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From Deregulated Genes to a Broader Understanding of Tumour Physiology

G. Kroemer, D. Mumberg, H. Keun, B. Riefke,
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Preface

There is strong evidence for a common metabolic phenotype associated with cancer, observed both in vivo and in vitro, across species, and across a wide range of primary and secondary tumour sites. Already in 1920s, Otto Warburg described the phenomenon of "aerobic glycolysis", the apparently greater tendency of tumour cells to convert glucose to lactate in the presence of normal oxygen conditions. At his time, Warburg's hypothesis that cancer was caused by altered metabolism found no wide acceptance even though other observations that growth of tumour cells in culture is often unusually dependent on the availability of common substrates, such as glutamine, arginine, methionine and cysteine, support the idea of a tumour metabolic phenotype. To what extent this metabolic phenotype of cancer is causal or consequential to carcinogenesis and disease progression is still not clear, but important evidence exists to suggest that it confers selective growth advantages to transformed cells.

While the debate still continues as to the significance of the Warburg effect, at least one aspect of the phenotype, namely, increased glucose uptake, is already being exploited clinically by PET imaging. Although the Warburg effect has been demonstrated and confirmed in most human tumours, the advent of molecular biology and the discovery of oncogenes and tumour suppressor genes in the 1970s have shifted the scientific interest in tumour metabolism towards the search for the genetic basis for cancer. To date, the focus in the cancer molecular profiling

community has been in serum proteomics for diagnostic markers, and in tissue/cell transcriptomics for prognostic markers. However, metabolites have a key advantage as biomarkers: they are highly translatable from laboratory work to the clinic. This is in part due to the fact that a metabolite is the same chemical entity irrespective of whether it is observed in a cell, organelle, tissue or biofluid, whichever individual, sex or species is being observed at the time. The same is not true for genes or proteins, which undergo alternative splicing and translational modifications in addition to sequence variation. Today technologies are available to rapidly analyse broad varieties of metabolites in various tissues and body fluids (metabonomics) and interpret the data. Additionally, it could be shown that a variety of oncogenes exert their transforming activities largely by modulating central metabolic pathways, such as glycolysis. Particularly, those oncogenes protecting cancer cells from naturally occurring programmed cell death (apoptosis) appear to act predominantly by ensuring sufficient nutrient supply and energy production for the malignant cancer cells. Thus, we are starting to unravel how oncogenic signal transduction is connected to the metabolism, survival and growth of tumour cells. Obviously, these findings will have tremendous consequences for the understanding of the molecular mechanisms leading to cancer. Furthermore, they open new opportunities for the development of new therapeutic drugs and diagnostic tools for the treatment of cancer, as exemplified by the recent finding that LDH (lactate dehydrogenase) may be a predictive marker for the response of tumours to anti-angiogenic therapies.

A long-awaited promise of the post-genomic era was the use of biomolecular profiling, particularly genetic profiling, to tailor the therapy of each individual to their specific needs and susceptibilities. This goal of personalized medicine has already begun in oncology by the selective application of drugs such as Herceptin that only benefit a subpopulation of patients based on the genetic makeup of their tumour (over-expression of HER2). Metabonomics has enormous potential in this area, not only because metabolic biomarkers can act as phenotypic indicators for expression of genetic differences as described above, but also because essentially the same analytical protocol and platform used to discover a metabolic biomarker in an experimental model can be applied in subsequent preclinical efficacy and safety studies, as well as

in later clinical trials. Therefore metabonomics, whether based on mass spectrometry or nuclear magnetic resonance, can be considered as an ideal technology to detect translational biomarkers.

The Workshop "Oncogenes Meet Metabolism—From Deregulated Genes to a Broader Understanding of Tumour Physiology" was organized in order to discuss the recent advances and controversies in this fast-moving research area. We tried to bring together many of the internationally recognized experts who, through a variety of approaches, have made seminal contributions, thus leading to major strides forward. We are grateful to all of them for their excellent presentations and lively discussions, and also for their contributions to this book. We are convinced that the proceedings of the workshop will allow a better understanding of important aspects of the metabolism of tumours and will help in the future development of more effective and selective cancer diagnostics and treatments.

Finally, we would like to express our gratitude to the Ernst Schering Foundation for its generous support and superb organization, which allowed us to hold this workshop in the best possible conditions.

