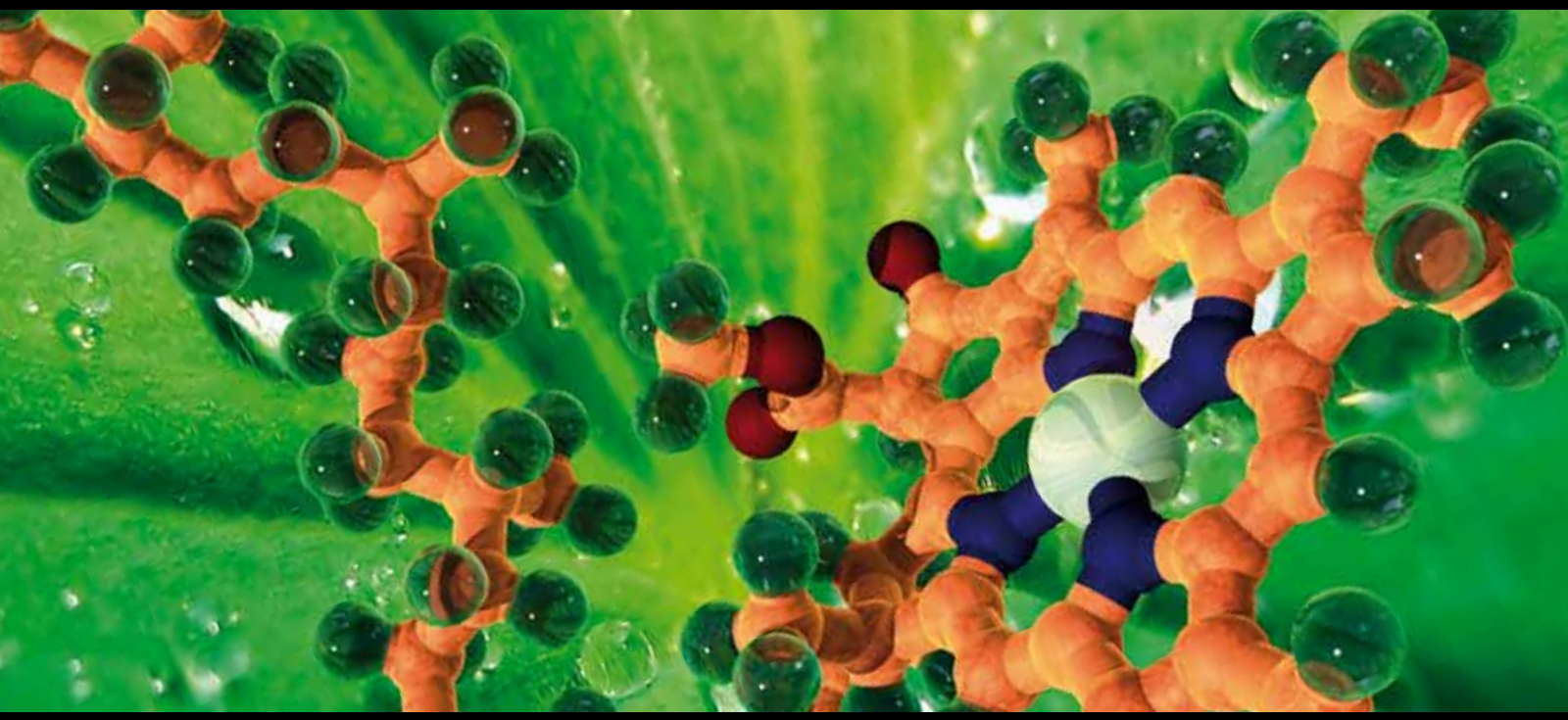
Biochemistry Research International

Mitochondria and Cytoprotection

Guest Editors: Catherine Brenner, Renée Ventura-Clapier, and Etienne Jacotot



Mitochondria and Cytoprotection

Biochemistry Research International

Mitochondria and Cytoprotection

Guest Editors: Catherine Brenner, Renée Ventura-Clapier, and Etienne Jacotot

Editorial Board

Contents

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 351264, 2 pages
doi:10.1155/2012/351264

Editorial
Mitochondria and Cytoprotection

Catherine Brenner,[1,2] Renée Ventura-Clapier,[1,2] and Etienne Jacotot[3,4,5]

[1] *Inserm UMR-S 769, LabEx LERMIT, Châtenay-Malabry, F-92296, France*
[2] *University Paris-Sud, Châtenay-Malabry, F-92296, France*
[3] *Inserm UMR 676, Hôspital Robert Debré, F-75019 Paris, France*
[4] *Faculté de Médecine Denis Diderot, Université Paris 7, IFR02, F-75019 Paris, France*
[5] *Faculty of Medicine, Institute of Reproductive and Developmental Biology, Hammersmith Hospital, Imperial College London, London W12 0NN, UK*

Correspondence should be addressed to Catherine Brenner, catherine.brenner-jan@u-psud.fr
and Etienne Jacotot, etienne.jacotot@inserm.fr

Received 11 July 2012; Accepted 11 July 2012

Mitochondrion is an eukaryotic double-membrane bound organelle that arose from a bacterial endosymbiont. Its circular DNA is small, as many genes were transferred to nucleus during evolution, and almost exclusively maternally inherited. Mitochondria harbor highly regulated biogenesis activities (division and fusion) and motility, establish interorganellar contacts, and perform/house multiple and interconnected cellular functions, as diverse as ATP production (via oxidative phosphorylation), parts of pyrimidine and lipid biosynthetic pathways (including the fatty acid β-oxidation pathway), reactive oxygen species production and detoxification, and metal metabolism (synthesizing heme and Fe–S clusters). Mitochondria also participate in calcium homeostasis, metabolic sensing, innate immunity, autophagy, and cell death regulation. The coordination of these functions contributes to the cell and life balance and mitochondrial dysfunction is consequently associated with many human diseases.

On the one hand, discovery of the major role of mitochondria in cell death signaling and fate has led to important conceptual changes in oncology and has enriched anticancer strategies. On the other hand, structurally and functionally abnormal mitochondria have been described in nonalcoholic fatty liver diseases, as well as many neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. Mitochondrial failure has been shown to be central to ischemia/reperfusion damages. An energetic imbalance involving impaired mitochondrial respiration and substrate utilization has been demonstrated in cardiopathies in rodents as well as in human.

Increasing evidence indicates that cytoprotection via preservation of mitochondrial function is a promising therapeutic strategy to limit the severity of the diseases and their progression. In this special issue, we have invited reviews that discuss the interest and the modalities of cytoprotection in diverse conditions or experimental settings.

At the crossroad of physiology, metabolism, and toxicology, liver mitochondria have been intensively studied. Their particular importance in chronic liver disease is discussed by D. Esposti and coworkers. Mitochondria also play a key role in glucose-stimulated insulin secretion. Human pancreas islet transplantation is a promising therapy for type I diabetes mellitus. However, innovative methods are required for β-cell preservation against oxidative stress, inflammation, and cell death signaling. Y. Wang et al. present a detailed review of the recent progress in mitochondrial cytoprotection in each step of the islet isolation and transplantation process.

Prevention of mitochondrial membrane permeabilization to block cell death is a powerful concept, with many therapeutic implications in organoprotection (e.g., heart, brain, liver, and kidneys). In a number of acute conditions like ischemia-reperfusion, the so-called mitochondrial permeability transition (MPT) is an operational checkpoint to

cell death. C. Martel et al. discuss how MPT is regulated and can be manipulated to reduce cell death.

