research revealed that morphological changes of the mitochondria, both overall organization and internal structure, are intimately linked with bioenergetics and apoptosis (for reviews, see (4) and (5), respectively). The additional discovery of pathogenic mutations in genes that are essential for fusion and fission of the mitochondrial network has added complexity to the physiopathological mechanisms of these diseases and the regulation of organellar functions as diverse as energetics, signaling, biosyntheses or apoptosis (6).

Prior to discussing the abnormalities of cancer cells energetics it is necessary to give a brief introduction on the metabolic pathways by which glucose is converted in ATP, the only form of energy considered by the cell (Figure 4). Briefly, glucose enters the cells through specific transporters and becomes phosphorylated by hexokinase to G_6P which is further cleaved in two molecules of glyceraldehyde-3-phosphate and dephosphorylated by the pyruvate kinase in the presence of ADP to yield pyruvate. These reactions produce two molecules of ATP and are collectively known as glycolysis (anaerobic and cytosolic) in contrast with respiration (aerobic and mitochondrial). The end product of glycolysis pyruvate can be transformed in lactate by the lactate dehydrogenase in the presence of an excess of NADH + H^+ during poor respiration, or it can enter the mitochondria to undergo an oxidative decarboxylation in the presence of coenzymes (NAD^+, FAD) which will fuel the respiratory chain to consume oxygen and yield ATP and H_2O. Inside the mitochondrion, the intermediate metabolism encompasses the Krebs cycle and the beta-oxidation which provide acyl-CoA and acetyl-CoA but also NAD (P)H + H^+ and $FADH_2$. Mitochondria contain other enzymes involved in catabolism (urea cycle) or anabolism with the synthesis of amino acids and uridine. They also play a pivotal role in the phosphorylation of nucleotide diphosphates, notably thymidine. The five complexes of the respiratory chain are embedded within the inner boundary membrane and the cristae membrane (ratio 1 to 6). Only 13 out of the hundred of proteins of the entire OXPHOS system (Figure 5) are encoded by the mtDNA which uses a specific genetic code. The inner membrane is impermeable to protons so that a proton electrochemical gradient can be formed upon the proton extrusion from the matrix to the intermal membrane space by the respiratory chain complexes I, III and IV. According to the chemiosmotic theory of P. Mitchell (7), the resulting electric membrane potential (deltaPsi) and pH difference (deltapH) caused by this process create a strong proton-motive force that is used for the condensation of ADP and Pi by the ATP synthetase to produce ATP. ATP is then exported to the cytoplasm by the adenine nucleotide translocator (ANT) in exchange with cytosolic ADP. Aerobic respiration from glucose produces 38 moles of ATP, which is 19 times more than what glycolysis can produce. However, the rate of ATP production by the OXPHOS system is slower than glycolysis (8).

3. METABOLIC REMODELLING IN CANCER CELLS

O. Warburg noticed that a significant number of tumors use glycolysis more than respiration to produce the vital ATP, even under normal O_2 pressure. As a result the extracellular medium is acidified. The genetic, biochemical and molecular basis of this phenomenon, which is called the "Warburg effect", still remain puzzling. Its occurrence

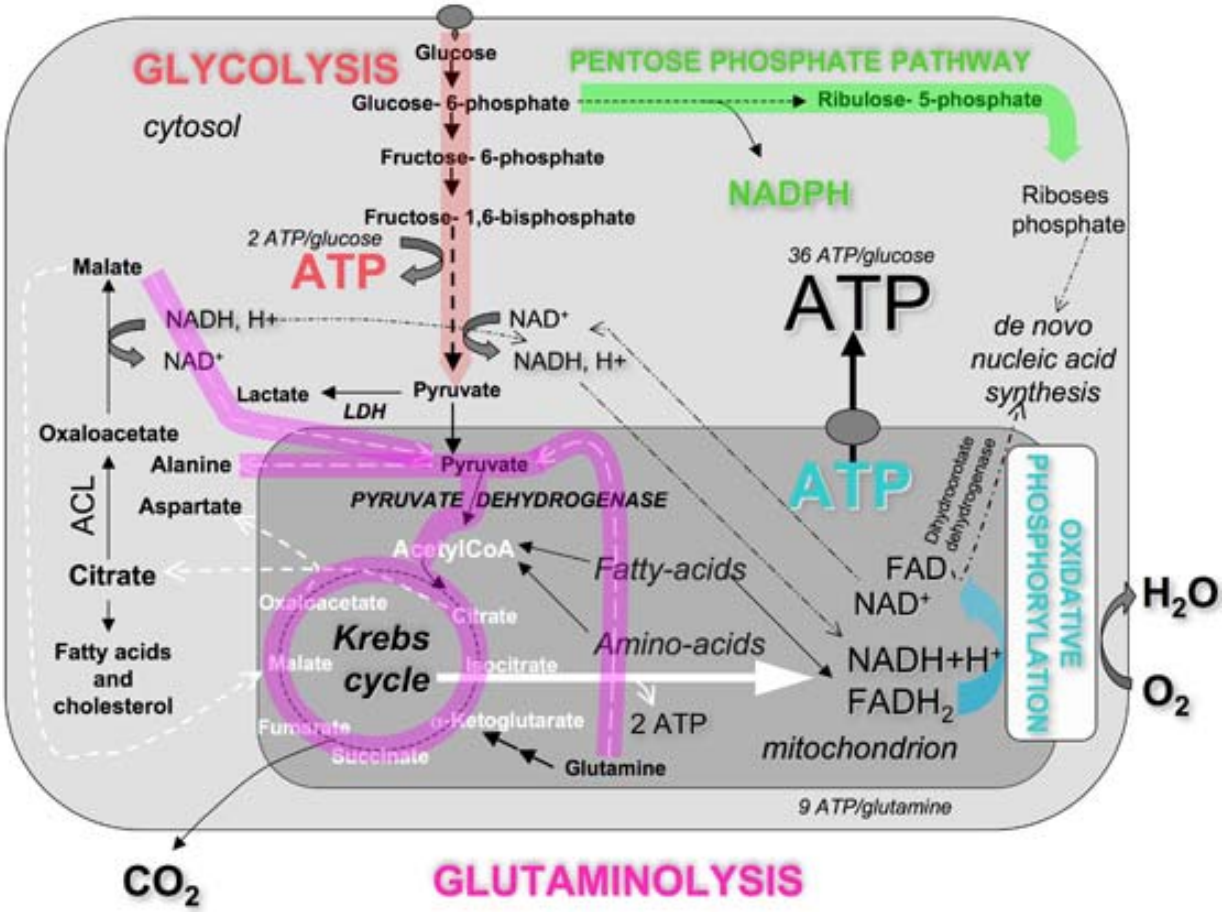

Figure 4. Main pathways of cellular and mitochondrial energy metabolism. The two main metabolic pathways, *i.e* glycolysis and oxidative phosphorylation are linked by the enzyme complex pyruvate dehydrogenase. Briefly, glucose is transported inside the cell and oxidized to pyruvate. Under aerobic conditions, the complete oxidation of pyruvate occurs through the TCA cycle to produce NADH, H^+ and/or $FADH_2$. These reduced equivalents are oxidized further by the mitochondrial respiratory chain. In a large variety of solid tumors very little of the pyruvate enters the mitochondrion, and most is tranformed in lactate by the lactate dehydrogenase despite the presence of oxygen (Warburg effect). On the figure we also figured in pink the glutamine oxidation pathway, as observed in cancer cells.

