Published in final edited form as:
*Chin J Cancer*. 2011 August ; 30(8): 526–539. doi:10.5732/cjc.011.10018.

# Mitochondria in cancer: at the crossroads of life and death

**Vanessa C. Fogg**, **Nathan J. Lanning**, and **Jeffrey P. MacKeigan**
Laboratory of Systems Biology, Van Andel Research Institute, Grand Rapids, MI 49503, USA.

## Abstract

Mitochondrial processes play an important role in tumor initiation and progression. In this review, we focus on three critical processes by which mitochondrial function may contribute to cancer: through alterations in glucose metabolism, the production of reactive oxygen species (ROS) and compromise of intrinsic apoptotic function. Alterations in cancer glucose metabolism include the Warburg effect, leading to a shift in metabolism away from aerobic respiration toward glycolysis, even when sufficient oxygen is present to support respiration. Such alterations in cellular metabolism may favor tumor cell growth by increasing the availability of biosynthetic intermediates needed for cellular growth and proliferation. Mutations in specific metabolic enzymes, namely succinate dehydrogenase, fumarate hydratase and the isocitrate dehydrogenases, have been linked to human cancer. Mitochondrial ROS may contribute to cancer via DNA damage and the activation of aberrant signaling pathways. ROS-dependent stabilization of the transcription factor hypoxia-inducible factor (HIF) may be a particularly important event for tumorigenesis. Compromised function of intrinsic apoptosis removes an important cellular safeguard against cancer and has been implicated in tumorigenesis, tumor metastasis, and chemoresistance. Each of the major mitochondrial processes is linked. In this review, we outline the connections between them and address ways these mitochondrial pathways may be targeted for cancer therapy.

### Keywords

Mitochondria; cancer; metabolism; apoptosis; reactive oxygen species

Mitochondria are often described as the “powerhouse” of the cell. While their role in ATP production is critical, mitochondria house numerous other biochemical reactions and lie at the intersection of multiple physiological processes including catabolic and anabolic metabolism, signaling, generation of reactive oxygen species (ROS) and apoptosis. Numerous studies indicate these mitochondrial processes may play an important role in tumor initiation and progression. In this review, we focus on three major processes by which mitochondrial function may contribute to cancer: through alterations in glucose metabolism, the production of ROS and through the compromise of apoptosis.

## Key Aspects of Mitochondrial Biology

Mitochondria are believed to have evolved from an ancient endosymbiotic bacterium which was engulfed by a eukaryotic ancestor more than a billion years ago [1]. Reflecting this origin, mitochondria have a double-membrane structure and possess their own independent genome, as well as an independent transcription and translation machinery. Over the course of evolution, an extensive transfer of mitochondrial genes has apparently occurred into nuclear DNA. In humans, the mitochondrial genome consists of a circular double-stranded

**Corresponding Author:** Jeffrey P. MacKeigan, Laboratory of Systems Biology, Van Andel Research Institute, 333 Bostwick Ave NE, Grand Rapids, MI 49503, USA. Tel: +1-616-234-5682; Fax: +616-234-5733; jeff.mackeigan@vai.org.

DNA molecule of 16 569 base pairs encoding only 37 genes: 2 rRNAs, 22 tRNAs, and 13 genes encoding subunits of the oxidative phosphorylation machinery [2]. All other mitochondrially localized proteins are encoded by nuclear DNA and imported into the mitochondria. Recently, Pagliarini *et al.* [3] undertook a systematic identification of mitochondrially localized proteins using a combination of mass spectrometry, GFP tagging, and computational methods to create a protein compendium consisting of 1098 mitochondrial mammalian proteins. Interestingly, nearly 300 of these identified gene products were of unknown biological function.

The number of mitochondria per cell varies with the cell type and under different physiological conditions, can range up to thousands per cell [4]. Mitochondria also show a very dynamic morphology. In the cell they can form an interconnected reticulum which is dynamically remodeled by frequent fission and fusion events. Mouse knockout models of the key mitochondrial fusion genes Mfn1, Mfn2 or OPA1, and knockout of the fission gene Drp1, results in embryonic lethality [5–8]. Nevertheless, the exact functional significance of mitochondrial fusion and fission remains unclear. In addition to undergoing dynamic fusion and fission, mitochondria are motile and actively recruited to specific subcellular sites, such as the axonal and dendritic processes of neurons. The trafficking of mitochondria into these neuronal processes is thought to be critical in providing energy for neuronal function [9]. Fusion and fission events appear important in regulating mitochondrial motility and cellular distribution, as well as in the maintenance of mitochondrial bioenergetics and function [10–12]. Interestingly, defects in mitochondrial dynamics have been linked to a number of human neurodegenerative diseases, including Parkinson's, Alzheimer's, and Huntington's [9]. This link between neurodegenerative disease and mitochondrial dynamics likely reflects the high energy demand of neurons and their dependence on proper mitochondrial trafficking into neural processes.

The double-membrane structure of mitochondria creates two separate compartments: an internal matrix surrounded by the inner mitochondrial membrane and a narrower intermembrane space surrounded by the outer mitochondrial membrane. The mitochondrial matrix and inner membrane are the sites of numerous metabolic enzymes, including those involved in the citric acid cycle and oxidative phosphorylation (OXPHOS). While mitochondria are perhaps best known for their role in ATP production via OXPHOS, it is recognized they play critical roles in a diverse range of physiological processes, including catabolic and anabolic metabolism, the maintenance of calcium homeostasis, the generation of ROS, cell signaling and apoptosis. Key processes detailed in this review are shown schematically in Figure 1.

## Cancer's Sweet Tooth: Alterations in Glucose Metabolism

### Overview of normal glucose metabolism

One of the most vital mitochondrial functions is energy production in the form of ATP. In normal differentiated cells, the bulk of ATP is produced in the mitochondria through the process of OXPHOS. Although various fuel substrates can be metabolized to produce ATP, glucose is the major fuel substrate for most cells and the focus of this review. Glucose is taken up by glucose transporters on the cell surface and metabolized to pyruvate through the cytosolic reactions of glycolysis. Pyruvate is then transported into the mitochondrial matrix, where it is converted to acetyl-CoA and oxidized via the citric acid/tricarboxylic (TCA) cycle to $CO_2$ and high-energy electrons in the form of the carriers NADH and FADH2. These high-energy electrons are passed along the electron transport chain, a set of specialized protein complexes in the inner mitochondrial membrane. As electrons are passed along the electron transport chain, energy is released, driving the formation of an electrochemical proton gradient across the inner mitochondrial membrane. The potential

energy of this gradient is used to drive generation of ATP by ATP synthase. Molecular oxygen ($O_2$) is necessary for this process, since it acts as the terminal electron acceptor and is reduced to water. The coupling of the electron transport chain redox reactions with electrochemical gradient-driven ATP production is termed OXPHOS. Small amounts of ATP are generated by substrate-level phosphorylation during the reactions of glycolysis and the TCA cycle; however, OXPHOS provides the majority of ATP derived from glucose metabolism in normal cells (Figures 1 and 2).