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Mitochondria and Cancer

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Abstract. Mitochondria contained in cancer cells exhibit two major alterations. First, they are often relatively resistant to the induction of mitochondrial membrane permeabilization (MMP), which is the rate-limiting step of the intrinsic pathway of apoptosis. The mechanisms of MMP resistance have come under close scrutiny because apoptosis resistance constitutes one of the essential hallmarks of cancer. Second, cancer cell mitochondria often exhibit a reduced oxidative phosphorylation, meaning that ATP is generated through the conversion of glucose to pyruvate and excess pyruvate is then eliminated as the waste product lactate. This glycolytic mode of energy production is even observed in conditions of high oxygen tension and is hence called anaerobic glycolysis. Here, we discuss the molecular mechanisms accounting for inhibition of the mitochondrial apoptosis pathway in neoplasia and discuss possible mechanistic links between MMP resistance and anaerobic glycolysis.

1 Introduction

When cells are kept in a glucose-rich milieu and are cultured first in a hypoxic environment and then in a normoxic one, they manifest the so-called Pasteur phenomenon, that is a reduction in glucose consumption, concomitant with a decrease in lactate production and an increase in oxygen consumption. In contrast, cancer cells behave differently and continue high glycolysis and lactate production, even in conditions of high oxygen tension. This phenomenon is referred to as anaerobic glycolysis and was discovered in the 1920s by the late Nobel Prize winner Otto Warburg as the first biochemical hallmark of cancer (Warburg et al. 1924, 1926). Nonetheless, Warburg was unable to demonstrate that the Warburg phenomenon would account for oncogenesis or participate in tumor progression as a causative factor. Indeed, this hypothesis was dismissed, and the study of intermediate metabolism and oxidative phosphorylation (which is decreased in cancer cells) was abandoned with the advent of molecular biology and the discovery of oncogenes and tumor suppressor genes that have captured most if not all of the attention of cancer biologists over the last three decades.

As a result, cancer biologists and medical oncologists have been considering the university courses in which they were taught that mitochondrial metabolism, including the tricarboxylic acid cycle (TCA) and oxidative phosphorylation (OXPHOS), were a useless and time-consuming effort that they discretely abhorred. Nonetheless, there was a sudden and unexpected renaissance of mitochondrial biology when it was discovered that these organelles control cell death (Kroemer et al. 2007; Liu et al. 1996; Zamzami et al. 1996). Indeed, it appears that mitochondrial membrane permeabilization (MMP) is often the decisive event that marks the frontier between survival and death, irrespective of the morphological features of end-stage cell death (which may be apoptotic, necrotic, autophagic or mitotic). In a way, mitochondrial membranes make up the battleground on which opposing vital and lethal signals combat to seal the cell's fate. Local players that modulate the propensity to MMP include the pro- and anti-apoptotic members of the Bcl-2 family (Adams and Cory 2007b), proteins from the mitochondrial permeability transition pore complex (PTPC), as well as a cornucopia of interacting partners including mitochondrial lipids (Zamzami and Kroe-

mer 2001). Intermediate metabolites, redox reactions, sphingolipids, ion gradients, transcription factors, as well as kinases and phosphatases, link survival or death signals emanating from distinct subcellular compartments to mitochondria. Thus, mitochondria have the capacity to integrate multiple pro- and anti-apoptotic signals. Once MMP has been triggered, it causes the release of catabolic hydrolases and activators of such enzymes (including those of caspases) from mitochondria. These catabolic enzymes as well as the cessation of the bioenergetic and redox-detoxifying functions of mitochondria finally cause cellular demise, implying that mitochondria coordinate the late stage of cell death. In tumor cells, MMP is inhibited at the level of mitochondria or upstream thereof, at the level of premitochondrial pro-apoptotic signal transduction pathways. Induction of MMP in transformed cells constitutes the goal of anti-cancer chemotherapy (Kroemer et al. 2007).

The purpose of the present review is to briefly discuss the mechanisms of MMP inhibition in tumor cells and to establish hypothetical links between MMP resistance and anaerobic glycolysis.

2 Mitochondrial Control of Apoptosis

Apoptosis is morphologically defined as a type of cell death in which the cell and, in particular, the nucleus shrinks (Kroemer et al. 2005). Chromatin condensation (pyknosis) and nuclear fragmentation (karyorrhexis) are the two hallmarks that define apoptosis. Although there have been attempts to define apoptosis biochemically (for instance, as cell death with caspase activation or cell death with phosphatidylserine exposure on the outer leaflet of the plasma membrane), these attempts have failed, for the simple reason that the alleged specific hallmarks of apoptotic cell death are not truly specific (thus, phosphatidylserine exposure and caspase activation can occur during T cell activation without cell death) (Galluzzi et al. 2007; Kroemer et al. 2005). Given that the morphology of nuclei changes in a much more characteristic (and specific) fashion than that of any other organelle, in apoptosis, at the beginning it was thought that these changes would reflect the essence of the apoptotic process and that the point-of-no-return, the frontier between

death and life, would be determined by alterations in nuclear morphology related to degradation of nuclear DNA (chromatinolysis).