The clinical use of anthracyclines in anticancer therapies is severely limited by the development of a progressive dose-dependent cardiomyopathy that irreversibly evolves toward congestive heart failure. D. Montaigne and coworkers review cytoprotection strategies to reduce the cardiotoxicity of anthracyclines.

The paper by J. Kang and S. Pervaiz addresses the central issue of redox metabolism, and the role of mitochondria as regulators of redox homeostasis and cell fate. They describe how dysfunction of mitochondria and the ensuring oxidative stress is one of the causal factors of a variety of diseases including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancer. From the current understanding of reactive oxygen/nitrogen species generation and regulation in the mitochondria and of their impact on cell fate, the authors propose some clues on how redox metabolism could be targeted for therapeutic purposes.

Oxydative stress, mitochondrial signaling, and dysfunction can also be induced through exposure to pollution particles (for instance generated by combustion of wood or diesel oil) and to gaseous ligands capable of binding to either hemoglobin or mitochondrial cytochrome *c* oxidase (e.g., NO, CO, and H_2S). K. Andreau et al. discuss how air pollutants impact redox status, metabolic, and apoptotic functions of mitochondria, and thereby have major consequences on cell death and health, with societal burden. Finally, C. S. F. Queiroga et al., focused on carbon monoxide, a toxic gas, which can be used, however, as a therapeutic agent.

In conclusion, this special issue discusses some aspects of cytoprotection through mitochondrial function preservation. Several of these reviews and our own past and present research suggest that this strategy might help to delineate efficient preventive or therapeutic strategies.

Catherine Brenner
Renée Ventura-Clapier
Etienne Jacotot

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 387626, 16 pages
doi:10.1155/2012/387626

Review Article

Mitochondrial Roles and Cytoprotection in Chronic Liver Injury

Davide Degli Esposti,[1,2] Jocelyne Hamelin,[1,2] Nelly Bosselut,[1,2] Raphaël Saffroy,[1,2] Mylène Sebagh,[3] Alban Pommier,[1,2] Cécile Martel,[2] and Antoinette Lemoine[1,2,4]

[1] *AP-HP, Hôpital Paul Brousse, Service de Biochimie et Biologie Moléculaire, 14 Avenue Paul Vaillant Couturier, 94804 Villejuif Cedex, France*
[2] *Inserm U1004, Université Paris 11, Institut André Lwoff, PRES Universud-Paris, Institut du Foie/Liver Institute, 14 Avenue Paul Vaillant Couturier, 94804 Villejuif, France*
[3] *AP-HP, Inserm U785, Hôpital Paul Brousse, Service d'Anatomie Pathologique, 14 Avenue Paul Vaillant Couturier, 94804 Villejuif Cedex, France*
[4] *Université Paris Sud 11, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry Cedex, France*

Correspondence should be addressed to Antoinette Lemoine, antoinette.lemoine@pbr.aphp.fr

Received 11 January 2012; Revised 20 March 2012; Accepted 11 April 2012

Academic Editor: Etienne Jacotot

The liver is one of the richest organs in terms of number and density of mitochondria. Most chronic liver diseases are associated with the accumulation of damaged mitochondria. Hepatic mitochondria have unique features compared to other organs' mitochondria, since they are the hub that integrates hepatic metabolism of carbohydrates, lipids and proteins. Mitochondria are also essential in hepatocyte survival as mediator of apoptosis and necrosis. Hepatocytes have developed different mechanisms to keep mitochondrial integrity or to prevent the effects of mitochondrial lesions, in particular regulating organelle biogenesis and degradation. In this paper, we will focus on the role of mitochondria in liver physiology, such as hepatic metabolism, reactive oxygen species homeostasis and cell survival. We will also focus on chronic liver pathologies, especially those linked to alcohol, virus, drugs or metabolic syndrome and we will discuss how mitochondria could provide a promising therapeutic target in these contexts.

1. Introduction

Mitochondria are intracellular double membrane-bound structures that provide energy (ATP) for intracellular metabolism. The intramitochondrial metabolism includes Krebs cycle and beta-oxidation. Mitochondria are also essential for assembly of iron sulfur clusters and regulation of calcium homeostasis. However, mitochondria are not only the cell's powerhouse, organelles whose particular architecture and biochemical composition enable the maximization of energy production by oxidative phosphorylation (OXPHOS), but they also have a second crucial function, namely, the control of cell death following activation of intracellular signaling cascades or death receptor-mediated pathways [1]. Indeed, the mitochondrial membrane permeabilization (MMP) is the decisive event that marks the transition from survival to death. Thus, mitochondrial membranes integrate proapoptotic and antiapoptotic signals coming from microenvironment or from other intracellular organelles, such as endoplasmic reticulum or lysosomes, defining the ultimate cell fate [1, 2]. The number and functions of mitochondria can vary depending on age, sex, organ, and physiological or pathological conditions that are still unknown [3–5].

Mitochondrial dysfunctions are frequently described as early and initiating events in various chronic pathological conditions in different tissues and organs, such as liver, brain, or heart [6–8]. Most forms of chronic liver diseases are associated with the accumulation of damaged mitochondria responsible for abnormal reactive oxygen species (ROS) formation, glutathione (GSH) depletion, protein alkylation, and respiratory complex alterations. Depending on their nature and severity, the mitochondrial alterations may induce lipid accumulation, apoptosis, and/or necrosis

leading to hepatic cytolysis and inflammation. These pathological events can correspond to different clinical features, such as lactacidosis, hypoglycemia, elevated serum transaminases, higher conjugated bilirubinemia, and hyperammonemia. However, a growing body of literature has also shown that demised cells with damaged mitochondria can develop cytoprotective mechanisms to ensure cellular energy homeostasis and limit cell death [9–12]. These mechanisms consist in both activation of intracellular pathways targeting mitochondria function and intercellular and interorgan signaling to coordinate adaptive metabolic responses within the organism as a whole. The regulation of the mitochondrial biogenesis and/or turnover (by general autophagy or specific mitochondria-targeted mitophagy) plays an important role in the balance of cell survival and cell death [13]. This balance is importantly linked to the energy metabolism homeostasis, in particular with ATP synthesis, as it has been reported in some chronic liver pathologies, such as steatosis and nonalcoholic steatohepatitis (NASH) [14].

In this paper, we will focus on the role of mitochondria in liver physiology and pathologies, especially those linked to alcohol, virus, drugs, or metabolic syndrome and we will discuss how mitochondria could provide a promising therapeutic target in these contexts.

2. Mitochondria in Liver Physiology

The liver is one of the richest organs in terms of number and density of mitochondria. The density of mitochondria is different in various tissues depending upon numerous factors, mostly the demands of oxidative phosphorylation. A study showed that in nontumorous liver tissue the copy number of mitochondrial DNA (mtDNA) in male patients affected by hepatocellular carcinoma (HCC) was lower than that of the female patients (5308 ± 484 versus 8027 ± 969, $P < 0.05$) [4]. Since each mitochondrion can host from two to ten copies of mtDNA [5], we can assume that in the liver, the number of mitochondria could range from 500 to 4000 per hepatocyte.

In this chapter, we will review the role of mitochondria in hepatic metabolism, reactive oxygen species (ROS) homeostasis, and cell death regulation.

2.1. Mitochondria Are Essential in the Hepatic Metabolism. The liver is an essential life organ in all mammals and plays a central role in the homeostasis of carbohydrate, lipid, and protein metabolism of the organism. The liver is a main target of insulin and glucagon signaling and contributes to balancing glucose blood levels by regulating glycogen synthesis and gluconeogenesis in hepatocytes [15]. It is also a key organ in maintaining lipid homeostasis: it is the main site of fatty acid oxidation together with the muscle (mainly β-oxidation taking place into the mitochondria) and it is the sole organ able to synthesize fatty acids by *de novo* lipogenesis [16]. Finally, the liver is a key regulator of protein metabolism for the entire organism as hepatocytes synthesize essential proteins such as albumin and lipoproteins and allow ammonia detoxification through the urea cycle [17].