## Altered glucose metabolism in cancer cells: the Warburg effect

Many cancer cells metabolize glucose differently from normal cells, with an increase in the ratio of ATP produced by glycolysis versus OXPHOS [13]. Glucose uptake is greatly increased in cancer cells and the metabolic shift to glycolysis results in an increased percentage of pyruvate being diverted away from the TCA cycle and being converted to lactic acid, which is excreted as waste (Figure 2). Most cancer cells exhibit this shift toward glycolysis even when sufficient oxygen is present to support OXPHOS. Thus, this pattern of metabolism has been termed “aerobic glycolysis” and is commonly known as the “Warburg effect.” Warburg *et al.* [13] surmised that altered glucose metabolism plays an important role in carcinogenesis. This work was not fully appreciated at the time and research on the topic went virtually dormant until a resurgence of interest in cancer cell metabolism within the last decade. Although the causes and functional consequences of the Warburg effect remain debated, there is a growing consensus that the Warburg effect is not an inconsequential byproduct of carcinogenesis, but is vital for cancer cells to maintain their proliferative potential.

## Consequences of the Warburg effect

At first glance, the increased dependence of cancer cells on glycolysis for energy production appears energetically wasteful and would appear to represent a disadvantage for cell growth. Glycolysis yields only two moles of ATP per mole of glucose, as compared with approximately 36 moles of ATP per mole of glucose yielded by aerobic respiration [4,14]. Why would such a less efficient energy extraction process be selected for in cancer cells? One part of the answer may lie with important metabolic needs extending beyond ATP production. Rapidly proliferating cells require the synthesis of large amounts of biological molecules to generate new cells. Completely oxidizing glucose to $CO_2$ and $H_2O$ through the TCA cycle and electron transport chain disallows the use of glucose’s carbon skeleton for new biological molecule synthesis. In cancer cells, glucose metabolites are diverted away from complete oxidation and into biosynthetic pathways to produce lipids, amino acids, and nucleotides required for proliferating cells [15] (Figure 2). Additionally, these alternative biosynthetic pathways generate nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), which is a critical regulator of cellular redox potential (see discussion on ROS below). The Warburg effect may also contribute to cancer cell survival and progression in other ways. Although the Warburg effect appears to occur in the absence, or before the onset, of cellular hypoxia, it is possible the shift to aerobic glycolysis provides “pre-emptive ” protection against subsequent fluctuation in oxygen levels and cellular hypoxia, both of which are frequently observed in solid tumors[16]. Moreover, increased lactate production that occurs as a result of the Warburg effect creates an acidic environment which is toxic to normal cells and favors tumor cell invasion [17]. Finally, there is evidence the shift to glycolysis provides cancer cells with an acquired resistance to apoptosis. Although poorly understood, observation suggests glycolytic shift makes tumor cells less susceptible to mitochondrial outer membrane permeabilization (MOMP), a critical step in the intrinsic apoptosis pathway (see section on apoptosis below)[17,18].

Regardless of the mechanisms, studies have shown that alterations of glucose metabolism favor tumor cell growth. The inhibition of glycolysis and a forced shift to OXPHOS in cancer cells results in reduced cell proliferation and tumor growth [18,19]. The inhibition of biosynthetic pathways linked to glycolysis, such as ATP citrate lyase, a key enzyme in lipid synthesis, also results in reduced tumor growth, supporting the idea that proliferative consequences of the Warburg effect are connected to altered biosynthetic pathways[20].

## Regulation of metabolism by classic oncogenes and tumor suppressors

Warburg originally proposed that defects in mitochondrial OXPHOS machinery are central to enhanced glycolysis in cancer cells, forcing the cells to rely on a glycolytic metabolism even in the presence of oxygen. Defects in OXPHOS have been reported in some cancer cells and OXPHOS altering mutations in mitochondrial DNA have been implicated in tumorigenesis [21]. However, it has become clear that in many cancer cells the OXPHOS machinery remains intact [22]. Thus, mutations or alterations affecting other mechanisms must underlie the Warburg effect in many cells.

Interestingly, many well-known oncogenes and tumor suppressors are known to regulate cell metabolism. Myc, Ras, Akt, phosphoinositide 3-kinase (PI3K) and hypoxia-inducible factor (HIF) have all been implicated in enhanced glycolytic activity [23–25]. Myc transcription factor directly activates transcription of numerous glycolytic enzymes. PI3K signaling through Akt and mTOR also leads to an increased expression of glucose transporters and glycolytic enzymes. Oncogenic mutations in the PI3K signaling pathway converge upon activation of HIF, the “master sensor” of oxygen levels and a major mediator of the cellular hypoxia response. HIF transcription factors consist of two subunits: HIFα and HIFβ. While HIFβ is stable under conditions of normal oxygen tension, HIFα is hydroxylated by prolyl hydroxylases under normal oxygen levels, which targets HIFα for proteasome-mediated degradation. Under hypoxic conditions, however, HIFα is stabilized, allowing it to dimerize with HIFβ and regulate expression of the large number of genes involved in the hypoxic response. Target genes include those mediating angiogenesis, metastasis, and a metabolic shift toward glycolysis. For example, HIF activity results in an increased expression of glucose transporters and glycolytic enzymes, and the inhibition of metabolic pathways leading toward OXPHOS[26,27]. Thus, the aberrant activation of HIF under conditions of normal oxygen tension may be involved in instances of the Warburg effect [27,28]. The aberrant activation of HIF is tumorigenic, as occuring in the hereditary von Hippel-Landau (VHL) cancer syndrome[26,29].

Loss of tumor suppressors may also contribute to the Warburg effect. The loss of phosphatase and tensin homolog (PTEN) leads to enhanced PI3K signaling, which in turn enhances glycolysis [25]. Activity of p53 suppresses glycolysis through induction of the phosphofructokinase isoform TIGAR, and also directly stimulates mitochondrial respiration through activation of the SCO2 gene which is required for assembly of the cytochrome c oxidase (COX) complex (complex IV of the electron transport chain). Thus, the loss of p53 results in decreased mitochondrial respiration and increased glycolysis[25].

Myc and p53 also control factors regulating metabolism of the amino acid glutamine[30–32]. Like glucose, the import and metabolism of glutamine is dramatically upregulated in cancer cells [30]. Glutamine metabolism in cancer cells replenishes TCA cycle intermediates, contributes to biomolecule synthesis and ATP production, and appears to be a critical factor for oncogenic transformation[33,34].

## Metabolic TCA cycle enzymes as a new class of tumor suppressors

An exciting development has been the discovery that mutation in metabolic enzymes may be carcinogenic. Mutation in several TCA cycle enzymes have been linked to human cancer: succinate dehydrogenase (*SDH*), fumarate hydratase (*FH*), and the mitochondrial isocitrate dehydrogenase 2 (*IDH2*), as well as its cytosolic counterpart isocitrate dehydrogenase 1 (*IDH1*)[35]. SDH and FH catalyze successive reactions in the TCA cycle; SDH catalyzes the conversion of succinate to fumarate, whereas FH catalyzes the conversion of fumarate to malate. Loss-of-function mutation in these genes results in the build-up of their substrates, succinate and fumarate, respectively. SDH also functions as complex II in the electron transport chain, and loss-of-function mutations in SDH may also affect respiratory function. Current evidence suggests that SDH and FH function as tumor suppressors. Mutations in genes encoding the SDH subunits have also been linked to hereditary paranganglioma and pheochromocytomas[36–38], whereas mutation in FH have been linked to uterine and skin leiomyomas and papillary renal cancer[39]. The behavior of cancer-linked mutations in the IDH genes appears complex. IDH1 and IDH2 both function to convert isocitrate to α-ketoglutarate, an important TCA intermediate. Heterozygous point mutations affecting one of several residues in IDH1 (or the corresponding residues in IDH2) are prevalent in gliomas and acute myeloid leukemia (*AML*)[40]. Remarkably, these mutations confer a neomorphic activity upon the IDH enzymes, converting them to proteins that reduce α-ketoglutarate to a new metabolite, D-2-hydroxyglutarate (2-HG) [41]. Implications of this new activity are not fully understood and may affect multiple cellular processes, including epigenetic programming through the inhibition of the TET2 DNA methylase [42]. A common tumorigenic mechanism underlying mutations in these three metabolic enzymes appears to be the aberrant buildup of metabolites with oncogenic potential. The metabolic products of mutant SDH, FH, and IDH, namely succinate, fumarate, and 2HG, are believed to inhibit activity of a class of α-ketoglutarate-dependent enzymes, which may lead to wide-ranging effects, including alterations in DNA methylation (as demonstrated by Figueroa *et al*.[42] for IDH). Interestingly, prolyl hydroxylase enzymes, which normally target HIF for degradation, are among the α-ketoglutarte-dependent enzymes which may be inhibited by mutant SDH, FH, and IDH. Mutant SDH, FH, and IDH1 have all been shown to induce pseudohypoxia and HIF activity [35]. Thus, oncogenic alterations in metabolism appear to converge upon HIF, although other pathways are most likely also involved. Regardless of the exact mechanism, these discoveries demonstrate that altered mitochondrial metabolism can be a key aspect of carcinogenesis.