in all tumors is also challenged by the discovery of cancers that utilize more the mitochondrion to produce ATP. An elevated uptake of glucose (by a factor of 10) is generally observed in human living tumoral tissues by positron-emission tomography (PET) using the isotope analog glucose tracer 2- (^{18}F) fluoro-2-deoxy-D-glucose (FDG). This index was further correlated with increased malignancy and invasiveness (9, 10); for some tumors, a close correlation was observed between the degree of differentiation, growth rate and glucose accumulation (1, 11). Such clinical observations suggest that a large amount of glucose enters tumors, but the contribution of glycolysis and OXPHOS to glucose catabolism cannot be determined with this technique. To do so, bioenergetic studies are required on tissue sample or tumor derived cell lines. In this manner the extent of ATP produced by glycolysis was measured on various cancer cell lines and revealed that Warburg predictions were not systematic. For instance the bioenergetic analysis of two human lung cancer cell lines verified Warburg's theory but also precised that OXPHOS is not inactive *per se*: it operates at a low-capacity and is repressed by the presence of glucose (Crabtree effect) (12). In striking contrast, an analysis of MCF-7 cells originating from a mammary gland epithelial adenocarcinoma revealed that ATP production is 80 % oxidative in glucose medium (13). Likewise, in hepatoma cells mitochondrial respiration was found to be coupled to ADP phosphorylation and produced 40% of the total cellular ATP in glucose medium (14). More recently, Rodriguez-Enriquez *et al.* revealed that in HeLa and Hek293 young-spheroids, the contribution of OXPHOS to the total ATP supply was 60% (15). They further studied this feature and evidenced a class of tumor cell lines in which the oxidative metabolism prevails over glycolysis. This situation has been extensively reviewed in (16), and the authors even conclude that "high glycolysis" is not a prerequisite of all cancer cells but could be acquired during the highest proliferative activity and/or in response to stringent micro-environmental conditions, such

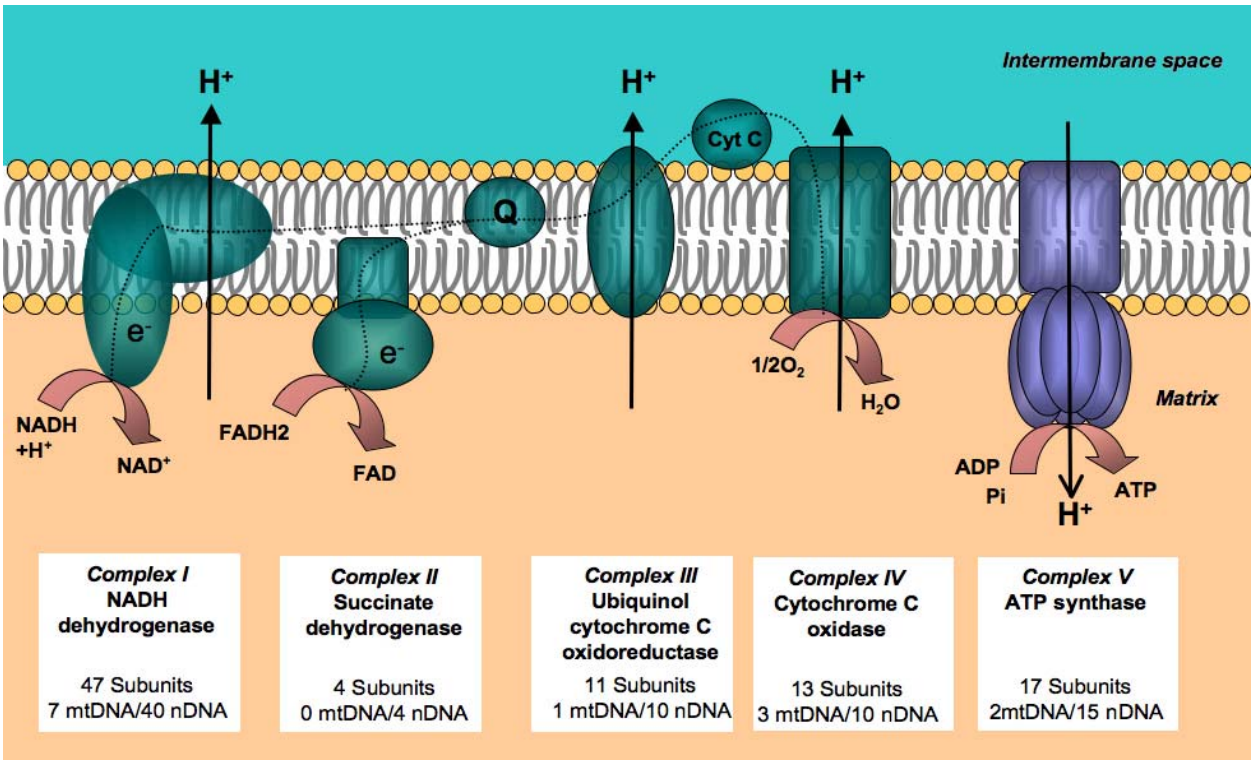

Figure 5. Mitochondrial respiratory chain. For mammals, the respiratory chain consists of four enzyme complexes (complexes I – IV) and two intermediary substrates (coenzyme Q and cytochrome c). The $NADH+H^+$ and $FADH_2$ produced by the intermediate metabolism are oxidized further by the mitochondrial respiratory chain to establish an electrochemical gradient of protons, which is finally used by the F1F0-ATP synthase (complex V) to produce ATP, the only form of energy used by the cell. In this simple representation of the respiratory chain, the supramolecular organization (supercomplexes, dimers) is not shown.

as intermittent hypoxia and/or glucose limitation. Accordingly, a critical review of numerous studies comparing cancer cells with normal tissues concluded that several tumors derive most of their ATP from mitochondrial oxidative phosphorylation, in striking contrast to Warburg's hypothesis (17). These divergent observations highlight the fundamental question of how variable is the metabolic reprogramming of cancer cells (Figure 6) and what are the relative contributions of oncogenesis, tumor environment, proliferative activity and experimental conditions. The examination of cancer cells metabolic profile should be considered for tumor caracterisation and classification as specific alterations in mitochondrial and glycolytic protein expression were reported in association with changes in cancer cell bioenergetics or apoptosis (18-20). In a large variety of tumors, extensive modifications of the mitochondrion have further been described, including a decrease in organellar biogenesis (11, 21), the alteration of respiratory chain complexes specific activity (22, 23), inhibition of the pyruvate dehydrogenase (PDH) complex (24), truncation of the Krebs cycle with citrate extrusion (25), an increase in the binding of hexokinase II to the mitochondrion (26), variegated changes in organellar shape and size (27) and the accumulation of diverse mutations in mitochondrial DNA (28, 29). More recently, it was proposed that metabolic reprogramming could be used to enhance tumors cells biosynthetic pathway, as required by the deregulated growth and needs. This is exemplified by the preferential expression of the glycolytic enzyme pyruvate kinase M2 isoform (PKM2) in cancer cells (30, 31). The lower capacity of this enzyme leads to the upstream accumulation of glycolytic metabolites which are diverted in part to various biosyntheses. Remarkably, switching to the M1 (adult) isoform leads to the reversal of the Warburg effect in these cells. This demonstrates that PKM2 expression might provide a growth advantage to tumor cells *in vivo*. Hence, the predominance of glycolysis, which was observed in numerous cancer cell lines, might be advantageous not only for fast ATP synthesis, despite unfavorable stoichiometry, but also for associated by-processes including the utilization of lactic acid for tumor invasion (32) or the redirection of excess pyruvate toward lipid synthesis (25). Another example where catabolism is diverted to anabolism concerns the remodeling (truncation) of the Kreb's cycle in tumors whereby the citrate is exported outside the mitochondrion and serves for fatty acids and cholesterol synthesis. In this case, the key enzyme is the ATP citrate lyase (ACL), which catalyzes the conversion of citrate to cytosolic acetyl-CoA. Lastly, another feature of energy metabolism reprograming in cancer cells is the extensive utilization of the pentose phosphate pathway (PPP) (Figure 4). In tumor cells, glucose is highly metabolized through the PPP, which produces ribose for *de novo* nucleic acid synthesis. PPP is also thought to be the primary source of the reducing