In this context, the mitochondria provide the hub that integrates these pathways, serving as a critical site for the production and exchange of metabolic intermediates (Figure 1). It plays a critical role in orchestrating these complex metabolic networks in order to maintain proper homeostasis.

Mitochondria are largely involved in glucose metabolism, as the pyruvate dehydrogenase (PDH) complex is expressed in the mitochondrial matrix. It is composed by 5 subunits (pyruvate dehydrogenase, E1 alpha and E1 beta; dihydrolipoamide S-acetyl transferase, E2; and lipoamide dehydrogenase, E3 and E3BP). It catalyzes the conversion of pyruvate, the last metabolite of aerobic glycolysis, to Acetyl-CoA and CO_2. In the last ten years evidence has accumulated showing an important involvement of liver mitochondria in insulin resistance. In insulin resistant states, alterations in mitochondrial function, structure, and organization have been described [18]. In particular, a decrease in respiration and ATP production has been frequently described and the decreased efficiency is often attributed to excessive mitochondrial ROS production inducing respiratory chain protein oxidation [14, 18, 19].

Concerning lipid metabolism, few mitochondrial proteins play key roles in catabolism as well as in anabolism. The carnitine palmityl transferases I and II (CPT I-II) are expressed at the mitochondrial outer membrane (MOM) and mitochondrial inner membrane (MIM), respectively, and are essential for acyl-CoA transport and subsequent fatty acid β-oxidation in liver and muscle. A mitochondrial transport protein, the citrate transport protein (CTP), allows acetyl-CoA to be transported from mitochondria to the cytosol in the form of citrate in order to be used as building block in hepatic *de novo* lipogenesis [20]. Hepatic mitochondria are essential also in protein metabolism. Nitrogen enters the liver as free ammonia and amino acids, mostly glutamine and alanine [17]. Enzymes involved in ammonia detoxification and urea synthesis (glutamate dehydrogenase, carbamoyl phosphate synthetase I and ornithine transcarbamylase) are exclusively expressed in the hepatocyte mitochondria. Indeed, the first step in the urea cycle for ammonia detoxification and disposal is located at mitochondria and mediated by the enzyme carbamoyl phosphate synthetase 1 (CPSI). CPSI is allosterically regulated by cytosolic N-acetyl-L-glutamate (NAG) [21]. Ammonia can be also converted to glutamine by the glutamine synthetase (GS) catalyzing the condensation of glutamate and ammonia and, *vice versa*, ammonia can be generated by glutaminase. Therefore, an increase in blood ammonia depends on the activity of the enzyme glutamine synthetase, the glutamine/glutamate cycle, and the tissue capacity to eliminate toxic ammonia. Mitochondria represent a major site of glutamine metabolism, as both glutaminase and GS are mitochondrial processes in the liver. Interestingly, in absence of glucose but with high glutamine concentrations, mitochondrial structure and dynamics change towards a more condensed configuration and extended reticulum [22]. Moreover, urea and glutamine metabolism are differently distributed in the hepatic acinus. Ammonia is taken up by periportal hepatocytes, metabolized to urea via the urea cycle

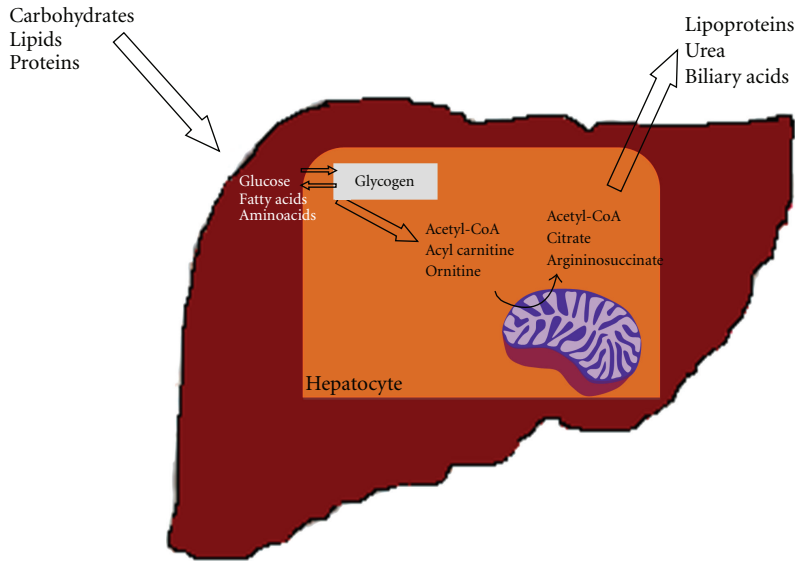


Figure 1: The role of hepatocyte mitochondria in liver metabolism. The liver is a central organ for the homeostasis of carbohydrates, lipids and proteins metabolism. In this context, hepatocyte mitochondria are essential in regulating the flux of metabolites in the cell in order to adjust energetic demand, ammonia detoxification, or anabolic pathways. Energy demand is met by complete oxidation of acetyl groups coming from glycolysis through tricarboxyilic acid cycle or of acyl groups coming from lipolysis through β-oxidation. Moreover, mitochondria are a unique site for metabolizing ammonia into the less toxic urea. Then, mitochondria provide shuttle proteins that allow specific addressing to anabolic pathways, as in the case of citrate transport protein (CTP) (see text for details).

and excreted through the kidneys. Any ammonia escaping detoxification is usually trapped by perivenous hepatocytes, where it is converted to glutamine via glutamine synthetase [23]. Indeed, urea synthesis enzymes and glutaminase are expressed in periportal hepatocytes, while glutamine synthetase is expressed in perivenous hepatocytes [17]. Then, the periportal region has a low affinity but a high capacity for ammonia detoxification. Hepatic GS allows ammonia scavenging, and when liver impairment is present, a diminished detoxification capacity is observed. GS has a short half-life and its activity is regulated and modulated by several mediators and hormones. The brain also uses glutamine synthesis for metabolizing ammonia and for deamination in the presynaptic terminals to produce glutamate, an important excitatory neurotransmitter. When it accumulates, it is taken up by the astrocytes and recycled back to glutamine, the "storage centre" for free ammonia [24, 25]. It is interesting to note that the different subcellular localization of GS (mitochondrial in hepatocytes and cytoplasmic in astrocytes) has been considered a partial explanation to the higher toxicity of ammonia in the brain than in the liver [26]. However, the exact role of mitochondrial dysfunctions in hyperammonemia still needs to be addressed, in particular for chronic liver disease. However, liver and mitochondria metabolism are directly involved in the homeostatic balance of brain ammonia, glutamine, and glutamate.