This concept was invalidated, initially by a cell-free system in which cellular constituents (organelles and cytosol) were admixed in vitro to recapitulate the process culminating in nuclear alterations (pyknosis, karyorrhexis, chromatinolysis). Using this system, we discovered that the most reproducible way to induce apoptosis in vitro was the following: in a first step, cells were treated with an apoptosis inducer. After a short incubation, the cytosol that contained accumulating MMP inducers was purified. In the second step, this cytosol was then mixed with mitochondria from healthy cells, resulting in MMP and hence the release of pro-apoptotic effector molecules through the permeabilized outer mitochondrial membrane. In the third and final step, these effector molecules were added to healthy nuclei to induce apoptotic changes (Susin et al. 1996, 1997; Zamzami et al. 1996).

Using this system, we purified and identified apoptosis-inducing factor, a caspase-independent death effector that acts on purified nuclei to cause peripheral chromatin condensation and large-scale DNA fragmentation to approximately 50 kbp (Susin et al. 1999). Using a slightly different cell-free system, Xiadong Wang and colleagues purified and identified all the constituents of the postmitochondrial caspase activation pathway, namely, cytochrome *c* (which leaks out from the mitochondrial intermembrane space), Apaf-1 (an ATP-dependent adaptor) and caspase-9 (the apical caspase of a cascade culminating in the activation of the effector caspases-2, -6 and -7) (Li et al. 1997; Liu et al. 1996; Zou et al. 1997). Importantly, the anti-apoptotic action of the oncoprotein Bcl-2 was mapped by several groups (Kluck et al. 1997; Susin et al. 1996; Yang et al. 1997) at the mitochondrial level, meaning that Bcl-2 interrupts the apoptotic process by sealing the mitochondrial membranes and by preventing MMP.

These results as well as other experiments transposed the nucleocentric world view of apoptosis to a mitochondriocentric one (Fig. 1). The mitochondrion, and in particular, MMP would determine the decision of committing apoptotic suicide, acting as the central integration point of the apoptotic process and then as the coordinator of the catabolic process that leads to ordered cellular dismantling (Kroemer et al. 1995). Obviously, this concept had far-reaching implications for the concep-

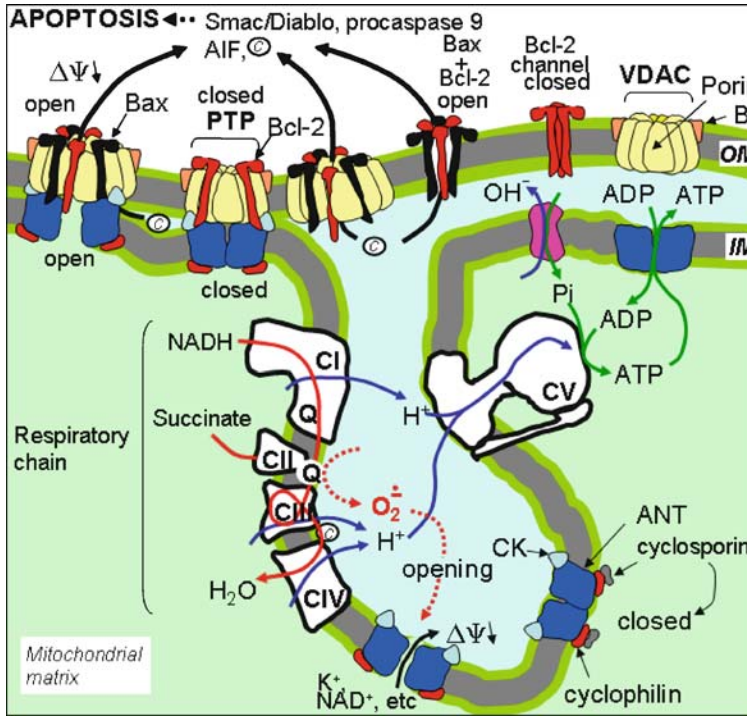

Fig. 1. Mitochondrial pathways to apoptosis. The release of intermembrane space components (such as AIF, or cytochrome *c*) and/or the loss of membrane potential ($\Delta\Psi$) as affected by numerous stimuli trigger caspase activation and cell commitment to die through an apoptotic process. Loss of membrane potential and of various matrix cofactors can result from the opening of the mitochondrial permeability transition pore (*PTP*). The balance between the pro- and anti-apoptotic members of the Bcl-2 family controls the opening of the pore. Alternatively, the members of this family can form channels that may also allow for the release of proapoptotic components present in the intermembrane space. PTP is a complex formed between the voltage-dependent anion channel (*VDAC*) of the outer membrane and the adenylate nucleotide translocase (*ANT*) of the inner membrane associated with several additional proteins. *AIF*, apoptosis-inducing factor; *Br,* benzodiazepine-receptor; *CI–CV,* the various complexes of the respiratory chain; *c,* cytochrome *c*; *IM,* inner membrane; *OM,* outer membrane

tion, detection and manipulation of cell death at several levels (Jiang and Wang 2004; Kroemer et al. 2007). This can be easily illustrated by the pathophysiological implications of MMP-mediated cell death control:

- Many different signals can induce (or inhibit) MMP, linking different types of cellular stress and damage to mitochondria. This underscores the potential of mitochondria to function as general cell death sensors and to integrate many distinct lethal triggers (Brenner and Kroemer 2000).
- MMP is not simply induced (or inhibited) by a single class of molecules. Rather, several alternative, complementary and intertwined modes of MMP exist that are mediated by distinct classes of proteins and modulators (Zamzami and Kroemer 2001). This introduces some sort of redundancy into the system that regulates cell death, hence preventing a mutation completely suppressing cell death, an event that would be intrinsically oncogenic.
- When MMP has trespassed a critical threshold, its biochemical consequences possibly encompass further permeabilization of adjacent and distant mitochondria, thereby resulting in a rapid self-amplifying phenomenon, which occurs prominently in an all-or-nothing fashion. This implies that the detection of MMP indeed predicts imminent cell death.
- Once MMP has occurred, it triggers cell death rapidly and efficiently, through a plethora of independent and redundant mechanisms. These include the activation of caspases and caspase-independent death effectors, as well as irreversible metabolic changes at the bioenergetic and redox levels.
- If cytoprotection is the therapeutic goal, it is indispensable to prevent MMP or the upstream events leading to MMP. In contrast, cellular demise cannot be avoided by inhibiting the post-mitochondrial phase of apoptosis, which comprises biochemical changes occurring after the point of no return has been trespassed (postmortem events). This is essential for the design of neuro-, hepato-, nephro- or cardioprotective therapies.
- Pathological MMP contributes to the unwarranted loss of post-mitotic cells in the brain and heart. Pharmacological agents that

target specific mitochondrial ion channels or proteins that contribute to MMP may be useful for the therapeutic suppression of acute cell death.

- Cancer cells are often relatively resistant to MMP induction, and the therapeutic induction of MMP constitutes a therapeutic goal in anti-cancer chemotherapy or radiotherapy. The inhibition of MMP-inhibitory proteins (such as Bcl-2-like proteins) can sensitize tumor cells to apoptosis induction (Kroemer et al. 2007). This latter point will be discussed in more detail in the following section.

3 Therapeutic Interventions for the Restoration of Mitochondrial Apoptosis in Cancer Cells

The inhibition of cell death is one of the hallmarks of cancer. Apoptosis is inhibited in carcinomas, sarcomas, melanomas and hematopoietic malignancies, either upstream or at the mitochondria. One of the most prominent examples of apoptosis inhibition acting at the mitochondrial level is the overexpression of anti-apoptotic proteins of the Bcl-2 family such as Bcl-2 or its close homologues Bcl-X_L and Mcl-1 (Adams and Cory 2007a,b). Bcl-2, Bcl-X_L and Mcl-1 are multidomain proteins and carry four distinct Bcl-2 homology (BH) domains that are labeled BH1–BH4. The branch of pro-apoptotic multidomain proteins of the Bcl-2 family comprises Bax and Bak, which both possess BH1, BH2 and BH3 domains, yet lack a BH4 domain. Finally, a vast group of at least a dozen different proteins makes up the so-called BH3-only branch of the Bcl-2 family. Together, these proteins can regulate MMP induction in many instances, and cancer cells can be resistant to MMP stimulation due to the overexpression of anti-apoptotic Bcl-2 proteins or the absence of pro-apoptotic Bcl-2 family proteins (Deng et al. 2007). As a result, one of the most specific therapeutic interventions that can be created, on theoretical grounds, is a specific ligand that inhibits anti-apoptotic or activates pro-apoptotic proteins of the Bcl-2 family. For example, ABT737 has been designed as a specific ligand that inactivates Bcl-2 (and Bcl-XL) (Oltersdorf et al. 2005), and ABT737 derivatives

with improved pharmacokinetic properties are currently under clinical evaluation.