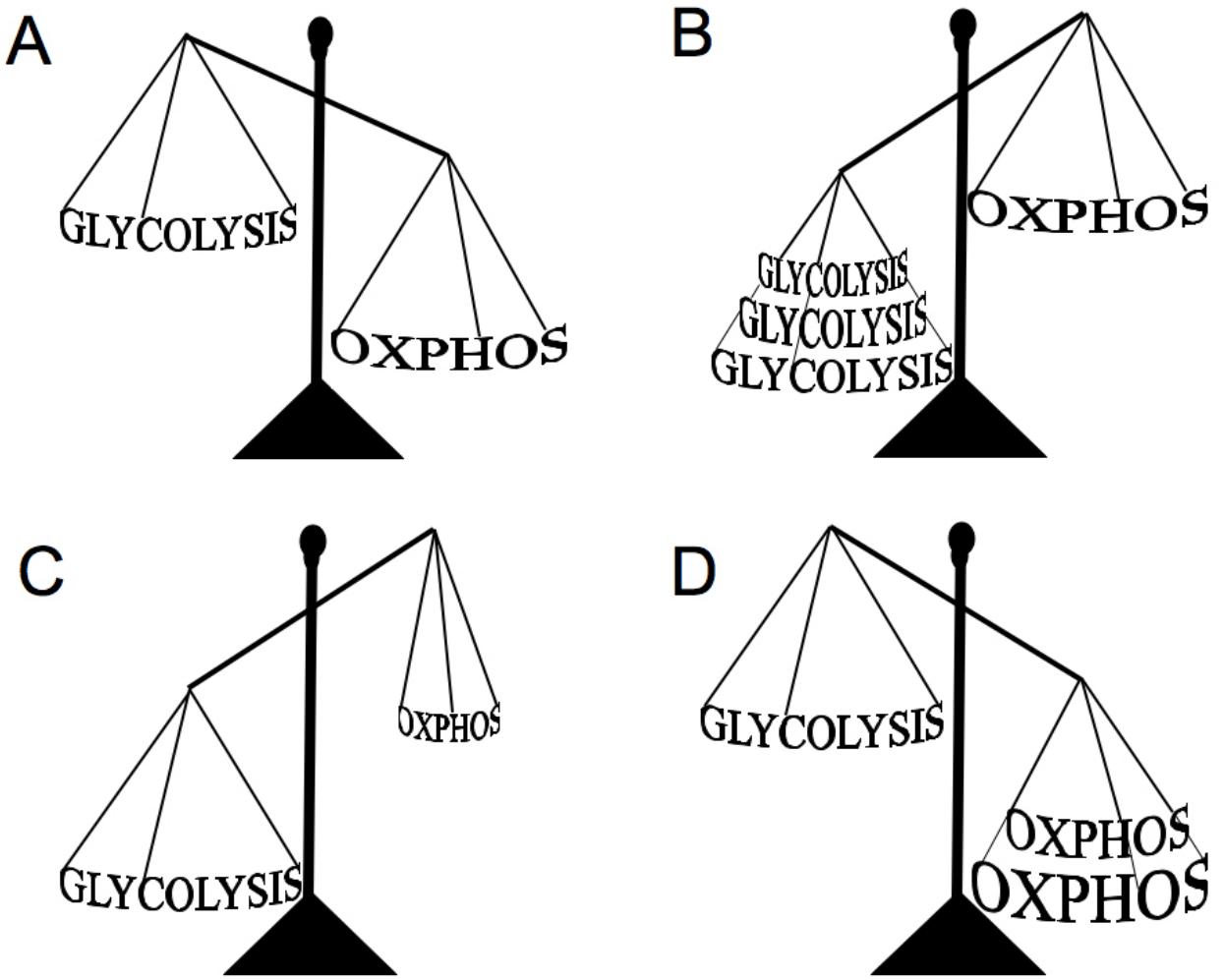

Figure 6. Illustration of the variability of metabolic remodeling reported in cancer cells. Various changes of cancer cells energetic machinery have been reported (B, C, D) as compared to normal cells (A). These include the "Highly glycolytic" (B), the "OXPHOS deficient" (C), and the "OXPHOS enhanced" (D).

equivalent (NADPH) that is necessary for producing nucleotides (33). Taken together, these observations indicate that the remodeling of energy metabolic pathways encountered in cancer cells go beyond the enhancement of energy production and might underscore a more profound need for biosyntheses and regulatory events.

All of the above listed alterations of the metabolic profile of cancer cells might combine to varying degrees to explain the variability observed in the metabolic profile of tumors and cancer cell lines. Another extensively documented feature of cancer cells in the production of lactic acid. For instance, HeLa and Osteosarcoma cells cultured in glucose medium produce 40 times more lactic acid than normal cells (34). A close stoichiometry between glucose utilization and lactate production in CHO cells was revealed during the monitoring of glucose and lactate concentrations (35). This indicates that the conversion of pyruvate to lactate is almost total in these cancer cells, suggesting that the enzyme lactate dehyrogenase (LDH) works with a high efficiency in the opposite direction of what normally occurs in non-cancer tissues (Figure 4). According to Pedersen (11), the NADH "shuttle" mechanism operates in highly glycolytic cells at a rate that is sufficient to reoxidize all of the NADH produced during glycolysis, so that the high lactate production observed in tumor-derived cell lines could only result from an imbalance between a limited oxidative capacity of the mitochondria confronted to an enhanced capacity of glycolysis. A blockade of mitochondrial biogenesis could explain this lowered OXPHOS capacity in cancer cells as a lower amount of mitochondria was reported in various tumors (11). In renal cell carcinoma (RCC), the level of impairment of mitochondrial function correlates positively with disease severity and negatively with the expression level of OXPHOS complexes. Meierhofer *et al.* found that in some renal carcinoma tissues, there is indeed a reduction in activity for all of the mitochondrial enzymes, including complex V, as well as a reduction in mtDNA content (37). They concluded that this downregulation is an early event in the formation of most renal carcinomas which could remain throughout the progression of the disease (37). In

non-small-cell lung cancer, non-angiogenic tumors express a higher level of genes that are involved in mitochondrial metabolism than angiogenic tumors (38). In rat gliomas, the mitochondrial content is also decreased (39). A recent study of renal tumors confirmed that mitochondrial biogenesis was inhibited in cancer cells and proposed a mechanism whereby HIF and ROS (down)regulate the expression levels of OXPHOS proteins (40). Alternatively, it has been reported that in breast cancer, the proliferator-activated receptor gamma coactivator 1 (PGC1alpha) was expressed at lower levels as compared to normal background tissue (36). This could suggest an important role for this factor in refining the cancer cell's metabolic profile since PGC1alpha is a strong regulator of miochondrial biogenesis. A therapeutic gain might be obtained from the reactivation of mitochondrial biogenesis in cancer cells. In addition to biogenesis reduction the low efficiency of the OXPHOS system observed in cancer cells could be explained by the alteration of respiratory chain complexes specific activity, possibly caused by cancer specific regulations such as post-translational modifications or ROS-induced alterations. Yet little is known on this particular aspect of OXPHOS dysregulation and only one study reported a functional alteration of the respiratory chain complex I in cancer (23). Furthermore, abnormalities in the overall morphology of the mitochondrial network and its internal organization have been reported in numerous cancers, including human astrocytomas (27), carcinomas (41), Warthin's tumor (42), xenografted gliomas (39), malignant glioma cells (43) and HeLa cells (44). Most of these studies reported heterogenous ultrastructural abnormalities, such as organellar swelling with a disorganization and distorsion of the cristae and partial or even total cristolysis. Those might underline an acivity defect of the OXPHOS system as such ultrastructural alterations of the mitochondrion are commonly found in mitochondrial diseases. Abnormalities in mitochondrial fusion-fission were also proposed from the analysis of astrocytomas sections by electron microscopy (27). Such structural defects in the mitochondria are frequently seen in cancer cells, although their precise origin and participation in tumor progression are not well understood. An analysis of mitochondrial morphogenesis in cancer cells could help to clarify this question.