2.2. Mitochondria Are Essential in Reactive Oxygen Species Homeostasis. Mitochondria are the intracellular organelles devolved to energy (ATP) production in all eukaryotic cells through oxidative phosphorylation (OXPHOS). OXPHOS is allowed by the four multiprotein complexes of the mitochondrial respiratory chain (MRC) and by the ATP synthase. OXPHOS physiologically produces reactive oxygen species (ROS) and *in vitro* estimations lead to considering that up to 2% of oxygen consumption results in superoxide anion generation [27]. Thus, mitochondria are a main source of ROS (Figure 2). ROS are produced during oxidative metabolism mainly by the complexes I, III, or IV of the electron transport chain, where electrons can prematurely reduce oxygen, resulting in the formation of superoxide radical [27–30]. In the normal state, most of the ROS generated by the MRC are detoxified by the mitochondrial antioxidant enzymes, such as SOD2/MnSOD, which convert superoxide to hydrogen peroxide, subsequently detoxified by GSH peroxidase. The remaining nondetoxified ROS diffuse out of mitochondria and serve as signaling molecules vital for normal cellular functions [31]. These physiological ROS are involved in specific cellular pathway aimed to adapt global metabolism to transient or chronic stress conditions. It is interesting to note that ATP synthase may also have a regulating role in ROS production. Actually, in the experimental model of aging provided by the fungus Podospora anserine, characterized by mitochondrial etiology of aging, the alpha subunit of ATP synthase functions as a sensor of oxidative stress and provides an intramolecular quencher (at the residue Trp503) for ROS [32]. Moreover, a recent mechanism that seems to buffer ROS excess has been described in physiological and pathological conditions. The expression of uncoupling proteins (UPCs) promotes a controlled uncoupling of proton flux from the ATP synthase and could lead to decreased ROS production [33].

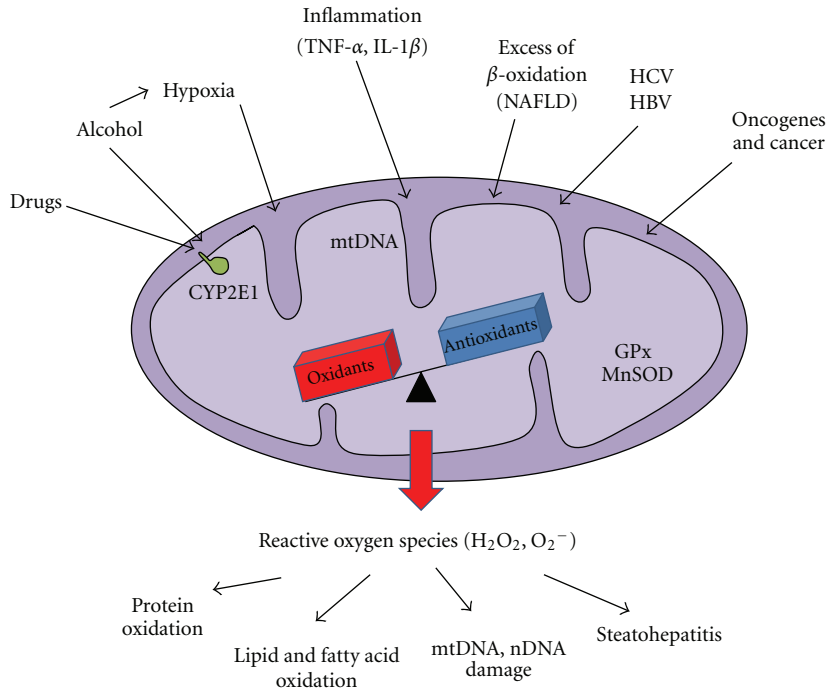


Figure 2: The role of hepatocyte mitochondria in reactive oxygen species homeostasis. Mitochondria are a physiological source of reactive oxygen species (ROS). In this context, ROS exert a signaling role in cell proliferation and differentiation. However, different types of stress can target directly or indirectly hepatocyte mitochondria, such as drugs, virus, hypoxia, inflammatory cytokines, excess of β-oxidation, ectopic expression of cytochromes P450. In this case, overproduction of ROS may damage both mitochondrial and other cellular components, such as OXPHOS protein subunits, lipid membranes, mitochondrial, or nuclear DNA. These cellular lesions can favor the development of tissue lesions, such as steatohepatitis or hepatocellular carcinoma.

2.3. Mitochondria Are Essential in Cell Survival. Mitochondria are the essential actor in keeping the balance between cell survival and cell death, in particular in hepatocytes, where they trigger the intrinsic pathway of apoptosis and are also involved in necrotic cell death. The regulation of membrane permeability is the main mechanism that makes the cells shift from survival to cell death. The MOM is permeable to solutes of molecular mass (MM) $\approx$ 6 kDa due to the presence of channels, such as the voltage-dependent anion channel (VDAC), which belongs to the porin subfamily. However, with an estimated pore diameter about 2.6–3 nm, VDAC would not allow the passage of a folded protein like cytochrome c. In contrast, the MIM is almost totally impermeable and transport of ions and solutes is granted by mitochondrial carrier proteins. Most mitochondrial proteins exhibit dual functions, a vital metabolic function, and a lethal pro-apoptotic function. This applies to various channels: voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT), Bax, *t*-Bid, Bak; receptors (e.g., TOM22); chaperones (cyclophilin D, CypD), as well as oxidoreductases (apoptosis-inducing factor, AIF).

During apoptosis, many signals can converge to the mitochondrion to MMP, the rate-limiting step in the execution of the death process [1]. MMP is regulated mainly by the members of Bcl-2 family, members of the PTP complex (VDAC, ANT, CypD) and lipids [1]. Bcl-2 family is composed of pro-apoptotic proteins (e.g., Bax, Bak, Bid, Bik, Bnip3) and anti-apoptotic members (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, A1). Pro-apoptotic proteins favor MMP by translocating to MOM and forming mega channels, mainly by oligomerization (e.g., Bax-Bak oligomers or Bax-VDAC complexes), while anti-apoptotic members stabilize MOM and tend to prevent MMP [1, 34–36]. Accumulation of modified lipids (e.g., oxidized cardiolipin, ceramide) and ions (e.g., Ca^{2+}) in the mitochondrion can also influence MMP [37]. Moreover, the intracellular milieu, such as pH, ROS, and ATP levels can contribute to define a permissive environment for MMP execution [1]. Multiple mechanisms can mediate MMP, depending on the cell type and the death stimuli. They can affect either the MOM, or both mitochondrial membranes (MOM+MIM). In the MOM model, intermembrane space proteins are released into the cytosol by passage through large proteic/lipidic channels while, in the MOM+MIM model, intermembrane space proteins are freely released into the cytosol through the MOM ruptures. Nevertheless, these two models can coexist in conditions involving on the one hand the translocation of the truncated form of Bid (tBid) to mitochondria, and in the other hand mitochondrial Ca^{2+} accumulation and ROS increase, as observed in conditions of endoplasmic reticulum stress [1]. In the MOM+MIM model, the contribution of the permeability transition pore (PTP) seems to play an important role. The PTP consists of a multiprotein complex (PTPC) and various proteins are involved in its opening. Long lasting opening of PTPC increases MIM permeability and, in the presence of adequate amounts of ATP, would

lead to apoptotic cell death [1]. PTPC opening is highly sensitive to Ca^{2+}, prooxidant agents, pro-apoptotic Bcl-2 family members and some chemotherapeutics agents [38]. However, Ca^{2+}-induced PTP opening has been also reported to induce necrotic cell death, in particular when intracellular ATP levels are too low to allow apoptosis execution [39].

Once initiated, MMP leads to the release into the cytosol of caspase-dependent proteins (i.e., cytochrome c or Smac/DIABLO) and caspase-independent proteins (such as apoptosis-inducing factor, AIF, or EndoG) with consequent coordinated cell degradation [40]. Concomitantly, MMP provokes a mitochondrial failure with dissipation of the inner membrane potential ($\Delta\Psi m$), subsequent arrest of OXPHOS and ATP synthesis, and increased ROS level. Therefore, MMP constitutes a point of no return of the activation cascade of cell death [41].