## Targeting metabolic pathways for cancer treatment

The altered metabolism of cancer cells suggests new therapeutic strategies. One major s trategy is to inhibit glycolysis in cancer cells and to promote OXPHOS, forcing the cell into a more “normal” metabolism which would presumably disadvantage cancer cell survival and growth. To this end, various agents and strategies have been explored to regulate key metabolic control points (Figure 2). The direct inhibition of glycolytic enzymes is one such approach, and may be useful in combination with conventional chemotherapy. Drugs targeting hexokinase, the first enzyme in the glycolytic pathway, have shown promise. 2-deoxyglycose (2-DG) and lonidamine, have entered clinical trials for a variety of solid tumors. In addition, Cap-232/TLN-232, an agent which targets the last step of glycolysis by inhibiting pyruvate kinase, has entered clinical trials [43,44]. Other agents target metabolic steps downstream of glycolysis which control the fate of pyruvate. Dichloroacetate (DCA) indirectly stimulates the activity of pyruvate dehydrogenase, thus stimulating the entry of pyruvate into the TCA cycle. DCA does this by inhibiting pyruvate dehydrogenase kinase (PDK), an enzyme which suppresses pyruvate dehydrogenase. In preclinical studies, DCA has shown remarkable anti-tumor activity, and is currently being tested in phase-I and -II clinical trials against metastatic solid tumors, gliomas, and glioblastoma

multiforme [18,43,44]. Inhibition of the enzyme lactate dehydrogenase (LDH) may also be a promising approach. LDH catalyzes the conversion of pyruvate to lactate. The inhibition of this reaction would thus help drive the entry of pyruvate into the TCA cycle and OXPHOS. Downregulation of LDH-A activity by shRNA has been shown to stimulate mitochondrial respiration and significantly reduce tumor cell growth *in vitro* and in a xenograft animal model [19,45]. A major caveat with glycolysis-targeting agents is that cancer cells exhibit changes to multiple metabolic pathways, and may demonstrate remarkable metabolic flexibility; they may be able to switch to alternative fuel sources and pathways if glycolysis is inhibited. Other metabolic alterations in cancer cells, such as changes in amino acid metabolism, may also provide targets for cancer therapy. In the end, combinatorial targeting of multiple metabolic pathways may be required to prevent resistance.

## Contributions of Mitochondrial ROS to Cancer

### Oxidative phosphorylation and the generation of ROS

As discussed above, the final and most complete steps of catabolic fuel metabolism in eukaryotic cells occurs through the process of oxidative phosphorylation. As a byproduct of OXPHOS, ROS are generated. These occur through the incomplete reduction of oxygen, as electrons pass through the electron transport chain. The ROS superoxide anion ($O_2^-$) is directly produced by such a "leaky" transfer of a single electron to molecular oxygen during OXPHOS (Figure 3). It has been estimated that under physiological conditions, 1% to 2% of the molecular oxygen consumed by mitochondria is converted to ROS molecules [47].

### Properties of mitochondrial ROS

As indicated by their name, ROS are highly reactive molecules and behave as oxidants which can extract electrons from DNA, proteins, lipids, and other molecules. Although ROS can be generated through non-mitochondrial mechanisms (notably by the plasma membrane NAPDH oxidase), mitochondria are the main intracellular source of ROS in most tissues. $O_2^-$ generated as a byproduct of OXPHOS is the precursor to other major forms of ROS, including hydrogen peroxide ($H_2O_2$) and hydroxyl radical (OH–) (Figure 3). $O_2^-$ displays a high reactivity toward iron-sulfur (Fe-S) clusters. Due to its negative charge, $O_2^-$ does not diffuse freely across membranes. However, there is evidence that mitochondrial $O_2^-$ may enter the cytosol through specialized mitochondrial channels, such as the voltage-gated anion channel (VDAC) and other as yet unidentified channels[48]. *In vivo* it is quickly converted by mitochondrial or cytosolic superoxide dismutases to $H_2O_2$, a ROS molecule which diffuses freely across membranes. $H_2O_2$ displays high reactivity to select cysteine residues on target proteins, depending upon the cysteine environment. $H_2O_2$ toxicity arises chiefly when it interacts with $O_2^-$ in a metal-catalyzed reaction (metal-catalyzed Haber-Weiss, or Fenton, reaction) to form the highly reactive and dangerous ROS, hydroxyl radical (OH–). OH– reacts indiscriminately with any and all surrounding macromolecules, including proteins, nucleic acids, carbohydrates and lipids. Due to its extremely high reactivity, OH– has a short half-life and its diffusion is limited to its sites of production[49].

Uncontrolled ROS activity can result in oxidative damage to proteins, lipids, nucleic acids, and other biological molecules. Such damage may ultimately result in the inactivation of proteins, injury to the integrity of biological membranes and genotoxicity. Sufficiently high levels of ROS induce cell death by apoptotic and/or necrotic mechanisms. However, studies show that low levels of ROS can also act as signaling molecules in the cell. There is evidence that both ROS-mediated genotoxicity and ROS-mediated signaling may contribute to tumor initiation and progression.

## ROS and genotoxicity

As mentioned above, ROS can damage nucleic acids, resulting in mutations and genomic instability, thus setting the stage for tumorigenesis. Although nuclear DNA is susceptible to ROS-mediated damage, mitochondrial DNA presents an even more vulnerable target. Mitochondrial DNA lies in close proximity to the electron transport chain, the source of mitochondrial ROS. Mitochondrial DNA also lacks protective histones and has a limited DNA repair capacity. This sensitivity to ROS-mediated damage may contribute to the high mutation rate of mitochondrial DNA. It has been estimated that the mutation rate for mitochondrial DNA is at least two orders of magnitude higher than that for nuclear DNA [50]. Mutations in mitochondrial DNA have been observed in a majority of cancers, although the functional significance of most of these mutations is unknown [21,51]. Of note, mutations in mitochondrial DNA which encode components of the OXPHOS machinery have been observed in cancer [21,50,52]. Such mutations have been observed to result in OXPHOS dysfunction, which may in turn promote a metabolic shift toward glycolysis.