Warburg proposed that cancer could originate from the sole inhibition of respiration in hman cells, but are the mitochondria of cancer cells dysfunctional ? Different studies have indicated that OXPHOS is capable of synthesizing ATP in cancer cells, albeit with a low efficiency (12). To survive under conditions of glucose limitation, tumor cells use OXPHOS to derive energy from the amino-acids, glutamine and serine. This process (glutaminolysis) has been demonstrated to occur in multiple types of cancer cells (45-48). In healthy cells, glutamine degradation is catabolized by mitochondrial glutamase to produce ammonia and glutamate, which is further transaminated by glutamate dehydrogenase into alpha-ketoglutarate to feed the Krebs cycle (cf. Figure 4). The major degradation product of glutamine metabolism is ammonia, which is secreted into the medium of the cell culture (49). However, two metabolic pathways leading to different end products have been identified for the complete oxidation of glutamine, depending on the presence of malate in the cytosol (cf. Figure 4). In the first route, glutamate is converted in alpha-ketoglutarate (by the glutamate dehydrogenase) which enters the Krebs cycle to produce malate and then oxaloacetate, using the malate dehydrogenase. Oxaloacetate is then converted in aspartate which is extruded from the mitochondrion. In the other pathway, glutamate oxidation proceeds entirely via transamination, whereby the alpha-amino group of glutamate is transaminated primarily to pyruvate to form alanine, which is extruded from the mitochondrion; the resulting alpha-ketoglutarate is oxidized, ultimately to citrate (which is also extruded from the mitochondrion), via the enzyme malate dehydrogenase, with the aid of acetyl-coA generated from pyruvate by external or cytosolic malate using the malic enyme. It was proposed that tumors use more the latter pathway for glutamate oxidation (50) since the former is rendered inefficient by the inhibition of glutamate dehydrogenase, possibly by a negative allosteric effector such as GTP. Moreover, the citrate released by the second pathway serves for fatty acid and cholesterol biosynthesis in cancer cells, while the released alanine is used for cytosolic amino acid transformations and protein synthesis. In this view, the oxidation of glutamine not only serves for ATP synthesis, but also generates important intermediates for biosynthetic activities as discussed above for the glycolysis with PKM2.

4. METABOLIC SIGNALING IN CANCER CELLS

We define mitochondrial metabolic signaling (MMS) as the regulation of cellular gene expression by various (energy) metabolites and by-products that accumulate upstream or downstream of the mitochondrion. A list of signaling metabolites and their genetic targets is given in Figure 7. Accordingly, the remodeling of mitochondrial metabolic pathways that was described above alters, *de facto*, the metabolome; as a result, gene expression is altered by the modulation of transcription factors (HIF-1) and co-activators (PGC1alpha) activity. This feedback process occurs under physiological conditions and participates in the regulation of cell homeostasis. We hypothesize that in various pathologies MMS is altered by the abnormal accumulation of signaling metabolites, which interferes with physiological processes and participates to the progression of the disease as well as phenotypic variability. As mitochondria host a large number of cellular metabolic reactions, this organelle is central to metabolic signaling. For example, a lowered activity of mitochondrial complex two could trigger an increase in cytosolic succinate concentration which may lead to the stabilization of the transcription factor HIF-1alpha; this protein activates the expression of several genes, including erythropoietin, vascular endothelial growth factor, glucose transporter 3 and aldolase A, which are involved in the promotion of tumor proliferation through a redefinition of the metabolic profile. In human paragangliomas, this cascade is initiated by mutations in the succinate dehydrogenase gene that triggers the abnormal accumulation of succinate and the subsequent

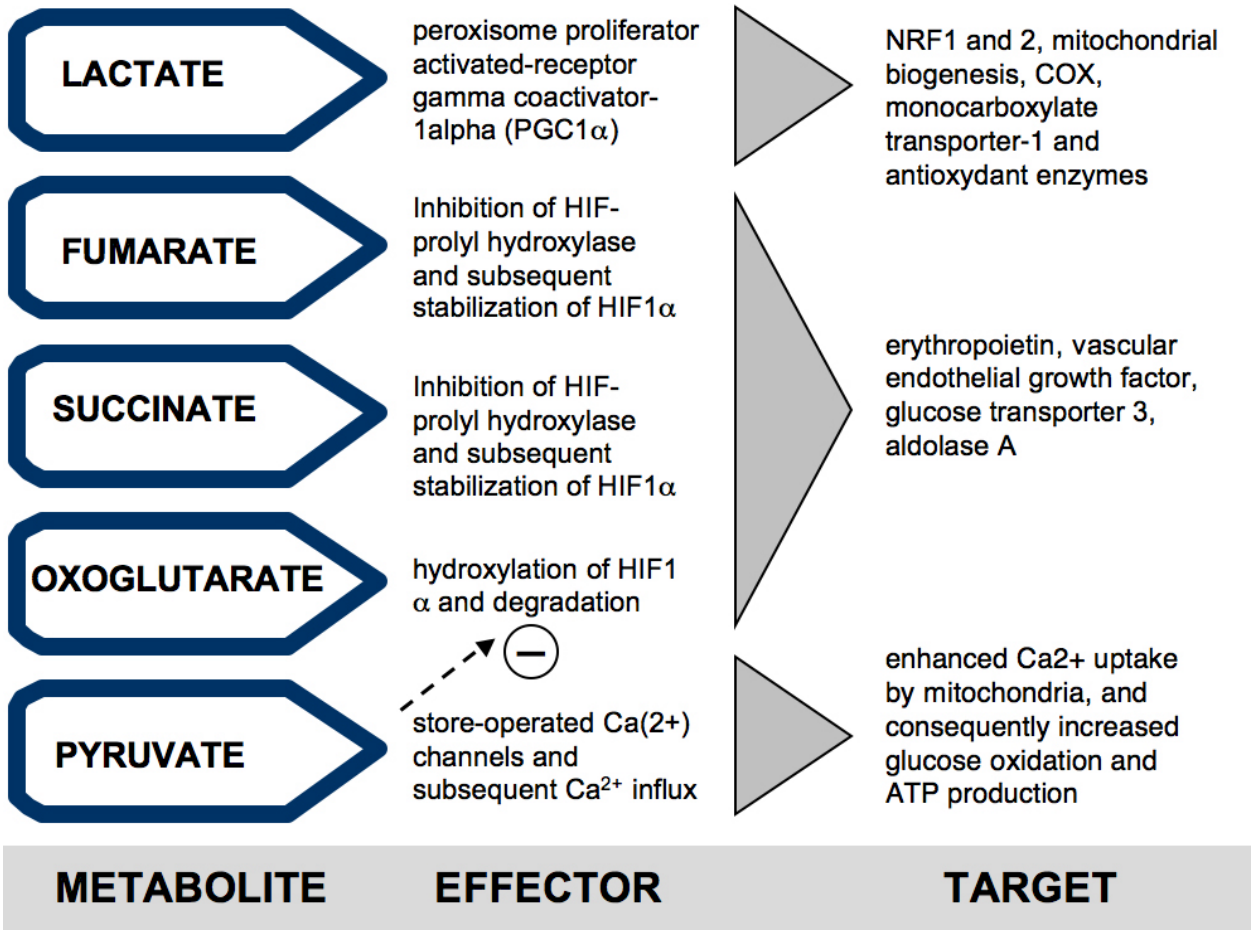

Figure 7. Metabolic signalling in cancer cells. In addition their role of intermediates in glucose metabolism, pyruvate, lactate, oxoglutarate, succinate and fumarate participate to the reglation of cellular gene expression through the activation or the inhibition of effectors such as HIF-1alpha or PGC1alpha. The concept of mitochondrial metabolic signalling aims to study these interactions and to elucidate the biological impact of metabolomic modifications.

activation of HIF-1 despite the presence of oxygen (51). In this case, one observes a pseudo-hypoxic activation of HIF. Likewise, mutations in the fumarate dehydrogenase gene are involved in the tumorigenesis of leiomyomas and renal cell cancer by similar mechanisms (51). It has been proposed that increased levels of succinate and/or fumarate stabilize HIF-1alpha by inhibiting HIF prolyl hydroxylase. Lactate can also be considered as a signaling metabolite, since it can activate mitochondrial biogenesis and the production of antioxidant enzymes (52). In cancer cells, the large amount of lactate that is produced by the conversion of accumulated pyruvate (due to the glycolysis / OXPHOS imbalance) could affect signaling during the cancer cell's metabolic reprogramming. Recent work also suggests that pyruvate, the end product of glycolysis and a precursor substrate for the Krebs cycle, regulates Ca^{2+} influx into the cell, which leads to physiological changes through the activation of transcription factors and gene expression (53). Fructose-2,6-bisphosphate is also a metabolic signaling molecule, as it regulates the gluconeogenic and glycolytic pathways of hepatoma cells (54). The accumulation of 2-oxoglutarate permits the continuous hydroxylation of the HIF-1 transcription factor alpha and activates the degradation of this protein, thus preventing the transcriptional activation of several genes involved in energy metabolism (55). Lastly, pyruvate and oxaloacetate also regulate this mechanism, as they can bind to the 2-oxoglutarate site of prolyl hydroxylases. These observations led to propose a model whereby HIF prolyl hydroxylases are regulated by oxygen availability or by reversible inactivation (55). Accordingly, the above listed metabolites, in addition to regulating HIF activity and being involved in related pseudo-hypoxic pathways (56), can participate in maintaining tumorogenesis in other ways. Reactive oxygen species (Figure 8), which are produced by the respiratory chain also participate to mitochondrial metabolic signaling in cancer cells. Although ROS cannot be strictly described as metabolites, they can leak into the cytoplasm and undergo inactivation by catalase and Mg SOD (superoxide dismutase). In some conditions, $O_2^{\bullet}$ can diffuse in the cytoplasm and induce damage or serve as sensor for other effector (s), notably HIF-1alpha. For instance, it was recently shown that UVB induces a biphasic variation of HIF-1alpha expression level through