3. Mitochondria in Liver Pathology

Most liver pathologies, including alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), drug-induced hepatotoxicity, viral hepatitis, and HCC, are characterized by mitochondrial dysfunctions. Moreover, during liver surgery, liver cells, in particular hepatocytes and endothelial cells suffer ischemia/reperfusion (I/R) injury. In the liver, as well as in other organs such as brain and heart, I/R injury involved mitochondrial permeability transition [1]. Since these abnormalities affect all the aforementioned physiological functions of mitochondria, we will review their roles in liver pathologies with a particular focus on the aspects of cell death regulation, alteration of hepatocyte metabolism, and disruption of ROS homeostasis.

3.1. Mitochondria in Cell Death Regulation. Mitochondria are key organelles in the development of liver diseases characterized by hepatocyte death and subsequent inflammation (Figure 3). Actually, increased hepatocyte apoptosis has been correlated with inflammation, fibrosis, and cell turnover, conditions that are permissive for the development of HCC [2]. Hepatocyte mitochondria are essential in making effective the extrinsic pathway activated by many ligands, such as Fas, TRAIL or TNF-α [2]. Moreover, constitutive expression of both anti-apoptotic proteins Bcl-x_L and Mcl-1, belonging to the Bcl-2 family, is required to avoid spontaneous caspase 3/7 activation, suggesting essential cytoprotective functions of these proteins in the hepatocyte [42, 43]. Bcl-2 is not constitutively expressed in the liver; however, it can be induced in order to cope with I/R, as shown in ischemic preconditioning during partial hepatectomy [44, 45].

Fas- and TRAIL-mediated apoptosis are involved in viral hepatitis, playing a crucial role in the elimination of infected cells and the hepatitis viral core protein binds Mcl-1 impairing its cytoprotective function [46–48]. TNF-α is secreted by infiltrating cytotoxic T lymphocytes during HBV infection and its apoptotic effect seems to be mediated by HBVx protein [2]. Mitochondrial apoptosis is also involved in the pathogenesis of NAFLD and in NASH [15]. In an experimental model using mice fed with a methionine and choline deficient diet, apoptosis was induced by an increase hepatic expression of functional p53, with a concomitant increase in the cleavage of Bid to tBid and a decrease expression of Bcl-x_L [49]. Moreover, p53 was also responsible for TRAIL receptor expression, linking intrinsic and extrinsic apoptosis pathway in NASH [49]. Recently, saturated free fatty acids have been shown to activate the proapoptotic proteins Bim and Bax via JNK, thus inducing MMP, and also increase ROS production [50].

Hepatocyte necrosis is usually considered an accidental (nonprogrammed) form of cell death, resulting from metabolic failure and consequent rapid ATP depletion [51]. It has been firstly described during I/R injury following liver transplantation or hepatectomy, but it is also described in NASH. In fact, hepatocytes necrosis is associated with significant inflammatory response, due to the liberation of IL1-β, TNF-α and other newly described proinflammatory proteins, namely, damage-associated molecular-pattern (DAMP) molecules, such as HMGB1, that activate innate immunity response, such liver resident macrophages (Kupffer cells) and polymorphonuclear cells [45, 51–54]. Recently, accumulated evidence indicates that necrosis can also occur in a regulated manner and that the liberation of cytokines from dying cells can function as a sentinel signal alerting to the need for defensive response [51]. This regulated or programmed necrosis (necroptosis) is initiated by death receptors, like apoptosis, but requires activation of specific kinases (receptor interacting proteins 1 and 3) and its execution involves the active disintegration of mitochondrial, lysosomal, and plasma membranes [55]. Interestingly, in the context of I/R injury, the PTPC opening seems to be a common event anticipating both necrotic cell death and apoptosis, reinforcing the idea that programmed necrosis may be involved in clinical and pathological contexts. In an experimental model of orthotopic liver transplantation in rats, inhibition of PTP by cyclosporine A or acidic pH improved mitochondrial and hepatocellular functions, in particular decreasing the percentage of apoptotic cells but not of necrotic cells [56, 57]. These results seem to confirm the concept that apoptosis is typically an early event in hepatocyte injury. Importantly, in steatotic livers submitted to ischemia/reperfusion, necrosis is predominant compared with normal liver in which apoptosis is the main form of cell death [52, 53]. This difference has been partially linked to the metabolic/energetic difference between steatotic livers and normal liver [52] since fatty liver mitochondria have a decreased content of cytochromes c oxidase, produce superoxide anion and H_2O_2 at increased rate and have an increase content in UPC-2 compared with normal livers, resulting in decreased ATP production that affects apoptosis execution, and favors necrosis [58].

3.2. Mitochondria in Alteration of Hepatocyte Metabolism. The aforementioned data suggest that mitochondria may be the convergence point between various metabolic stresses and cell death in hepatocyte. In this context, it merits noting that the cytosolic glucokinase, or hexokinase IV, the