As discussed previously, enhanced glycolysis is important for tumor growth. Moreover, mutations which disrupt the OXPHOS machinery, whether in nuclear DNA or mitochondrial DNA, may result in increased ROS production, potentially leading to a vicious cycle of increasing DNA damage and mitochondrial dysfunction, as well as the induction of tumorigenic ROS-mediated signaling pathways. Recently, Ishikawa *et al.*[52] has shown that mitochondrial mutations which compromise respiratory function and increase ROS production are able to induce tumor metastasis.

## ROS and signaling

There has been an increased focus on the role of ROS signaling in tumor formation and progression. It is now recognized that ROS play an important role as signaling molecules which mediate changes in cell proliferation, differentiation, migration, invasiveness and large-scale changes in gene transcription[53,54].

How are ROS able to act as signaling molecules? The answer lies with redox-sensitive proteins that act as exquisite “sensors” of ROS levels. In many cases, these sensors rely upon reversible oxidation of specific cysteine residues. For example, protein tyrosine phosphatases contain a redox-sensitive cysteine in the active site which can be reversibly oxidized [55]. Oxidation of the cysteine sulfhydryl group by $H_2O_2$ results in inactivation of the phosphatase; cellular mechanisms exist to reduce the oxidized cysteine residue and regenerate its original state, restoring activity of the phosphatase. Similar cycles of cysteine oxidation/reduction regulate the activity of many other ROS-sensitive proteins, including kinases and transcription factors [53]. During such cycles of reversible cysteine oxidation, the glutaredoxin and thioredoxin redox control systems play a critical role in the reduction of oxidized cysteine and the maintenance of redox homeostasis [56]. Although $H_2O_2$ is seen as the major ROS signaling molecule, $O_2^-$ has also been implicated in signaling although its mechanisms are not as well understood[49,57].

Tumor cells generally exhibit higher levels of ROS than normal cells [53]. These increased levels of ROS may lead to DNA damage (as discussed above) and/or to direct activation of signaling networks promoting tumorigenesis and metastasis. For instance, ROS have been shown to activate MAP kinase and phosphoinositide 3-kinase pathways important for cell proliferation and survival [53]. ROS have also been shown to both activate and upregulate the expression of proteins involved in epithelial-to-mesenchymal transition and metastasis, including matrix metalloproteinases (MMPs) and Snail [58]. Interestingly, oncogene activation has been shown to induce the production of mitochondrial ROS and there is growing evidence that in at least some cases, mitochondrial ROS are required for oncogene-

mediated cell transformation. In a mouse model of lung cancer, it was shown that mitochondrial ROS are increased by K-Ras and are required for K-ras induced tumorigenesis [59]. Mitochondrial ROS have also been implicated in mediating Myc-induced tumorigenesis [60,61]. In addition to activating MMP-3, mitochondrial ROS have also been shown to act downstream of MMP-3 to mediate MMP-3 induced cell transformation[62]. In summary, a number of studies now place aberrant ROS production at the heart of various tumorigenic pathways.

## ROS and the regulation of glucose metabolism: the Warburg effect strikes again

One signaling pathway which may be particularly important to ROS-mediated tumorigenesis involves the activation of HIF. As discussed previously, HIF transcription factors act as master sensors of oxygen levels and mediators of the cellular hypoxia response. A number of studies have now established that mitochondrial ROS are involved in the stabilization and activation of HIF under hypoxic conditions, likely through the deactivation of prolyl hydroxylase enzymes which normally modify and target HIFα for proteasome-mediated degradation[63–67]. Interestingly, ROS also appear to act downstream of some oncogenes to stabilize HIF under conditions of normal oxygen tension, leading to an aberrant activation of HIF and promotion of tumorigenesis. In xenograft models, ROS-mediated HIF-stabilization appears critical for MYC-mediated tumorigenesis [61]. Increased ROS levels and ROS-dependent stabilization of HIF have also been reported in a Rac1-driven mouse model of Kaposi's sarcoma [68]. The increased production of ROS and ROS-mediated stabilization of HIF may also play a role in cancers caused by mutations in the SdhB subunit of succinate dehydrogenase (SDH) [69]. The role of HIF in ROS-mediated tumorigenesis again underscores the importance of metabolism in cancer growth. In addition to other important processes, HIF mediates the upregulation of glycolytic genes and a global shift in cellular metabolism from OXPHOS to glycolysis.

## Targeting mitochondrial ROS in cancer therapy

The involvement of ROS in tumorigenic pathways suggests the inhibition of ROS production may be a valuable approach to cancer prevention and treatment. Dietary antioxidant treatment has been shown to inhibit the growth of tumors in xenograft animal models [61,68]. However, several large-scale clinical trials have found no effect or inconsistent effects of antioxidant dietary supplementation on human cancer prevention[70], and the use of dietary antioxidant supplementation during cancer treatment is highly controversial[71]. Indeed, there is evidence that dietary antioxidants taken concurrently with conventional cancer treatment may actually do harm by protecting cancer cells from the pro-oxidant effects of chemotherapeutics and/or radiotherapy[71]. Nevertheless, research continues into the use of antioxidants in cancer prevention and therapy. One promising avenue may be the development of antioxidants targeted specifically to mitochondria[72]. Paradoxically, ROS-promoting treatments also represent an approach for cancer therapy. As mentioned previously, high levels of ROS are toxic to cells and induce death by apoptotic and/or necrotic mechanisms. Because tumor cells generally have higher levels of ROS than do normal cells, they are under conditions of increased oxidative stress and are sensitized to death by ROS-promoting agents. Indeed, many commonly used anti-cancer therapies (cisplatin, vinblastine, doxorubicin, ionizing radiation, and others) exert their action through ROS-mediated cell killing[73]. Numerous other therapies designed to promote ROS overproduction are currently under investigation for the selective killing of cancer cells[73–75].

# Mitochondrial Apoptosis and Cancer

## Mitochondria are mediators of both life and death

Mitochondria metabolize fuel to generate energy to sustain life. In response to various triggers mitochondria also unleash an active program of cell death. Although mitochondria have been implicated in various forms of cell death, they are best known for their role in mediating the intrinsic apoptosis pathway, also referred to as mitochondrial apoptosis. This pathway is activated by a variety of cell stress and damage signals, including DNA damage, growth factor deprivation, oncogene activation, oxidative stress and other forms of cell stress. By initiating such a controlled form of cell suicide, an organism ensures that defective cells are rapidly and safely removed before they become a burden or danger (e.g. by passing on damaged DNA to daughter cells). The cellular evasion of apoptosis is a classical hallmark of cancer, and the inhibition of normal apoptosis pathways is almost certainly necessary for tumorigenesis [76]. In addition to the intrinsic apoptosis pathway, apoptosis can be mediated through the external apoptosis pathway, which is triggered by an activation of cell-surface tumor necrosis factor family “death” receptors. Cross-talk can occur between the extrinsic and intrinsic apoptosis pathways. Although intrinsic apoptosis involves mitochondria whereas extrinsic apoptosis does not, both intrinsic and extrinsic apoptosis converge upon the activation of caspases, a family of cysteine-dependent aspartic acid-specific proteases which carry out the final “execution” steps of apoptosis through protease-mediated dismantling of the cell (Figure 4).