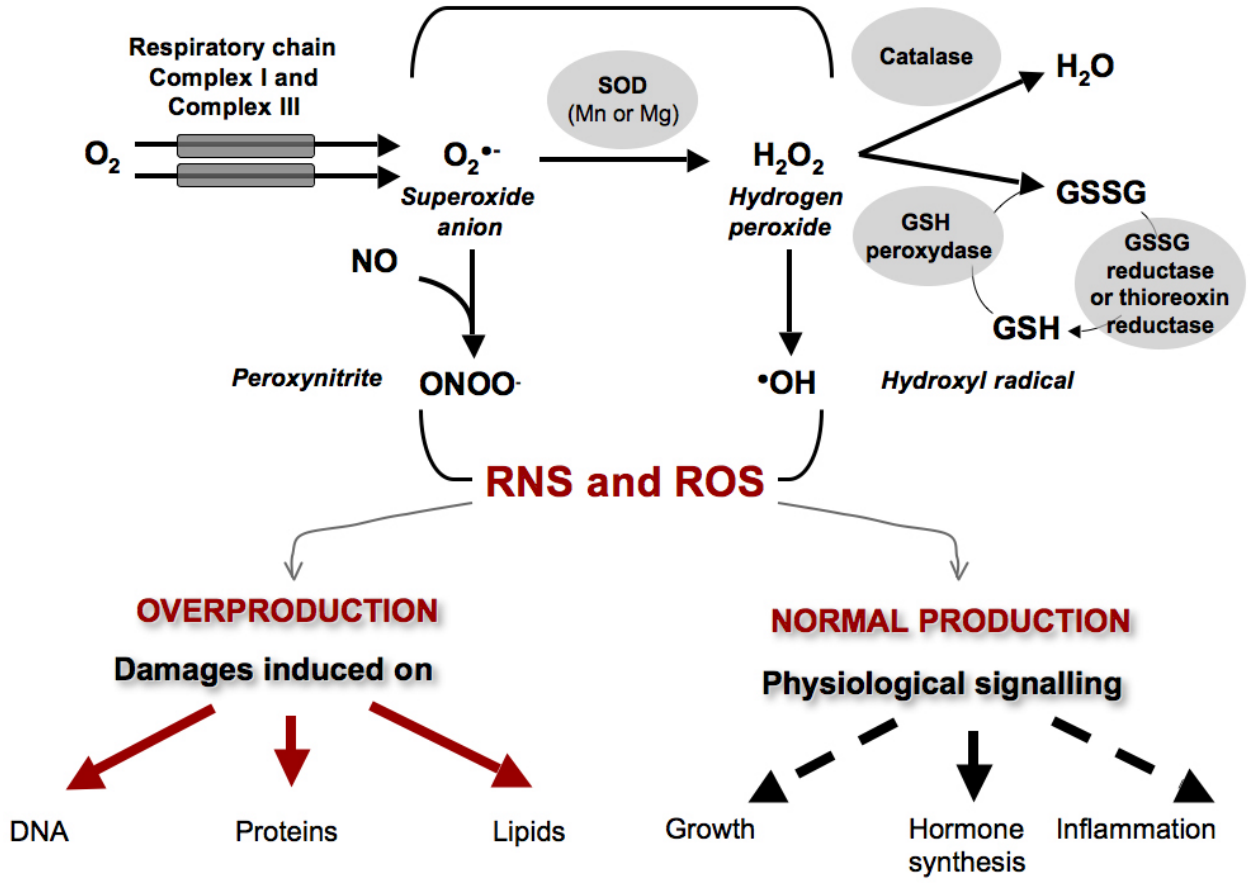

Figure 8. Mitochondrial signalization by ROS in cancer cells. The mitochondrion is implicated in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In most cells, the mitochondrial respiratory chain is recognized as the major site of ROS production in the form of superoxide, hydrogen peroxide and the hydroxyl free radical. These molecules can be considered as positive products that intervene in cell signaling. However, excessive amounts of ROS are deleterious for the cell, contributing to a variety of pathological processes. ROS production can result in the set up of a vicious cycle of oxidative damage causing a progressive alteration of mtDNA and mitochondrial functions that lead in turn to energy deprivation, redox imbalance and cell dysfunction.

the generation of reactive oxygen species (ROS) (57): a rapid production of cytoplasmic ROS downregulates HIF-1alpha expression and delays the mitochondrial generation of ROS, which in turn, triggers an upregulation of HIF-1alpha expression. In this study, HIF-1alpha was phosphorylated and accumulated after UVB irradiation, following the activation of p38 MAPK and JNK1 that was mediated by mitochondrial ROS. The amount of ROS in cancer cells is generally higher than that measured in normal cells, although more studies are required to confirmate this feature. Szatrowski and Nathan analyzed seven human tumor cell lines and observed an increased rate of H_2O_2 production (up to 0.5 nmol/10^4 cells/h) (58). This escalated production of ROS in cancer cells could serve as an endogenous source of DNA-damaging agents which may promote genetic instability and the development of drug resistance (59). This intrinsic oxidative stress is partly caused by oncogenic stimulation and a low level of mitochondrial respiration. Bioenergetic studies showed that mitochondrial ROS production is higher at state 4 (restng state) as compared to state 3 (phosphorylating state), so that inactivation of ATP synthesis while maintaining a basal respiration produces more ROS. Another condition for a high ROS generation is the presence of both pyruvate and succinate to allow a reverse electron flow from complex II to complex I. Current theories propose that ROS contribute to cancer initiation, maintenance and progression *in vivo* (60). In this view, the mtDNA mutations that occur in many human colorectal tumors and cancer cell lines could confer a cell growth advantage by inhibiting the ETC and causing higher levels of ROS production. The mitochondrial genome, given its low level of DNA repair mechanisms and its naked structure, is particularly sensitive to ROS. More examples of metabolic signaling can be found in normal cells, but the changes in these pathways that occur in the context of cancer are not known.

5. ORIGIN OF CANCER CELLS' METABOLIC REPROGRAMMING

The question that remains to be answered is the origin of metabolic reprogramming in cancer cells. Is it

induced by oncogenes during transformation? What is the role of the environment? Are pre-cancer cells selected for their already adapted metabolic features? Is it an experimental bias?

Concerning the environment, the vascular network that surrounds the tumor creates a chronic or intermittent hypoxia, which is thought to play a crucial role not only in cancer development and mitochondrial transformation (61) but also in the failure of radiotherapy and O_2-dependent chemotherapy (62). Interestingly, tumors are often observed in hypoxic regions of the tissues that are beyond the "diffusion limit" of oxygen. This fact might indicate that hypoxia could reshape the metabolic profile of pre-malignant cells that are located in this region, which can be detected by magnetic resonance imaging (63). This fact may also suggest, again, a role for hypoxia and the HIF factor in tumor initiation (Figure 9). Likewise, pseudo-hypoxic activation of HIF caused by mitochondrial alteration and subsequent MMS could be a determinant factor to predispose a cell to cancer. In this idea, mitotoxic drug might be considered as an additional risk factor for cancer, but this remain to be shown. Early studies by Gosálvez M revealed the possibility to create tumors by using rotenone, a potent inhibitor of mitochondrial complex I also used as an insecticide. More studies are required to assess the carconogenicity of OXPHOS inhibitors and to understand the associated biochemical mechanisms.