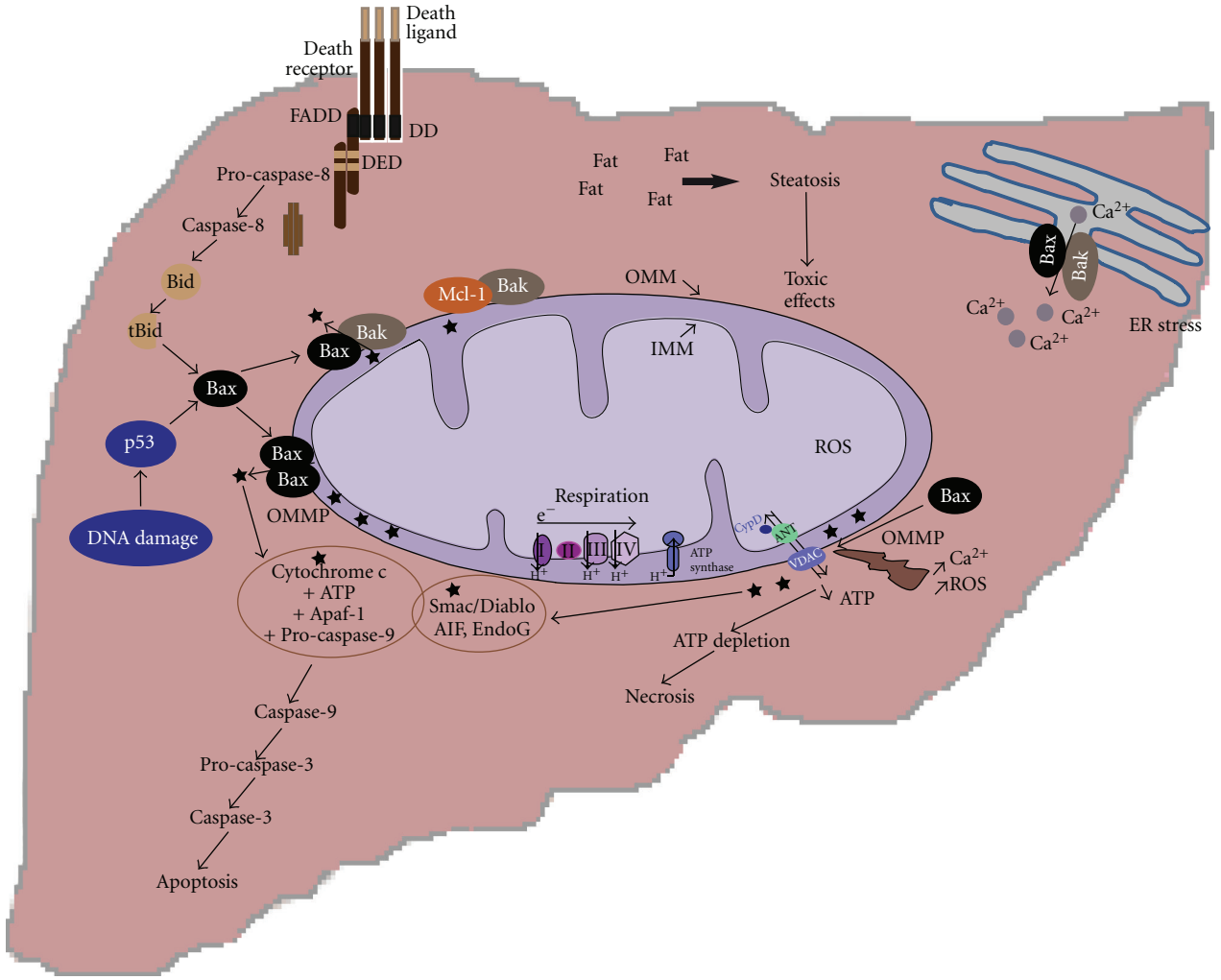


Figure 3: The mitochondria are central organelles in determining cell fate in liver diseases. Hepatocyte cell death is common to many liver diseases. Different stress stimuli can induce death signaling, such as toxic free fatty acids, DNA damage, endoplasmic reticulum (ER) stress observed in metabolic disease. In these contexts, mitochondria are essential to determine cell fate, as in hepatocyte the activation of the intrinsic pathway of apoptosis by cell death receptors is not usually sufficient to induce cell death and liberation of proapoptotic factors from mitochondria is a mostly necessary event. Moreover, previous alterations of mitochondrial function causing decreased ATP synthesis can induce a shift from apoptotic to necrotic cell death.

hepatic/pancreatic isoform of hexokinase, has been recently reported to be associated to mitochondrial proteins, such as Bad, at the MOM [59, 60]. The association of the pro-apoptotic protein Bad with the glucokinase suggests that a close integration exists between the pathways of glucose metabolism and apoptosis [59].

Many studies on obese, diabetic, or NASH patients have shown functional and structural abnormalities in hepatocyte mitochondria, such as OXPHOS impairment or megamitochondria [61]. Interestingly, both increased or decreased β-oxidation in insulin resistant hepatocytes has been reported as characteristic of liver steatosis and insulin resistance [16, 62, 63]. Decrease in β-oxidation activity induces diacylglycerol (DAG) accumulation and steatosis in the hepatocyte with concurrent activation of PKC pathway and inhibition of insulin signaling [62]. In insulin-resistant patients an increased activity of hepatic β-oxidation was observed and this was correlated to an increase in ROS production [61, 64]. Elevated β-oxidation could be an adaptive mechanism to limit free fatty acid lipotoxicity, thus providing large amounts of reduced equivalents (NADH) regardless of energetic requirements finally promoting ROS production due to impairment of respiratory chain [18]. These results linked mitochondrial metabolic dysfunctions to oxidative stress due to increased ROS production.

3.3. Mitochondria in Disruption of ROS Homeostasis. Increased ROS production has been described in most liver pathologies. Augmented generation of mitochondrial ROS has been reported in various animal models of NASH, namely, genetically obese-diabetic ob/ob mice [58] and rats

fed with a choline-deficient diet [65]. Moreover, mitochondria can be an ectopic site of cytochromes P450 2E1 expression [61, 66], where it can produce ROS and induce lipid peroxidation, as shown in the liver of an experimental model of diabetic rat [67].

Mitochondrial dysfunctions and ROS generation have been clearly shown in alcoholic liver disease [68]. Excessive ethanol consumption perturbs sinusoidal blood flow, inducing ischemia regions, and causes increased production of TNF-α, which induces inflammatory cell infiltration and an increase in hepatic O_2 consumption [69, 70]. Chronic ethanol consumption induces profound disruption in mitochondrial metabolism, in particular decreasing the rate of ATP synthesis, thus placing hepatocytes under bioenergetic stress [68]. Under alcohol feeding, mitochondria contribute to the production of ROS in hepatocytes through various mechanisms. Ethanol metabolism increases the availability of NADH, resulting in a more reduced state of complexes I and III of the respiratory chain with a consequent increased probability of superoxide ion production [71]. Moreover, chronic alcohol consumption decreases mitochondrial protein synthesis mainly due to alcohol-mediated damage to mtDNA, contributing to decreased functioning of the oxidative phosphorylation system [72–74].

Mitochondrial ROS also play an important role in viral hepatitis. HCV core protein directly interacts with mitochondria and inhibits complex I activity, inducing an increased production of mitochondrial ROS, reducing threshold for Ca^{2+} and ROS-induced MMP [75]. Moreover, it has been recently shown that during HBV infection, HBx protein interacts with mitochondria, increasing ROS production [76]. The increase in ROS production was necessary, although insufficient, to induce the proinflammatory enzyme cyclooxygenase 2 (COX-2), linking mitochondrial dysfunction with liver inflammation in HBV infection [76]. Numerous investigations have shown that mitochondrial dysfunction is a major mechanism of drug- (or drug-metabolite-) induced liver injury [77]. Different mechanisms of mitochondrial dysfunction have been described in drug-induced hepatotoxicity, including membrane permeabilization, OXPHOS impairment, inhibition of fatty acid oxidation, and mtDNA depletion, and it appears that overproduction of reactive oxygen species by the damaged mitochondria could play a major role [77]. Finally, there is evidence showing a role of ROS in hepatocarcinogenesis [78]. Chemical hepatocarcinogens, such as the mycotoxin aflatoxin B1 and 2-acetylaminofluorene (2-AAF), induced increased ROS production in hepatocytes. In particular, 2-AAF altered mitochondrial redox cycling and it activated NADPH oxidase, an important ROS producing enzyme, through PI3K/Akt pathway [79–81]. Growth factors and activated oncogenes also induce ROS overproduction. Cultured cells treated with epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) showed increased levels of H_2O_2 [82, 83]. Double transgenic mice bearing liver-targeted expression of transforming growth factor and the oncogene *c-myc* develop HCC as early as 4 and 8 months of age and elevated ROS levels associated with lipid peroxidation, mitochondrial damage and decreased GSH were already observed at 2-3 months of age [84].

Thus, it is clear that even a mild dysfunction of mitochondria in the liver could lead to hepatic and systemic pathological conditions and the identification of type and timing of mitochondrial lesions could allow major advancement in prevention, early diagnosis and treatment of systemic and liver diseases.

4. Mitochondria in the Cytoprotection of Damaged Liver Cells to Ensure Homeostasis in Chronic Liver Diseases

Mitochondrial dysfunction is described in various hepatic diseases or lesions, such as NAFLD, I/R injury, drug toxicity or hepatocellular carcinoma, and it is often detected as an early alteration, suggesting its causative effect [6, 85–87]. Cells have developed different mechanisms to keep mitochondrial integrity or to prevent the effects of mitochondrial lesions, such as disposal of damaged mitochondria by autophagy/mitophagy, increased biogenesis of mitochondria or regulation of signaling pathways to ensure energy metabolism and limit cell death and inflammatory response.