Another prominent collection of proteins that mediate MMP (or at least impinge on the probability of MMP induction) are the proteins contained in the so-called permeability transition pore complex (PTPC) (Zamzami and Kroemer 2001). Although the exact composition of the PTPC is still a matter of debate, it appears that this complex involves interactions between hexokinase (HK, an enzyme that catalyzes the initial step of glycolysis in the cytosol), the voltage-dependent anion channel (VDAC, a largely nonspecific pore in the outer mitochondrial membrane), the mitochondrial benzodiazepine receptor (in the outer membrane), the adenine-nucleotide translocator (ANT, the electrogenic antiporter of ATP and ADP on the inner mitochondrial membrane) and cyclophilin D (a prolyl *cis-trans* isomerase located in the mitochondrial matrix). Accordingly, inhibitors of the HK-VDAC interaction (Pedersen 2007), pharmacological components acting on VDAC (Yagoda et al. 2007), ligands of the mitochondrial benzodiazepine receptor (Decaudin et al. 2002), or knockdown of the ANT2 isoenzyme (Le Bras et al. 2006) may have apoptosis-inducing, antineoplastic effects.

A vast collection of pharmacological agents may exert direct MMP-inducing effects on isolated mitochondria, and the exact mode of action of these agents is often incompletely characterized (Costantini et al. 2000; Galluzzi et al. 2006). Prominent MMP inducers include lipophilic cations that enrich in cancer mitochondria (which are often hyperpolarized) and that trigger MMP, presumably through yet-to-be defined interactions with mitochondrial inner membrane proteins and/or lipids. Several among these agents are in preclinical development. On theoretical grounds, such direct MMP inducers may circumvent the apoptosis resistance that characterized transformed cells. We have published several reviews (Costantini et al. 2000; Galluzzi et al. 2006) on this important topic, enumerating the distinct compounds that can trigger MMP in a direct fashion not requiring the cell to generate MMP-inducing signal transducers. This strategy of cell death induction has the obvious advantage of readily bypassing mechanisms of apoptosis resistance that reside in the generation of MMP inducers (such as defects in the p53-dependent pro-apoptotic signal transduction pathway) or that affect the composition of mitochondrial membranes themselves.

4 Reduced Oxidative Phosphorylation and Carcinogenesis

Otto Warburg made the seminal observation that, under aerobic condition, cancer cells paradoxically shift their metabolism from an oxidative metabolism (through the mitochondrial respiratory chain) to a highly glycolytic metabolism, producing large amounts of lactate (Warburg et al. 1924, 1926). As a result, respiration would become secondary and the mitochondria status in tumor tissues would be somewhat irrelevant for tumor development. Recently, however, mutations in three of the four genes encoding respiratory chain complex II (succinate dehydrogenase, SDH) have been shown to cause paragangliomas (PGLs) or pheochromocytomas. PGLs are neuroendocrine tumors that may secrete catecholamines (Favier et al. 2005), which occur most frequently in the head, neck, adrenal medulla and extra-adrenal sympathetic ganglia. The hereditary form of PGLs, about 30% of cases, is usually characterized by an early onset and a more severe presentation than the sporadic form. In 2000, linkage analysis and positional cloning allowed Baysal et al. (2000) to report the first deleterious mutations in the SDHD gene. Subsequently, a candidate gene approach has led to the identification of mutations in SDHC and SDHB (Astuti et al. 2001; Niemann and Muller 2000). This fueled a strong debate on the mechanism linking SDH deficiency to tumor formation, initial observations suggesting that superoxide overproduction might be at the origin of increased cell proliferation (Rustin 2002). Indeed, the mev1 mutant of the worm *Caenorhabditis elegans*, defective in the cytochrome *b* subunit of CII, was found to have a reduced life span ascribed to overproduction of superoxides (Senoo-Matsuda et al. 2001). However, no hyperplasia or indications of abnormal cell proliferation were reported in the *mev1* mutant at variance with other *C. elegans* mutants for proteins which are prone to trigger tumorigenesis when mutated in the homologous protein in mammals, e.g., *cul1* mutant (Piva et al. 2002).