## Overview of the mitochondrial intrinsic apoptosis pathway

Numerous pro- and anti-apoptotic signals exist and vie for control within the cell. During the process of intrinsic apoptosis, these “pro-life ” and “pro-death” signals are integrated and converge at the level of the mitochondrial outer membrane[77]. Permeabilization of the mitochondrial outer membrane is the critical step which irreversibly commits a cell to apoptosis in the intrinsic pathway. Such mitochondrial outer membrane permeabilization (MOMP) results in the release of cytochrome c and other apoptogenic proteins (e.g. SMAC/Diablo, AIF) from the inner mitochondrial space into the cytosol. Cytochrome c is a key component of the electron transport chain and its function is vital to OXPHOS. However, once released into the cytosol, cytochrome c mediates apoptosis by triggering the irreversible activation of a cascade of caspase-mediated cell destruction. Excess release of cytochrome c also leads to an eventual loss of mitochondrial function and a bioenergetic crisis. Once the mitochondrial outer membranes of sufficient mitochondria have been breached in this way, cell death is almost always inevitable; thus, MOMP is often referred to as “the point of no return”[77,78].

The Bcl-2 family of proteins represents critical players in the regulation and induction of MOMP. This family is characterized by the presence of Bcl-2 homology (BH) domains, and consists of both pro-apoptotic and anti-apoptotic members. The pro-apoptotic Bcl-2 proteins can be divided into two groups: the “effector” proteins (BAK and BAX) which actively induce MOMP, and the “BH3-only ” proteins (e. g. BAD, BID, BIM, and others) which contain only one BH domain (BH3) and indirectly promote MOMP through the inhibition of anti-apoptotic proteins or through the activation of the effector Bcl-2 proteins. The anti-apoptotic or “pro-life” family members (Bcl-2, Bcl-xL, MCL-1, etc) bind to pro-apoptotic family members and inhibit their function. Thus, a complex set of interactions among Bcl-2 family members regulates the induction of MOMP and apoptosis[79,80]. Although the exact mechanics of MOMP induction remain controversial, it is now clear the effector BAX and BAK proteins are essential to the process. Studies on knockout cell lines have shown that BAX and BAK are functionally redundant. However, activity of at least one of these proteins is required for MOMP following triggers of intrinsic apoptosis [81]. BAK is

localized to the outer mitochondrial membrane, whereas BAX is cytosolic in unstimulated cells and translocates to the outer mitochondrial membrane in response to apoptogenic signals. Upon activation, both BAK and BAX can insert into the mitochondrial outer membrane, form homo-oligomers, and induce the formation of pores through which cytochrome c and other mitochondrial proteins are released. The exact molecular make-up of these pores remains a subject of debate, but in most models BAK and BAX are themselves key structural components [82]. Once released into the cytosol, cytochrome c interacts with the protein APAF1 to form a complex known as the apoptosome, which triggers activation of caspase-9 and a resulting cascade of caspase activation, leading to the final steps of cell death. Release of other mitochondrial proteins following MOMP also contributes to cell death[77,82].

## Mitochondrial ROS and regulation of cell death

As mentioned previously, mitochondrial ROS can trigger apoptosis. Multiple mechanisms may be involved. DNA damage induced by ROS can result in the activation of p53 and p53-mediated apoptosis [47]. ROS can also activate the kinase ASK1/JNK signaling pathway to trigger extrinsic or intrinsic apoptosis[56]. ROS have also been shown to interact with and induce opening of the mitochondrial permeability transition pore (MPTP) complex, a mitochondrial pore complex mediating the permeabilization of mitochondrial membranes, and has been proposed to contribute to apoptotic death as well as necrosis[47,83]. Finally, ROS can facilitate the release of cytochrome c from the inner mitochondrial membrane through the oxidative action on the inner mitochondrial membrane lipid, cardiolipin[84].

## Defects in the intrinsic apoptosis pathway in human cancer

The intrinsic apoptosis pathway provides an important safeguard against tumor formation. It eliminates cells with damaged DNA and cells expressing deregulated oncogene activation. Defects in intrinsic apoptosis compromise this safeguard, and allow for the continued growth of cells which would otherwise die, thus setting the stage for tumorigenesis. In addition, defects in intrinsic apoptosis play important roles in tumor metastasis and chemoresistance [85,86]. Accordingly, alterations in the molecular pathways regulating intrinsic apoptosis are commonly seen in human cancers. Compromised function of the intrinsic apoptosis pathway can occur by two major mechanisms: the overexpression or over-activation of anti-apoptotic proteins and the loss of expression/loss-of-function of pro-apoptotic proteins.

Bcl-2, the founding member of the Bcl-2 family, is an anti-apoptotic protein. The bcl-2 gene was first identified as a gene that is overexpressed in human B-cell follicular lymphoma due to a chromosomal translocation event which places Bcl-2 expression under the control of an immunoglobulin heavy chain enhancer (hence its name, which stands for “B-cell lymphoma-2”)[87–91]. The overexpression of Bcl-2 was first directly shown to be oncogenic in a mouse model of lymphoma [92]. Overexpression of Bcl-2 has since been detected in a number of hematopoietic malignancies, as well as in solid tumors including prostate, colorectal, lung and gastric cancers [85,86]. Overexpression of the related anti-apoptotic Bcl-2 proteins, Bcl-$X_L$ and MCL-1, has also been detected in a number of cancers[86]. Importantly, the elevated expression of Bcl-2 anti-apoptotic proteins has been correlated in some cases with increased tumor resistance to chemotherapy[86].

Conversely, loss of the pro-apoptotic Bcl-2 proteins has also been observed in human cancer. Inactivating mutations and impaired expression of the pro-apoptotic effectors Bak and Bax have been seen, most notably in gastric and colorectal cancers[78,86]. The loss of expression or function of the BH3-only pro-apoptotic proteins has also been reported in a number of human cancers. For example, loss of the BH3-only protein Bik (also known as

Blk or NBK) seems to be an important feature of clear-cell renal cell carcinoma [93]. Similar to the overexpression of anti-apoptotic proteins, the loss of expression of pro-apoptotic proteins in tumors has been linked to chemoresistance and poor prognosis[86].

Compromised function of the intrinsic apoptosis pathway can also occur through dysregulation of proteins upstream and downstream of the Bcl-2 proteins and MOMP. Alterations in upstream signaling pathways which regulate intrinsic apoptosis (such as the PI3K/Akt pathway, which phosphorylates and inactivates the pro-apoptotic protein Bad) are commonly seen in human cancer. The most well-known case of this may be exemplified by p53. p53 acts upstream of the Bcl-2 proteins to trigger apoptosis in response to DNA damage and other stressors and p53 is inactivated by mutation or other mechanisms in over 50% of all human cancers[94,95]. Inactivating mutations in the intrinsic apoptosis pathway downstream of Bcl-2 proteins appears more rare, but a loss of expression of the mitochondrial pro-apoptotic effector Smac/Diablo has been reported in renal cell carcinoma, and mutations in effector caspases have been reported in some cancers[78,96,97].

### Targeting the intrinsic apoptosis pathway for cancer therapy

Therapy targeting the intrinsic apoptosis pathway is one of the most exciting areas of cancer research to date. Many, if not all, current therapies act by inducing apoptosis. However, dysfunction of the intrinsic apoptosis pathway itself is a hallmark of cancer and contributes to chemoresistance. Thus, the direct targeting of elements of the intrinsic apoptosis pathway to restore apoptotic function is of great interest. Because deregulation of the Bcl-2 family of proteins and/or deregulation of signaling pathways upstream of Bcl-2 proteins are so often seen in human cancers, the Bcl-2 family represents a particularly attractive target. One promising class of targeting agents consists of the BH3 mimetics. These agents mimic the BH3 domains of Bcl-2–like proteins and act to bind and antagonize the action of the anti-apoptotic Bcl-2–like proteins[98]. One of the best characterized and most advanced of such agents is ABT-737. This small molecule inhibitor was identified in a screen for chemical compounds binding to the hydrophobic BH3-binding groove of the anti-apoptotic Bcl-$X_L$ protein. ABT-737 was shown to interact strongly with the anti-apoptotic Bcl-2 and Bcl-2w as well as Bcl-$X_L$, and has shown anti-tumor activity in several preclinical animal models of human cancer, including small cell lung cancer and hematologic malignancies [75,98,99]. An orally active derivative of ABT-737, known as ABT-263, recently became available, and is now in a variety of phase-I and -II clinical trials, including trials for solid tumors and hematopoietic cancers, both as a single agent and in combination therapy[100]. Other approaches to targeting the intrinsic apoptosis machinery include the direct activation of downstream effector caspases and the development of agents which mimic SMAC/DIABLO, a pro-apoptotic protein released from the mitochondria during MOMP[74,101].