Regarding the genetic link between oncogenesis and metabolic reprogramming, early hypotheses considered that the energy profile of a neoplastic cell mimics that seen during development and explored the idea that in such cases cancer cells were reverting to the fetal program of expression (64). This idea was originally proposed by Uriel J. after a study showing the existence of fetospecific antigens and fetal-type isozymes in adult malignant tumors (65). Likewise, there is a decreased expression of some OXPHOS proteins in tumors that can be explained by a genetic mechanism that has been documented in fetal tissues (21, 64). It is well known that anaerobic glycolysis is the major means of cellular ATP production in fetal organs (66) and that a rapid increase in mitochondrial respiratory capacity occurs during development to adapt fetal metabolism to sudden birth (67). It is possible that this process could be reversed in cancer cells, in which case similar bioenergetic profiles would exist in both cancer cells and fetal cells of the same tissue. This phenomenon might also explain the decrease of mitochondrial biogenesis in cancer cells. However, *in vitro* studies are limited by experimental bias, since the tissue culture itself can initiate a reversion from oxidative metabolism to glycolysis (68). Thiazolidine-4-carboxylic acid (thioproline, "norgamem") a compound found to act on the cell membrane of tumour cells, causing reverse transformation to normal cells was investigated in patients with advanced cancer. Histological studies showed involution and transformation into low-grade malignancies and disappearance of evidence of cancer.

6. MITOCHONDRIAL DNA (mtDNA) MUTATIONS AND GENE EXPRESSION IN CANCERS CELLS

In 1970, complex (head-to-tail) mtDNA were reported in leukemia (69). Subsequently, a number of mtDNA rearrangements and amplifications were reported in acute myeloid leukemia (70). Insertions in mtDNA were also found in the *c-myc* oncogene of HeLa cells (71). In renal cell carcinoma, a 261 base pair deletion was found in the *COXI* gene (72). Cells derived from hepatocarcinoma and chemically induced rat hepatocarcinoma display a much higher level of ND5 mRNA (73). Furthermore, an abnormal mtDNA sequence composed with ND6 and COI is also present in this tissue (74), although they are separated by 230 base pairs. Studies on oncocytic tumors showed a fivefold increase in mtDNA compared to corresponding controls (75). Unfortunately, no additional work has precisely linked these mtDNA rearrangements to cancer. More recent investigations using DNA sequencing have shown that 26% of oncocytic lesions have mis-sense or non-sense mutations in the complex I subunits (76) of thyroid oncocytic tumors (77). Complementary studies revealed that there is an increased expression of mtDNA-encoded subunit 5 of complex I, ATPase 6, Cyt B and Cox I and III (78); the expression of mitochondrial transcription factor mtTFA did not vary suggesting a possible regulation at the level of translation or mRNA stabilization. Of the 24 genes that are overexpressed in oncocytic carcinomas, 84% are involved in mitochondrial and cell metabolism, including those of the tricarboxylic cycle and of glycolysis (79). In this particular type of cancer, the activation of mitochondrial biogenesis can be seen as a compensatory response and resembles that observed in mitochondrial diseases. Accordingly, in the other types of tumors, where mitochondrial biogenesis is generally decreased, no alteration of respiratory chain complexes activity has been described.

Polyak *et al.* (80) sequenced the complete mtDNA genome of ten human colorectal cancer cell lines and found mutations in seven (70%). The majority of these mutations were transitions at purines, which is consistent with a ROS-related derivation. As pointed out by Brandon *et al.* (81), several of these mutations may be also related to the polymorphic mutations found in the normal population; their relevance to the transformed cell status remains unknown. Nevertheless, almost all of the reported mutations are transitions, but none are specific to any kind of tumor (81). Table 1 shows the affected tissue and the mutated genes. Table 2 presents their frequency. The D-Loop is proportionally the most mutated region (3.2%), followed by ND3 (2.2%); the ND2 regions represent only 0.4% of the mutants. The colon, pancreas and thyroid display wide spectra of mutations, whereas mutations in other tissues, such as the prostate, are essentially restricted to COX I. ND4 and ND4L are mutated in all tissues but not in the prostate and bladder. Interestingly, these studies can be performed on bodily fluids (82). Thus, the detection of mutations in mtDNA could help to refine the diagnosis of cancer. Interestingly, mammalian cells contain several hundred more copies of mtDNA than nuclear DNA, making such analysis more sensitive.