Soon after the discovery that SDH mutations can result in tumor formation, it was shown that these tumors were highly vascularized concomitantly with HIF stabilization and activation of the hypoxia pathway (Gimenez-Roqueplo et al. 2001). As a rule, under normoxic conditions, the HIF-α subunit is continuously ubiquitinated and subsequently de-

graded by the proteasome (Hickey and Simon 2006). The process of ubiquitination is started by their recognition by the von Hippel–Lindau (VHL) protein, which requires the hydroxylation of two proline residues on HIF-α (Kaelin 2005). The very first step of HIF-α degradation is dependent on this hydroxylation, which is catalyzed by HIF prolyl hydroxylases (PHDs). PHDs belong to the superfamily of the Fe(II)-dependant oxygenases and require reduced iron as a cofactor, α-KG and oxygen as co-substrates, with carbon dioxide and succinate being the products of the reaction (Lee et al. 2004). Under hypoxic conditions, the absence of oxygen prohibits PHD activity, and HIF-α is thus stabilized, allowing for its nuclear translocation and the subsequent activation of the target genes. The involvement of HIFs has been observed in numerous types of tumors, playing an active role in the progression of neoplasia (Gordan and Simon 2007). To make a long story short, it was established that a high intracellular succinate concentration, as measured in SDH-deficient cells and tumors, was responsible for the blockade of PHD activity with the consequent stabilization of the HIF1α protein (Briere et al. 2005a; Selak et al. 2005) (Fig. 2). Conversely, the addition of α-ketoglutarate, the substrate of the PHD, was shown to abolish the nuclear translocation of HIF1α in SDH-defective cells (Briere et al. 2005a). The abnormal organic acid balance thus provided a proficient mechanism linking SDH-deficiency to tumor formation.

Additional support in favor of this latter hypothesis came from the observation that a fumarase defect can lead to leiomyomatosis and renal cell cancer (HLRCC) syndrome (Tomlinson et al. 2002). In this latter case, fumarate, accumulated because of fumarase inactivation, was found to act as a competitive inhibitor of the PHD, thus inducing the abnormal stabilization of HIF-1α. Other structurally related organic acids can also inhibit PHD (MacKenzie et al. 2007). Therefore, a TCA cycle blockade may result in the induction of angiogenesis and tuning up glycolysis during tumorigenesis may be at the origin of the Warburg effect, rather than a blockade of the electron flow (potentially associated with all subtypes of RC defects) (Briere et al. 2005b). It would therefore be at least rather imprudent to invoke SDH mutations as general proof that a RC defect results in tumor formation.

To date, there is no strong evidence that a perturbation of the electron flow through the RC is sufficient to increase cell proliferation and

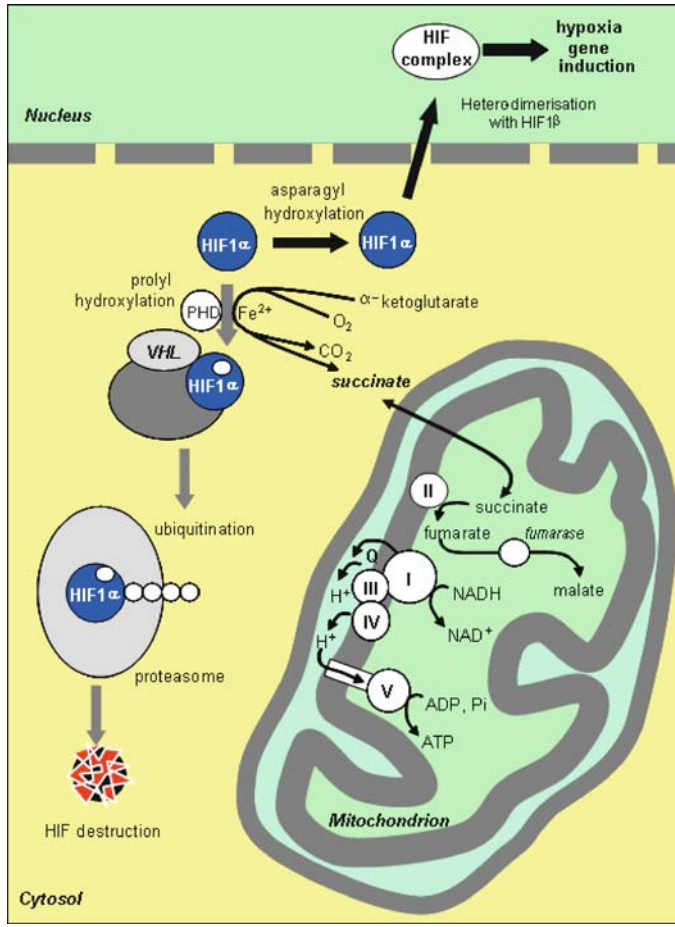

Fig. 2. Induction of the hypoxia pathway by succinate. Upon succinate dehydrogenase (complex II, *II*) blockade, succinate accumulates and is exported from mitochondria to the cytosol. There, it inhibits the prolyl hydroxylase (*PHD*), thus triggering Hif1α stabilization. The nuclear translocation of this latter factor induces an increased transcription of the hypoxia pathway components. *VHL*, Von Hippel–Lindau; *I–V,* the various respiratory chain complexes