4.1. Increased Biogenesis of Mitochondria. Regulation of mitochondria biogenesis is one of the mechanisms developed by cells to keep mitochondrial integrity or to prevent the effects of mitochondrial lesions. The peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) belongs to the family of PGC-1 transcriptional coactivators (PGC-1 alpha, PGC-1 beta and PRC), which have been shown to be master regulators of mitochondrial biogenesis, and cellular energy metabolism in many organs, including liver [88, 89]. PGC-1 alpha is present at low but inducible levels in the liver where it also regulates most of the metabolic pathways, including gluconeogenesis, fatty acid β-oxidation, ketogenesis and heme biosynthesis (Figure 4) [90–93]. Under stress conditions, such as low temperature, fasting or energy deprivation, PGC-1 alpha is activated both transcriptionally by cAMP response element binding protein (CREB) and post-traductionally by AMP-activated-protein-kinase- (AMPK-) induced phosphorylation and SIRT1-mediated deacetylation [89]. Following PGC-1 alpha activation, different nuclear factors are subsequently activated. In particular, an activation of the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) is observed and is followed by increased expression of multiple mitochondrial proteins. Moreover, PGC-1 alpha activates the nuclear receptors peroxisome proliferator-activated receptor alpha (PPAR alpha) and the estrogen-related receptor alpha (ERRalpha) both promoting the transcription of genes involved in β-oxidation, such as medium chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase-1A (CPT-1A) [94, 95]. The absence of adequate levels of PGC-1 alpha is correlated with mice developing fasting hypoglycemia and hepatic steatosis, while mouse models of type 1 and type 2 diabetes showed high hepatic levels of PGC-1 alpha [90]. However, it has been recently shown that the different tissue-specific

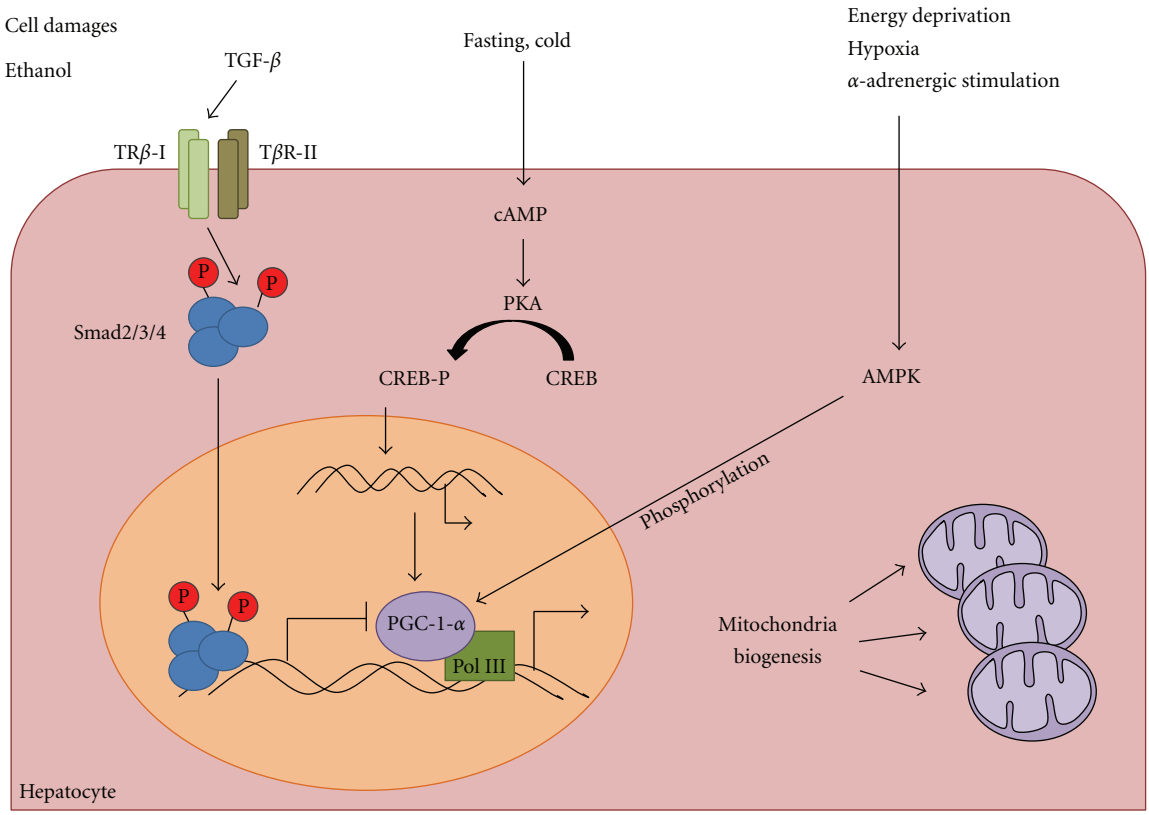


Figure 4: Mitochondria biogenesis allows tissue adaption under stress. Mitochondria biogenesis has been recently recognized as a central pathway in the adaptation of stress conditions in the liver, such as fasting, energy deprivation, hypoxia, or alcohol consumption. Different signaling pathways converge on the master regulator of mitochondria biogenesis, PGC1-alpha. In particular, AMPK and PKA signaling may activate gene transcription controlled by PGC1-alpha, while the TGF-β has been shown to inhibit PGC1-alpha-induced gene transcription.

functions of PGC-1 alpha are tightly and independently regulated [96]. In particular, S6 kinase-1 (S6K1), activated in the liver upon feeding, can phosphorylate PGC-1 alpha, decreasing its capacity to turn on genes of gluconeogenesis, while keeping the functions of activator of mitochondrial and fatty acid oxidation genes intact [96]. S6K1, liver kinase B1 and AMPK are key kinases in the regulation of energy metabolism in the liver. Actually, AMPK is emerging as a kinase that links energy metabolism to mitochondrial function and biogenesis since components downstream of AMPK may contribute to stabilize mitochondrial membrane potential for hepatocyte survival, strengthening the relationship between fuel metabolism and cell survival [10]. Actually, in the liver, the activation of AMPK has been shown to decrease gluconeogenesis and fatty acid synthesis, to increase fatty acid oxidation and mitochondrial biogenesis and this could be linked to PGC-1 alpha phosphorylation, as previously observed in skeletal muscle [97, 98]. Interestingly, the hepatitis B virus (HBV) uses the transcriptional machinery involved in the hepatic response to fasting for its own amplification, thus HBV life cycle is under the control of PGC-1 alpha that could be a new target for antiviral therapy [99]. The dynamic changes in mitochondrial morphology, connectivity, and subcellular distribution are also major mechanisms in cellular homeostasis. They are critically dependent on a highly regulated fusion and fission machinery. Mitochondrial function, dynamics, and quality control are vital for the maintenance of tissue integrity [100]. In the liver, it has been shown that specific protection against hepatocyte mitochondrial dysfunction plays a preventive role in early stages of fibrogenesis, delaying, but not avoiding, its onset [101]. In this context, it is interesting to note that TGF-β/Smad3 signaling pathway, known to be implicated in liver fibrogenesis, has been shown to regulate glucose and energy homeostasis. Smad3-deficient mice are protected from diet-induced obesity and diabetes and Smad3 acts as a repressor of PGC-1α expression, thus suggesting a link between failure in mitochondrial biogenesis, metabolic syndrome, and liver fibrosis [102].