## Summary and Conclusions

Much more than the “powerhouse” of the cell, mitochondria lie at the center of essential physiological processes. With this in mind, it is not a surprise that mitochondrial function and dysfunction should contribute to cancer initiation and progression in complex ways. In this review, we have touched upon three major mechanisms by which mitochondrial function may contribute to cancer: through alterations in glucose metabolism, through the generation of ROS and through compromised function of intrinsic apoptosis. Fascinating and complex links exist between all three of the tumorigenic mechanisms here outlined. Because mitochondrial ROS are generated as a byproduct of OXPHOS, alterations in glucose metabolism affecting OXPHOS will also affect the generation of mitochondrial ROS. Additionally, metabolic alterations in the Warburg effect have been suggested to provide an increased protection against oxidative stress through the increased generation of NADPH molecules which play an important role in cellular antioxidant “buffering”

systems [25]. ROS can produce multiple effects depending on the cellular context and the effects of such ROS regulation within the context of the Warburg effect are not clear. ROS may in turn act as signaling mediators to influence both glucose metabolism and intrinsic apoptosis. Availability of nutrients and bioenergetics also influences apoptosis. The unraveling and understanding of these pathways promises to keep researchers busy for years to come and will hopefully lead to a more integrated understanding of mitochondrial function and to the targeted development of new agents for cancer treatment.

## Acknowledgments

We thank Natalie Niemi for critical reading of this review. We also acknowledge the many researchers who have contributed to the field of mitochondrial biology and apologize to those whose work we were unable to cite due to space restrictions. This work was supported by Award Numbers R01CA138651 and R01CA138651S1 to J.P. MacKeigan and V.C. Fogg.

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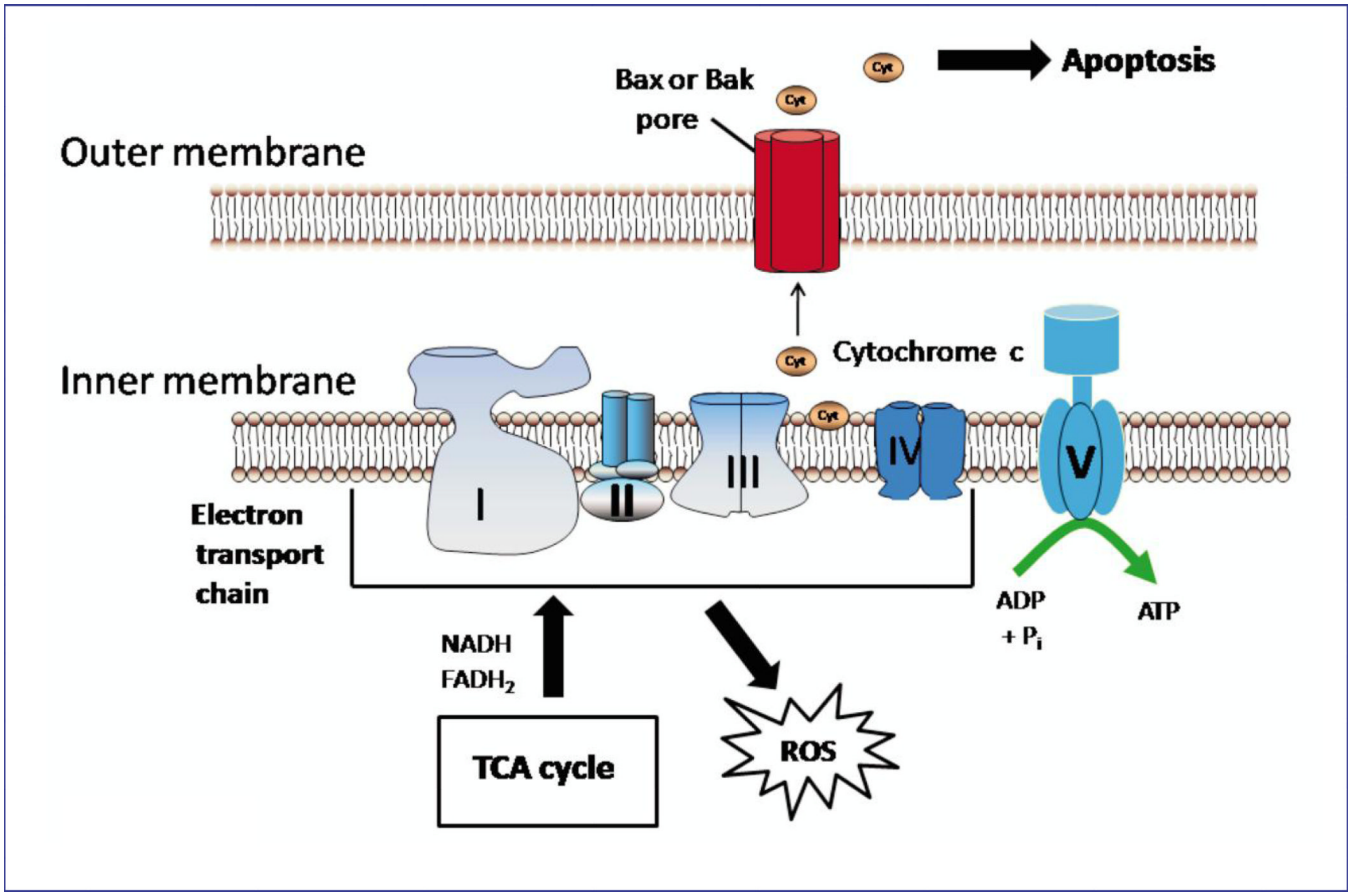


**Figure 1. Key physiological processes regulated by mitochondria**

Fuel substrates are metabolized by the citric acid/tricarboxylic (TCA) cycle in the mitochondrial matrix. High -energy electrons in the form of NADH and FADH2 are generated by the TCA cycle and fed into the electron transport chain in the mitochondrial inner membrane, ultimately resulting in the production of ATP. The major protein complexes of the electron transport chain are shown, labeled I to V (Complex V is also known as the F1F0-ATPase). Reactive oxygen species (ROS) are generated by the electron transport chain as a byproduct of respiration. Cytochrome c, an essential component of the electron transport chain, also plays a critical role in apoptosis when it is released into the cytosol through Bax/Bak pores.