constitutes the primary cause of tumor formation: on one hand, none of the nuclear genes encoding RC components or involved the building or maintenance of the chain has been demonstrated to be a tumor suppressor, with the exception of genes encoding RC complex II (or fumarase; see above) (Kroemer 2006). On the other hand, RC-specific poisons are not known as carcinogenic. Finally, patients harboring deleterious mutation in genes encoding RC components are not known to be particularly at risk for tumor formations. This is even true for mutations affecting RC proteins that result in high levels of superoxide production, such as ATPase (complex V) components (Geromel et al. 2001). Even more puzzling is the fact that in a subset of tumor cells (liver and pancreatic tumors), enhanced glycolysis might well require an active production of ATP by the RC. Using both ATP and glucose to produce ADP and glucose-6-phosphate, the hexokinase specifically expressed in most tumor cells is characterized by its poor affinity (high Km, about 10 mM) for glucose, similar to the hexokinase IV (glucokinase) found in normal hepatocytes (Brandon et al. 2006). It possibly allows for an additional capacity for glucose uptake from plasma and increased glucose phosphorylation, by displacement of the cell glucose equilibrium. Interestingly, while the isoform with a low affinity is expressed in most non-tumor cells, in the malignant hepatocellular carcinoma cells and in transformed pancreatic cells, it is largely replaced by a high affinity form (hexokinase II, HKII). This latter form can readily bind VDAC at the outer mitochondrial membrane and utilize the mitochondrial ATP to produce glucose-6-phosphate, thus favoring aerobic glycolysis (Bustamante and Pedersen 1977) as long as the electron transfer chain is working (Fig. 3). In this case, carcinogenesis would in fact require the preservation of the respiratory chain function, at least at a minimal level.

The possibility nevertheless exists that low oxygenation in tumors may secondarily affect mitochondrial function, thus favoring the formation of superoxides and peroxides by the RC. In principle, activated oxygen species might in turn signal both oncogene growth factors and their tyrosine kinase receptors, thus driving cell transformation (Aslan and Ozben 2003), simultaneously promoting HIF1α stabilization by inhibiting the prolyl hydroxylase. The suggested role of superoxides in triggering tumorigenesis has long been advocated in support to a therapeutic use of antioxidants (Nishikawa and Hashida 2006). However,

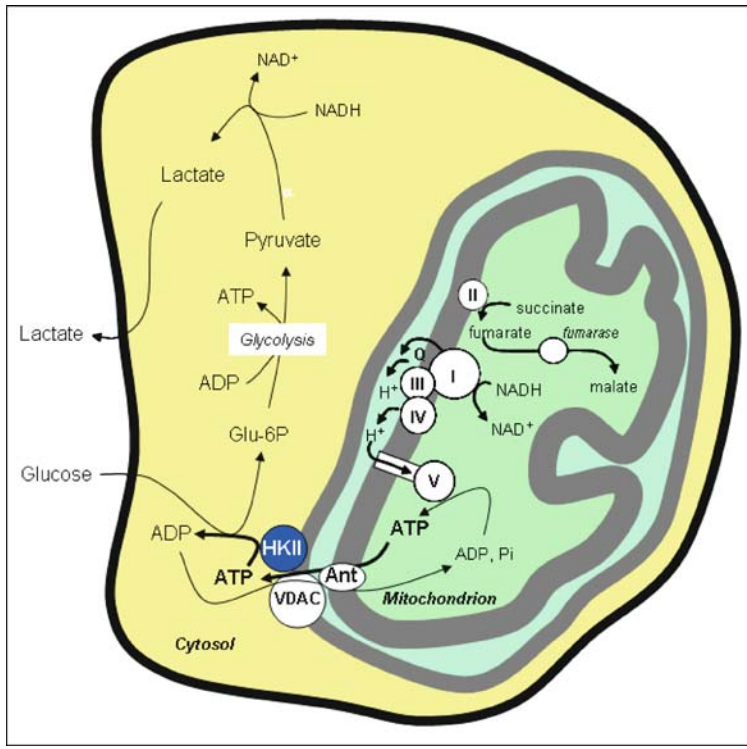

Fig. 3. Interaction between mitochondria and type II hexokinase. The channeling of mitochondrial ATP by type II hexokinase favors the production of glucose 6-phosphate (*G 6-P*) and ultimately glycolysis. *Ant,* adenylate carrier; *HKII,* type II hexokinase; *I–V,* the various complexes of the respiratory chain; *Q,* ubiquinone; *VDAC,* voltage-dependent anion channel

contrasting results from in vitro and in vivo experiments have raised some doubt about the ability of antioxidant enzymes (Lu et al. 1997; Welsh et al. 2002) or molecules to actually fight cancer by such a mechanism. Indeed, one should be aware that most antioxidant molecules also act as prooxidants, possibly accounting for a potential antitumoral activity. For instance, an antioxidant molecule such as melatonin exercises its antiproliferative effect on the growth of rat pituitary prolactin-

secreting tumor cells in vitro by damaging mitochondria rather than by quenching superoxides (Yang et al. 2007). Thus, even if increased superoxide production can be evidenced in a subset of cancer cells, we need more evidence to establish that, as a general rule, these superoxides are instrumental in triggering tumorigenesis.