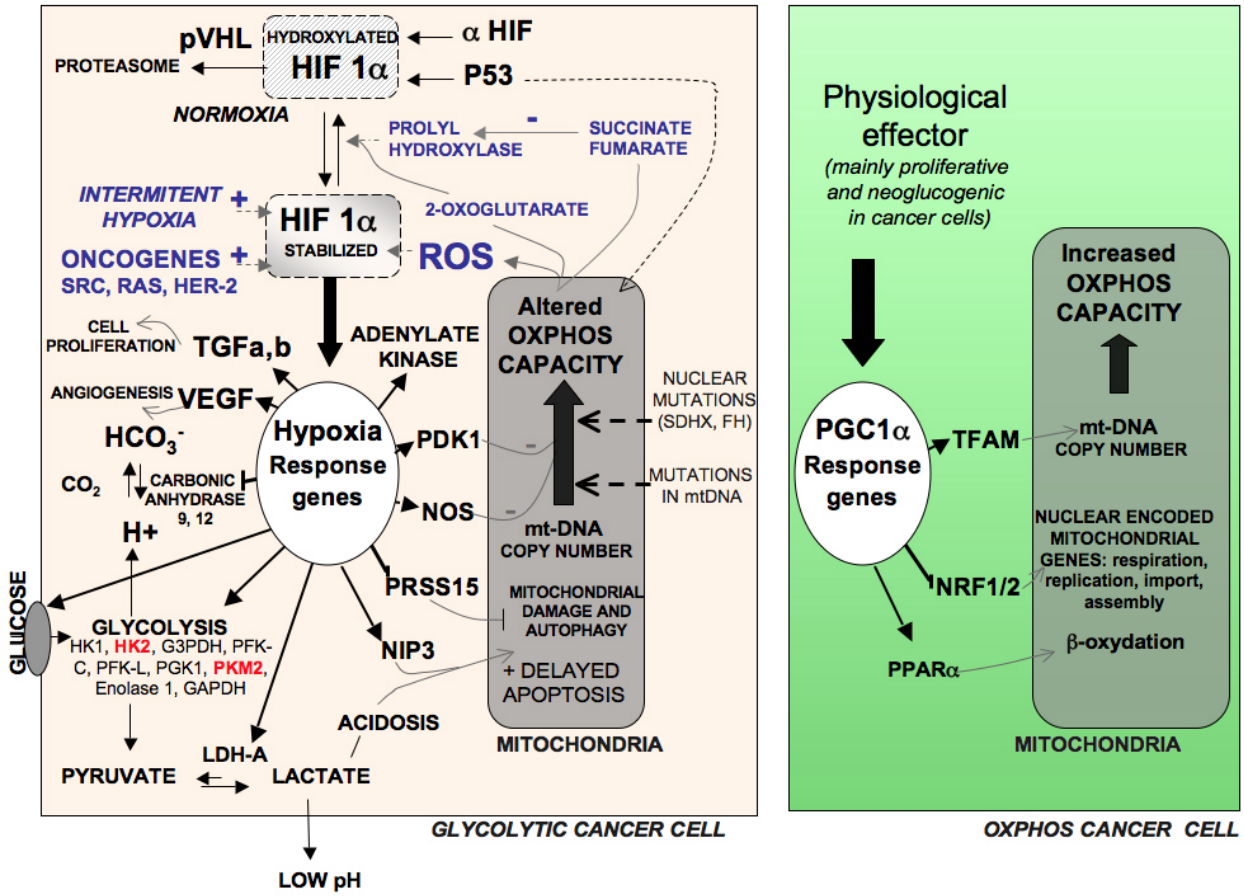

Figure 9. Role of HIF in cancer cell metabolic reprogramming. On the left panel is figured a "Glycolytic" cancer cell, on the right one an OXPHOS cancer cells. It was reported that HIF-1alpha plays a central role in the upregulation of glycolytic genes and the downregulation of mitochondrial energy genes. HIF is a heterodimeric complex composed of HIF-1alpha and HIF-1alpha subunits. Under normoxic conditions, the alpha subunit is degraded after two post-translational hydroxylations catalysed by the HIFalpha prolyl hydroxylase domains (PHD) proteins 1, 2, and 3 which require O_2, alpha keto-glutarate, ascorbate, and 2-oxoglutarate for their activities. The tumor suppressor von *Hippel-Lindau* gene product (pVHL) then mediates the ubiquitinylation of HIF-1 alpha yielding to its degradation by the proteasome. As PHD display a high Km for O_2, HIF-1alpha escapes hydroxylation under low oxygen pressure. HIF-1alpha then translocates to the nucleus, associates with HIF-1 alpha and triggers transcription of genes involved in glycolysis energy metabolism notably glucose transporters, hexokinase II, and angiogenesis (VEGF), allowing adaptation of cells to hypoxia. Other gene products such as c-Myc cooperate with HIF-1 alpha by inducing the metabolic switch from aerobic to glycolytic pathways via the expression of pyruvate dehydrogenase kinase 1. Lactate concentration thus increases notably around the cell. Mutations in succinate dehydrogenase (SDH) and fumarate hydratase (FH) of the Krebs cycle have been reported as associated with Paraganglioma (94) or pheochromocytoma (95). These mitochondrial proteins encoded by nuclear DNA therefore act as tumor suppressors (96). To account for these observations, accumulation of fumarate and succinate in the cytoplasm stabilize HIF through the PHD. Thus mutations in these enzymes activate HIF-1 which promotes transcription of genes involved in low O2 pressure adaptation by strongly amplifying the synthesis of mitochondrial hexokinase II and angiogenesis through production of VEGF. The heterodimeric complex HIF-1 binds to DNA at hypoxia responsive element (HRE) of target genes implicated in glycolysis (hexokinase1 (HK1) and 2 (HK2), Phosphofructokinase C and L (PFK-C, PFK-L), Pyruvate kinase M2 isoform (PKM2), Enolase1 and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)). HIF-1 complex is also implicated in angiogenesis (Vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS), cell proliferation (Transforming growth factor alpha and beta (TGF beta, TGF beta)) and metabolism (adenylate kinase, Lactate dehydrogenase A (LDH-A)). HIF-1 also induces the expression of nineteen kD interacting protein-3 (NIP3), a mitochondrial protein which trigger cell death. On the right panel is depicted an "OXPHOS cancer cell" where the level of PPARgamma co-activator-1alpha (PGC1alpha) is increased in response to proliferative signal or as part of a compensatory phenomenon. PGC1alpha induces the expression of mitochondrial transcription factor A (TFAM) and nuclear respiratory factors 1 and 2 (NRF1/2). As a result, both the mitochondrial content and activity are increased.

Table 1. Somatic mutations of mtDNA in cancer tissues

Malignant tissue	Gene (number of different mtDNA mutations reported)
Bladder	D-Loop (6). ND3 (3). Cyt b (2). ND 4 (7). ND 5 (1). 16S rRNA (3)
Breast	ND4 (1). ND5 (2)
Colon	ND1 (5). ND4L (2). ND5 (1). Cyt b (4). Cox I (2). Cox II (2). Cox III (2). 12 S rRNA (1), 16 S rRNA (3)
Head and neck	D Loop (6), ND4 (3), 16 S rRNA (1)
Lung	D Loop (7), 16 S rRNA (1), $tRNA^{trp}$ (1), $tRNA^{Leu}$ (1)
Medulloblastoma	ND4L (1)
Pancreas	ND1 (4). ND2 (1). ND3 (1). ND4 (3). ND6 (2). Cyt b (1). Cox I (2). CoxII (1). Cox III (1). ATPase 6 (2)
Prostate	ND1 (2). Cox I (20)
Thyroid	ND1 (3). ND2 (2). ND3 (4). ND4 (3). ND4L (1). ND5 (7). ND6 (6). Cyt b (3) Cox II (4). Cox III (5). ATPase 6 (3)

Constructed from the data reported in three different studies (28, 80, 82).

Table 2. Percentage of somatic mutations per mtDNA base pairs

Gene	% of mutations
D Loop	3.2%
rRNA	0.6%
ND1	1.3%
ND2	0.4%
ND3	2.2%
ND4	0.6%
ND4L	1%
ND5	0.7%
ND6	1.1%
Cyt b	0.8%
Cox I	1.3%
Cox II	1.1%
Cox III	1.1%
ATPase 6	0.7%
Mean value	***1.1%***

Calculated from (2, 28, 81, 82)

To understand the role of mtDNA mutations in cancer progression, cybrids were created to replace the mtDNA of a normal cell with the mtDNA from a cancer cell line (83). Upon fusion with the cytoplasm of the mutant mtDNA, cells form cybrids which can be injected into nude mice and then analysed for tumor induction properties. In an elegant experiment, S. Ohta designed the T8993G mutation in the mitochondrial gene of the ATPase 6 subunit, which causes a mitochondrial myopathy (NARP) by changing a Leucine to an Arginine. He also added an import sequence to the N terminus and a FLAG tag to the C terminus, to confirm its expression by immunodetection (84). Upon formation of the cybrid with the normal mtDNA, transformed cells consume less oxygen than normal cells. After transplantation of these cybrids into nude mice, the mutant T8993G ATPase localized to the nucleus, conferred a growth advantage and triggered the formation of tumors. This experiment demonstrates the functional importance of the mitochondrial ATPase, even though it is located in the nucleus of the transformed cell (85). More recently, the same technology was applied to study the link between mtDNA mutations and carcinogenesis (29). In this study, two mtDNA mutations, G13997A and 13885insC, in the gene that encodes the NADH (reduced form of nicotinamide adenine dinucleotide) dehydrogenase subunit 6 (ND6) conferred a high metastatic potential. These mutations produced a deficiency in the activity of the respiratory complex I and were associated with an overproduction of reactive oxygen species (ROS). These studies demonstrate that tumor growth can be modulated by changes in mitochondrial activity, as the generation of ROS depends on the magnitude of the electrochemical proton gradient that is generated by the respiratory chain. Uncoupling agents that target the mitochondria of cancer cells could decrease mitochondrial ROS production and slow tumor growth. These studies raise the question of the occurrence of cancer in patients with mitochondrial disease but so far no data are available concerning this link.

7. MITOCHONDRIA AS THERAPEUTIC TARGETS

A challenge in metabolic anti-cancer therapy is the development of pharmaceutical agents that could selectively target cancer cells. These efforts will undoubtedly benefit from fundamental studies on the structure and function of the mitochondrial network in cancer cells, as suggested by Otto Warburg in 1930: *"Can tumour cells be destroyed in the living animal through lack of energy? If the carcinoma problem is attacked in its relation to the physiology of metabolism the first question is: In what way does the metabolism of growing tissue differ from the metabolism of resting tissue?"* The main changes in mitochondrial function that occur in transformed cells are listed in table 3. It is conceivable that the Warburg effect could be prevented by inhibiting glycolysis with metabolic analogues, such as 2'-deoxyglucose or Ionidamine (86). Secondly, the binding of hexokinase II to VDAC (Voltage dependent anion channel) on the mitochondrial outer membrane can be inhibited by 3-bromopyruvate (26, 87). Another strategy would be to commit transformed cells to apoptosis through the mitochondrial outer membrane permeabilization pore (MOMP), to which pro-and anti- apoptotic factors bind, including the BH3 family proteins (88). Peptides that mimic the interfaces of the BH3 family may have great potential. More recently, a screen for inhibitors of HIF successfully identified small chemicals with IC_{50} values in

Table 3. Non-exclusive, frequently encountered differences involving mitochondria functions between normal and cancer cells

Normal	Transformed	Selected reference
Normal PO_2	Intermitent, low PO_2	(32)
Low HIF activation	Higher HIF activation	(97)
Glycolysis and respiration	High Glycolysis with lactic acidosis	(1)
Glutaminolysis	Glutaminolysis	(47)
Low Hexokinase II content	Higher level of HKII bound to mitochondria	(26)
Pyruvate kinase isoform 1	Pyruvate kinase isoform 2	(30)
Physiological level of ROS	Elevated ROS	(58)
Normal mitochondrial membrane potential	Elevated mitochondrial membrane potential	(98)
Normal mtDNA mutation rate	Higher mtDNA mutation rate	(81)
Normal Complex II	Some mutations in complex II	(51)
Normal pyruvate dehydrogenase (PDH)	PDH Inhibited by PDH kinase	(24)
BAX/ $Bcl-_2$	BAX/ $Bcl-_2$	(99)