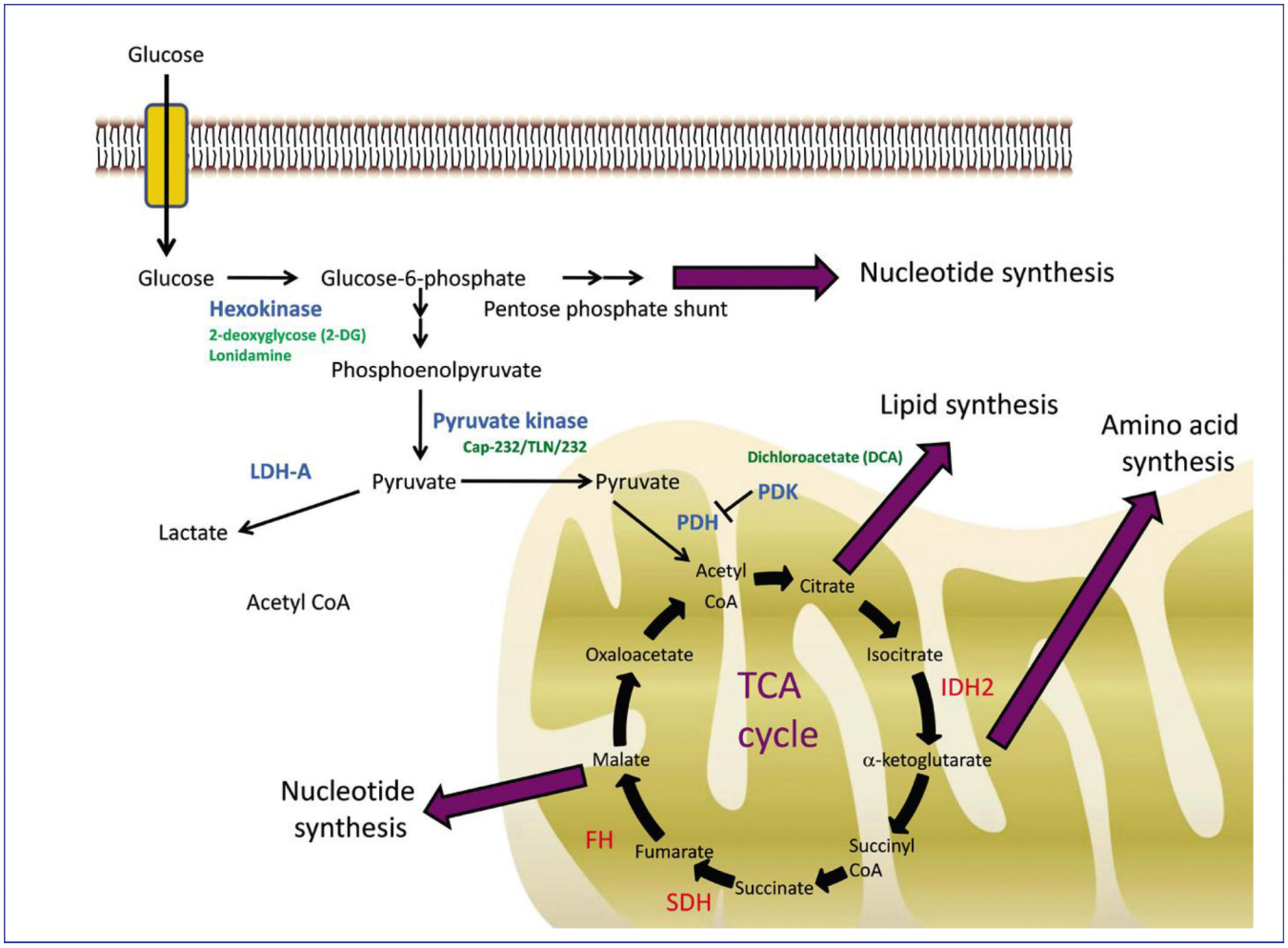


**Figure 2. Targeting glucose metabolism in tumor cells**

Key metabolic pathways and control points, which may serve as useful targets for cancer therapy, are shown schematically. Glucose is taken up into the cell by glucose transporters and metabolized by glycolysis to pyruvate in the cytosol. Pyruvate is either converted to lactate through the action of lactate dehydrogenase-A (LDH-A), or imported into the mitochondrial matrix where it is converted to acetyl CoA via the action of pyruvate dehydrogenase (PDH). Acetyl CoA can then enter the tricarboxylic acid (TCA) cycle. In cancer cells, pyruvate often enters a truncated TCA cycle and its metabolites are diverted away from complete oxidation and into various biosynthetic pathways (see purple arrows). It should be noted that the glycolytic intermediate glucose-6-phosphate can also be diverted into nucleotide synthesis pathways through the pentose phosphate shunt. Key enzymes which may be particularly promising targets for cancer therapy are shown in blue; drug inhibitors of these enzymes are shown in green. Pyruvate dehydrogenase kinase (PDK) suppresses activity of PDH and is itself inhibited by the drug dichloroacetate (DCA). TCA enzymes which are known to be mutated in cancer are shown in red: IDH2 (isocitrate dehydrogenase 2), SDH (succinate dehydrogenase), and FH (fumarate hydratase).