While it is not clear that a defective respiratory chain actually favors tumor formation, mitochondria might instead represent the Achilles tendon of cancer cells. As discussed above, mitochondria house several proapoptotic factors that are simultaneously components of (or closely associated with) the electron transfer chain. Targeting tumors with reagents susceptible to inducing the release of these components has become a fashionable idea (Galluzzi et al. 2006). Thus, cisplatin, one of the most important chemotherapeutic agents ever developed, has been shown to readily interact with mitochondria to trigger apoptosis (Cepeda et al. 2007). Resveratrol, a natural polyphenolic antioxidant, has been reported to possess a cancer chemopreventive potential that has been ascribed to its ability to trigger mitochondrial dysfunction and apoptosis (Fulda and Debatin 2006), mention yet another example.

5 Hypothetical Links Between Apoptosis Resistance and Anaerobic Glycolysis at the Mitochondrial Membrane

As summarized above, mitochondria from cancer cells are relatively resistant against MMP induction, thereby reducing the propensity to undergo apoptosis. In addition, tumor mitochondria are, to some extent, perturbed in their metabolism, often exhibiting reduced OXPHOS. Are these two phenomena mechanistically linked? Unfortunately, there is no simple answer to this question, because there may be multiple links, none of which is firmly established to contribute to oncogenesis or tumor progression.

A first explanation for simultaneous apoptosis inhibition and OXPHOS defects of cancer cells may reside in the composition of mitochondrial membranes. For example, Bcl-2 and Bcl-XL are prominent MMP inhibitors, yet also have direct effects on ATP synthesis in which they act as allosteric activators of ANT (Belzacq et al. 2003). Report-

edly, Bcl-2 and Bcl-XL can also inhibit the capacity of VDAC to exchange metabolites on the outer mitochondrial membrane, an effect that would reduce respiration (Tsujimoto and Shimizu 2007). A functional and structural Bcl-2 homolog, vMIA (for viral mitochondrial inhibitor of apoptosis), which is encoded by cytomegalovirus, acts as a strong inhibitor of apoptosis (via its capacity to inhibit Bax), yet is also an inhibitor of the phosphate carrier, one of the proteins of the ATP synthasome (Poncet et al. 2006). This implies that vMIA reduces ATP generation by OXPHOS, an effect that accounts for the cytopathic effect of cytomegalovirus. No such inhibitory effect was, however, found for Bcl-2 (P. Rustin and G. Kroemer, unpublished data).

Unfortunately, there are no systematic studies on the composition of the outer mitochondrial membrane of cancer cells. However, differences in the composition of the PTPC have been reported, and whether alterations in the abundance of VDAC or ANT isoforms account for dual apoptosis/OXPHOS defects of tumor cells remains to be investigated in detail. One PTPC component, hexokinase, has been shown to associate more vigorously with VDAC in tumor cells than in normal control cells (Pedersen 2007). When associated with VDAC, hexokinase may efficiently couple residual OXPHOS to the initial, rate-limiting step of glycolysis, and simultaneous inhibit MMP, presumably through an effect on the PTPC.

Other links between OXPHOS defects and inhibited apoptosis maybe more indirect. A hyperpolarization of the inner mitochondrial transmembrane potential, as is frequently seen in cancer cells (perhaps secondary to defects in the F1F0 ATPase), can intrinsically reduce the propensity of PTPC opening (Zoratti and Szabo 1995). Total inhibition of the respiratory chain inhibits the activation of the pro-apoptotic Bcl-2 proteins Bax and Bak (Tomiyama et al. 2006). In addition, OXPHOS defects (and in particular mtDNA mutations) might increase the production of ROS and hence activate, via HIF, a transcriptional program that reduces the propensity of the cells to succumb to stress-induced MMP. Major defects in respiratory chain complexes reduce electron flow on the inner mitochondrial membrane and reduce the capacity of certain xenobiotics to elicit ROS generation in mitochondria, thereby abolishing their pro-apoptotic effects. This latter mechanism may explain the fact that ρ° cells (cells that lack mitochondrial DNA and hence OX-