Table 4. Anti-cancer mitochondrial therapeutic targets

Cancer Cells Particularity	Therapeutic mode of action	Active molecule	Ref
Abnormal Metabolic systems (glycolytic and oxphos)	Inhibition of glycolysis	2-deoxy-D-glucose (2DG)	100)
		Lonidamide (inactivation of hexokinase)	101)
		3-bromopyruvate	(26)
	Inhibition of the metabolic reprogramming	HIF-1 inhibitors	(102)
	Inhibition of glutaminolysis	antisense mRNA for glutaminase	(103)
	Inhibition of the pentose phosphate pathway	6-amminonicotinamide	(104)
	Inhibition of OXPHOS and apoptosis induction	F16	(105)
	Inhibition of Krebs cycle enzyme	Casiopeina II-gly	(106)
	Activation of PDH via inhibition of PDH kinase (PDK1)	Dichloroacetate (DCA)	(107)
	Inhibition of mitochondrial oxidative phosphorylation leading to decrease of deltapsi and subsequent induction of apoptosis caused by ATP depletion and/or depolarization	Clofazimine	(108)
		MKT077	106)
		Laherradurin	109)
		Rhodamine 123	110)
		Angucycline landomycine E	111)
		All-trans retinoic acid	112)
Decreased sensitivity of Mitochondrial Apoptotic Pathway Induction	Induction of the mitochondrial apoptotic pathway	Resveratrol (caspase activation)	113)
		Sulindac (Bax induction)	114)
		Tributyrin (Bax induction)	115)
		Cisplatin (Bax induction)	116)
		Celecoxib (Bax induction)	117)
		Downregulation of bcl-2 with antisense	118)
		Ridaifen-B (downregulates bcl2)	119)
Higher level of reactive oxygen species	Increase the level of ROS generated by mitochondria over the death threshold to activate apoptosis. This is also achieved by mitochondrial antioxydants depletion.	Phthalocyanine 4 (Photodynamic therapy)	120)
		Artesunate	121)
		Cinnamaldehyde	122)
		Arsenic Trioxide with Ascorbic Acid	(123)

the nanomolar range (89). The authors of this work suggested that these compounds act by inhibiting mitochondrial ROS production. Several therapeutic anti-cancer approaches are currently being considered (summarized in Table 4). One can classify drugs into three classes by mechanism: (i) inhibition of energy metabolism, (ii) activation of the apoptotic machinery and (iii) proposed induction of reactive oxygen species production. The first class of drugs take advantage of the specificity of the cancer cell metabolic profile, particularly the higher dependence of cancer cells on glycolysis, the high mitochondrial membrane potential and the increased participation of glutaminolysis and the pentose phosphate pathway in cancer cell's metabolism. The second class of drugs induces programmed cell death in cancer cells; some of these molecules are currently being employed for anti-cancer therapy in clinical use. The third class proposes to exploit the higher levels of ROS in cancer cells, and focuses on killing tumors by further increasing their level over a threshold of tolerence. However, this class of drug could be hazardous for non-cancer cells in the long-term, as we detailled in a previous chapter (chapter 6) that sustained high level of reactive oxygen species can participate to tumorogenesis through mutations in mtDNA and HIF stabilization. Thus, it remains unclear and questionable to use such compounds for anti-cancer therapy since most studies were performed on cultured cells and no long-term investigations are available.

In the search for new therapeutic approaches, metabolic profiling and analysis of metabolic control can be used to identify effective targets for cancer therapy. By revealing tumor-specific metabolic shifts in tumor cells, metabolic profiling enables drug developers to identify the metabolic steps that control cell proliferation and thus aid in the identification of new anti-cancer targets and the screening of lead compounds with anti-proliferative metabolic effects (90). Metabolic control analysis (MCA) allows one to quantify the importance of each enzyme in a given metabolic network that functions at a given steady-state. This analysis, for example, determines the enzymatic steps that have the highest control over the system. Such enzymes are more likely to be chosen as therapeutic targets to block or activate the metabolic system that is involved in the disease process. The impact of this approach for designing anti-cancer therapies has been reviewed by Cascante *et al.* (91). As an example, transketolase of the

pentose cycle has been identified, as a key enzyme necessary for nucleic acid synthesis in tumor cells. Lastly, the concept of mitochondrial metabolic signaling that was presented in this review could also be used for developing innovative therapeutic approaches. These types of approaches would aim to change the intracellular concentration of various metabolites that are involved in tumor progression by modulating different metabolic pathways (upstream and downstream) with dedicated pharmacological effectors. More studies on the particularities of mitochondrial dynamics in cancer cells could also permit to evidence key regulatory points of organellar (dys)functions.

8. CONCLUSION

Numerous studies on cancer cell bioenergetics evidence a large variability in the relative contribution of glycolysis and OXPHOS to cellular ATP production. The corresponding differences in the capacities for glycolysis and OXPHOS demonstrate that there is a cancer-specific metabolic remodeling caused by a combination of genetic and environmental factors. The dogma that cancer cells solely use glycolysis is no longer valid; as such, a detailed bioenergetic characterization of each type of tumor must be performed in order to develop adequate treatments. HIF-1alpha and PGC1alpha have been identified as the two main factors involved in the determination of a cancer cell's metabolic profile, but more studies will be required to understand their interaction. In particular, the concept of metabolic signaling will have to be further investigated *in situ* with a combination of transcriptomic and metabolomic studies. Mutations found in mtDNA may contribute to the variability in the metabolic profiles of cancer cells, depending on how they affect metabolic signaling. They could trigger the accumulation of specific metabolites and/or the generation of reactive oxygen species, which further impacts the expression of different genes involved in tumor proliferation. A future challenge for cell biology will be to evaluate the relative importance of cell culture techniques, cell proliferation rates, medium composition, oxygen availability and the presence of various oncogenes in the determination of a cancer cell's bioenergetic profile. Despite the growing number of genetic analyses that are helping to identify biomarkers of the disease, more functional studies on human cancer surgical pieces will be required to fully understand the role that mitochondria play in cancer biology. An effort should also be made to look at the particularities of mitochondrial dynamics in tumors, since mitochondrial morphogenesis controls play a role in both energy metabolism and apoptotic processes. The growing interest in the mitochondrion in cancer research could lead to the development of adapted metabolic therapies that might also serve to treat mitochondrial diseases. The recent development of functionalized nano-particles directed to the mitochondrion could help to deliver active metabolites or compounds to the organelle to modulate ROS generation or complex II activity and ultimately slow cancer cell proliferation. Recent studies also indicate that the mitochondrial remodeling of cancer cells might be linked to their capacity to evade the immune response.

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Abbrevations: AIF, Apoptosis inducing factor; ACL, ATP citrate lyase, ANT , adenosine nucleotide transporter; ATP, adenosine triphosphate; CHO, Chinese hamster ovary cell; delta psi,..mitochondrial membrane potential; ETC, electron transport chain; $FADH_2$, reduced form of flavin adenine dinucleotide; FDG, 18-Fluorodeoxyglucose; G_6P, Glucose - 6 – phosphate; HIF1alpha,..Hypoxia inducible factor 1alpha; HKII, Hexokinase 2; LDH, Lactate dehydrogenase; MCA, Metabolic control analysis; MnSOD, Mangenese Superoxide dismutase; MOMP, mitochondrial outer membrane permeabilization; mt DNA, mitochondrial DNA; NADH, reduced form of Nicotinamide adenine dinucleotide; NGF, Nerve growth factor; nDNA, nuclear DNA; OXPHOS, oxidative phosphorylation; PDH, Pyruvate dehydrogenase; PDK1, Pyruvate dehydrogenase kinase 1; PET, Positron emission tomography; pVHL, von Hippel-Lindau tumor suppressor protein; ROS, Reactive oxygen species; TNF, tumor necrosis factor; TSA, Thiol-specific Antioxidant Enzyme; VEGF, Vascular endothelial growth factor.

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