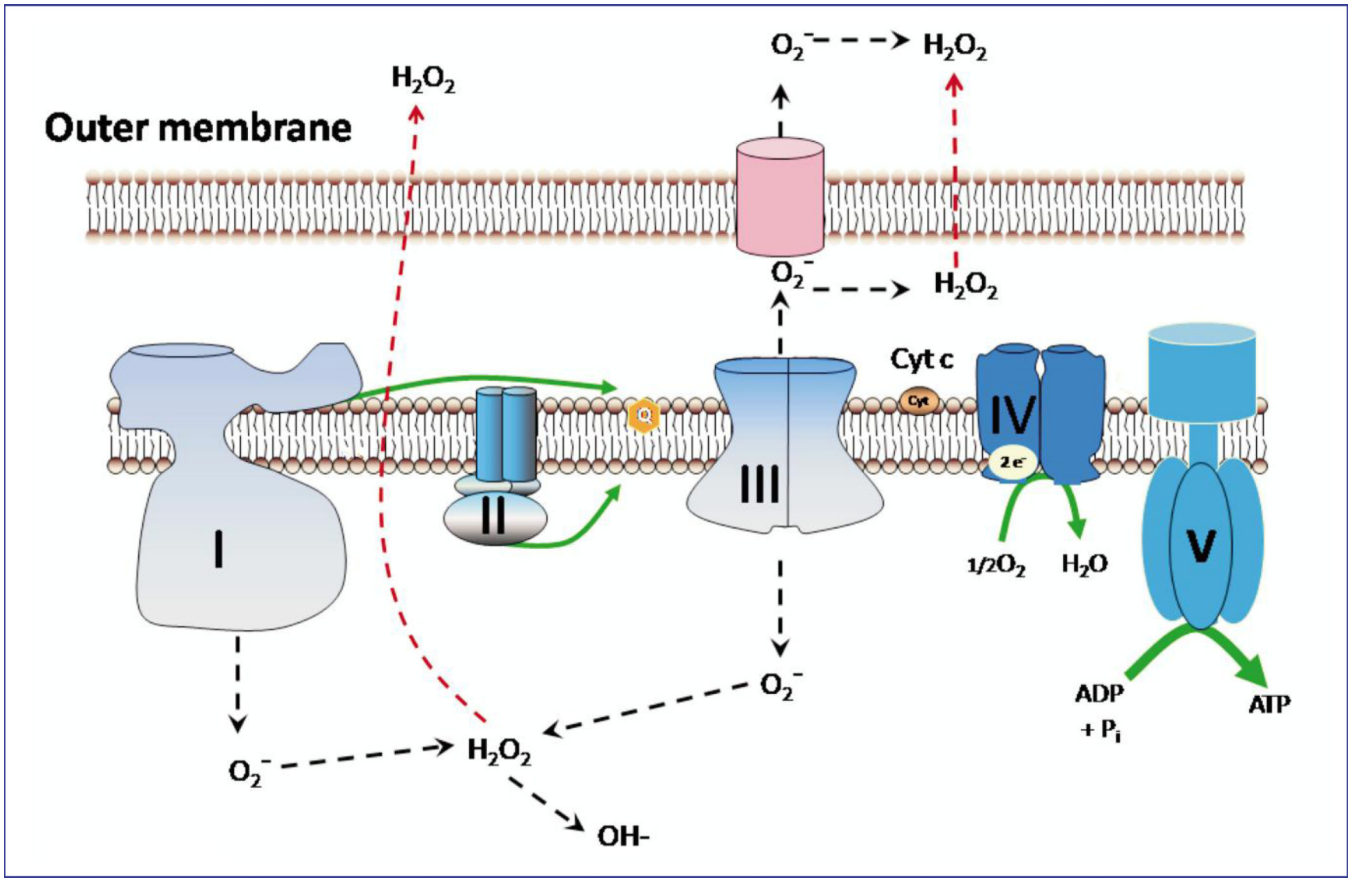


**Figure 3. Generation of mitochondrial ROS by the electron transport chain**

Superoxide is produced primarily by complex I on the matrix side of the inner mitochondrial membrane, and by complex III on both sides of the inner mitochondrial membrane[46]. Superoxide does not readily diffuse across membranes on its own; however, there is evidence that it may pass through mitochondrial membranes into the cytosol through specialized channels such as VDAC[48] (mitochondrial channel shown in pink). In the mitochondria, superoxide is rapidly converted to hydrogen peroxide by the action of both matrix and intermembrane superoxide dismutases. In the cytosol, superoxide is converted to hydrogen peroxide by cytosolic superoxide dismutase. Hydrogen peroxide can diffuse freely across membranes, and can also convert to hydroxyl radical through a Fenton/Haber-Weiss reaction. Both superoxide and hydrogen peroxide may act as signaling molecules.

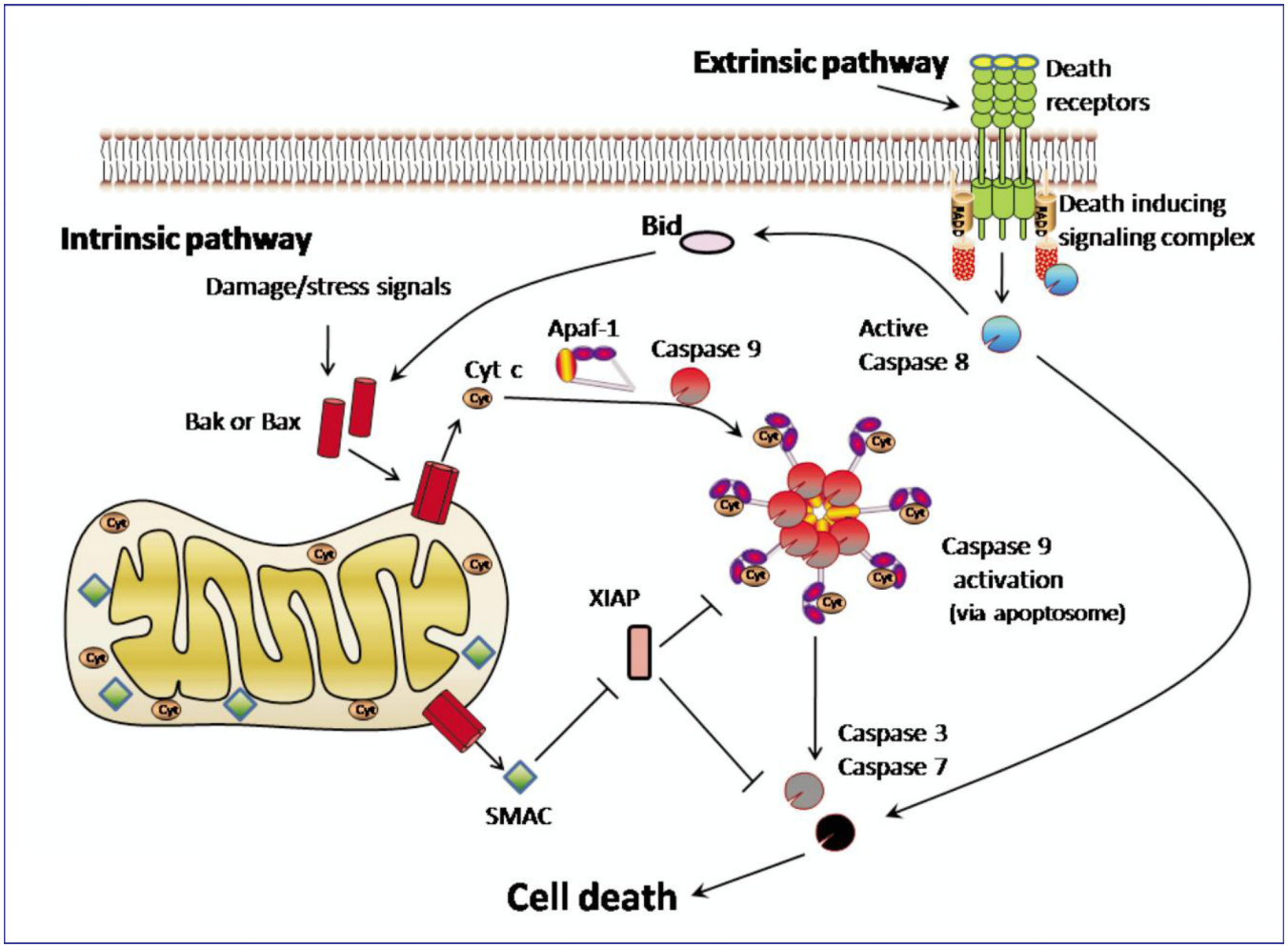


**Figure 4. Overview of apoptosis pathways**

Extrinsic apoptosis is triggered by cell-surface "death receptors" of the tumor necrosis factor family. Upon ligand-mediated activation, these receptors cluster and recruit adaptor proteins (including Fas-associated death domain or FADD proteins) and caspase 8 into a death-inducing signaling complex (DISC) which results in activation of caspase 8. Caspase 8 in turn activates the executioner caspases 3 and 7, resulting in cell death. The intrinsic apoptosis pathway is triggered by signals of intrinsic cell damage or stress. These stress/damage signals result in the activation of the pro-de ath Bcl-2 proteins, Bak and Bax, resulting in oligomerization of these proteins and formation of Bak or Bax-based pores in the outer mitochondrial membrane. Cytochrome c is released from the mitochondria into the cytosol via these pores. Once in the cytosol, cytochrome c assembles with the proteins Apaf-1 and caspase 9 to form a complex known as the apoptosome, which induces activation of caspase 9. The initiator caspase 9 in turn activates the executioner caspases 3 and 7, resulting in apoptosis. Cross-talk between the extrinsic and intrinsic apoptosis pathways occurs at the level of Bak/Bax activation. Caspase 8 cleaves and activates the pro-apoptotic Bcl-2 protein Bid, which in turn activates Bak/Bax. In addition to cytochrome c, other apoptogenic proteins are released from the mitochondria during the process of intrinsic apoptosis. Shown in the figure is the activity of one such protein, SMAC (also known as Diablo). SMAC promotes apoptosis by blocking the activity of XIAP, an inhibitor of caspases 9, 3, and 7.