4.2. Autophagy and Mitophagy as Mechanisms to Limit Mitochondrial Lesions. Autophagy is a cellular pathway by which cytoplasmic materials, including organelles, reach lysosomes for degradation. Autophagy may occur either as a general phenomenon, for instance, during nutrient deprivation, or it can specifically target distinct cellular structures, such as damaged mitochondria (mitophagy) [13]. An important interplay exists between induction of autophagy and mitochondria. Actually mitochondria seem to have a key role

in general autophagy as they may supply membranes for the biogenesis of autophagosomes during starvation [103]. Moreover, low ATP production or enhanced ROS generation by mitochondria induces general autophagy [104, 105]. The selective removal of mitochondria by mitophagy regulates mitochondrial number to match metabolic demand and is considered a form of quality control to remove damaged mitochondria [106]. Induction of general autophagy by a sublethal stress before a lethal stress can protect cells against cell death [13]. Indeed, we showed that ischemic preconditioning of livers previously treated by chemotherapy or steatotic livers induced autophagy and decreased necrosis without altering apoptosis. [45, 54]. The elimination of damaged mitochondria has been correlated to resistance of residual mitochondria to MMP and opening of PTP, two early events of apoptotic/necrotic cell death. This can be explained either by the removal of mitochondria that have a low threshold for permeabilization or by the fact that MMP or PTP opening occurs in a fraction of mitochondria and may activate autophagic disposal of depolarized mitochondria [106, 107]. Different mechanisms may regulate mitophagy. The dual system PINK1-Parkin is well described especially in neural tissues. The stabilization of the kinase PINK1 occurs at the surface of mitochondria with low $\Delta\Psi_{mito}$ with the subsequent recruitment of the ubiquitin ligase Parkin and ubiquitinylation of outer membrane proteins [108]. Mitophagy can be also stimulated by histone deacetylase 6, which is recruited to mitochondria and catalyzes proautophagic cytoplasmic deacetylation reactions [109].

Interestingly, although hepatic PINK1-expression is described [110], to our knowledge, no reports on PINK1 dependent mitophagy in the liver are published. Another mechanism of mitophagy involves the activation of AMPK [111]. AMPK phosphorylates and activates ULK1, one of the initiators of autophagy and the genetic loss of AMPK or ULK1 results in defective mitophagy in mammalian liver and *C. elegans*. These findings showed a conserved mechanism coupling nutrient status with autophagy and cell survival [111]. Interestingly, mitochondrial degradation by autophagy was also described in the liver of GFP-LC3 transgenic mice following nutrient deprivation, reinforcing the results linking AMPK regulation of mitophagy [112].

4.3. Mitochondria Can Integrate Energy, Nutrient Metabolism, and Oxidative Stress Responses Determining Cell Fate. Insulin, secreted by pancreatic beta cells upon nutrient stimulation, is one of the most important regulators of nutrient utilization and metabolic homeostasis in the liver. Insulin resistance, a hallmark of NASH and more generally of metabolic syndrome and type II diabetes, is accompanied by reduction of mitochondrial OXPHOS activity and increased ROS production [113]. On the other hand, ROS produced during mitochondrial OXPHOS promote insulin signaling through oxidation of insulin receptor and inhibition of phosphatases, such as PTP1B and PTEN [113]. Importantly, recent investigations pointed out a tight molecular crosstalk between cell survival or cell death pathways and energy metabolism. Using *ex vivo* multinuclear NMR-spectroscopy to study metabolic pathways of [U-(13)C] glucose in mouse liver during Fas-induced apoptosis, Gottschalk et al. found early upregulations in glucose metabolic pathways occurred prior to any visible signs of apoptosis, accompanied by an increased mitochondrial energy production and cellular glutathione synthesis [114]. This metabolic shift seems to potentially contribute to the initiation of apoptosis by mitochondrial energy production and cellular glutathione stores, thus orienting cell fate towards a less pro-inflammatory death. A biochemical analysis using liver mitochondria of two strains of mice (A/J and C57Bl/6, respectively, resistant and susceptible to high-fat diet-induced hepatosteatosis) confirmed a rapid increase by high-fat diet feeding of the respiration rate in A/J but not C57Bl/6 mice. Importantly, ATP production was the same in both types of mitochondria, indicating increased uncoupling of the A/J mitochondria [115]. These results suggest that livers can adapt to high-fat diet feeding by increasing the activity of the oxidative phosphorylation chain and its uncoupling to dissipate the excess of incoming metabolic energy and to reduce the production of ROS [115].

As we mentioned above, liver mitochondria are essential in ammonia detoxification following protein catabolism. In recent years, studies from several laboratories have uncovered a number of factors and pathways that appear to be critically involved in the pathogenesis of hepatic encephalopathy. Foremost is oxidative and nitrosative stress (ONS) and the MMP playing major roles in the mechanism of ammonia-induced astrocyte swelling [116]. The accumulation of intramitochondrial glutamine has been involved. Norenberg et al. [117] were first to describe that the newly synthesized glutamine could be toxic when subsequently metabolized in mitochondria by phosphate-activated glutaminase, yielding glutamate and ammonia. Thus, glutamine can be considered as a carrier of ammonia. The authors propose to consider the intramitochondrial glutamine as a Trojan horse that interferes with mitochondrial function giving rise to excessive production of free radicals and induction of the MPT, two phenomena known to bring about astrocyte dysfunction, including cell swelling.

Moreover, an ammonia-induced increase in intracellular Ca^{2+} has been described which activates a number of enzymes promoting the synthesis of reactive oxygen-nitrogen species, including constitutive nitric oxide synthase, NADPH oxidase and phospholipase A2. ONS subsequently induces the opening of PTP and activates mitogen-activated protein (MAP) kinases and the transcription factor nuclear factor-kappaB (NF-κB). These factors act to generate additional reactive oxygen-nitrogen species, to phosphorylate various proteins and transcription factors, and to cause mitochondrial dysfunction [26]. The pathways and factors described above provide attractive targets for identifying agents potentially useful in the therapy of HE and other hyperammonemic disorders. The most promising of them is the glutamate/glutamine cycle. Indeed, in hyperoxia, glutamine has been described to protect cellular structures, especially mitochondria, from damage. This has been attributed to the activity of the tricarboxylic acid cycle enzyme alpha-ketoglutarate dehydrogenase that was partially

protected by its indirect substrate, glutamine, indicating a mechanism of mitochondrial protection [118]. Glutamate dehydrogenase (GDH), a mitochondrial enzyme linking the Krebs cycle to the multifunctional amino acid glutamate could be also an interesting target. Indeed, GDH controls production and consumption of glutamate. GDH activity is under the control of several regulators, conferring to this enzyme energy-sensor property. Indeed, GDH directly depends on the provision of the cofactor NADH/NAD(+), rendering the enzyme sensitive to the redox status of the cell. Moreover, GDH is allosterically regulated by GTP and ADP. GDH is also regulated by ADP-ribosylation, mediated by a member of the energy-sensor family sirtuins, namely, SIRT4. In the brain, GDH ensures the cycling of the neurotransmitter glutamate between neurons and astrocytes. GDH also controls ammonia metabolism and detoxification, mainly in the liver and kidney. Eng and Abraham [119] have described that ammonia, generated from Gln deamination (glutaminolysis) in mitochondria, functions as an autocrine- and/or paracrine-acting stimulator of autophagic flux. Recently, Nissim et al. [120] reported a downregulation of hepatic urea synthesis by oxypurines. Indeed, xanthine and uric acid, both physiologically occurring oxypurines, inhibited the hepatic synthesis of N-acetylglutamate, the key regulator of the first step of mitochondrial urea cycle.

As discussed above, mitochondria are a main source of ROS in hepatocytes and ROS importantly contribute in liver health and disease. While ROS has been commonly associated to lipid, protein, and DNA oxidation and consequent cellular damage, recent studies have shown that mitochondria-generated ROS may be regulated and may regulate many signaling pathways [27, 121]. Oxidative stress may activate prosurvival pathways in hepatocytes, such as NF-κB and NRFs [122–124]. NF-κB regulates a complex network of pathways, as it is known to control the transcription of over 150 genes [122]. Depending on cell type, microenvironmental conditions and eventually costimulated pathways, NF-κB may exert either a prosurvival or a proapoptotic function [122, 125]. In the context of hepatic oxidative stress, it has been shown that NF-κB may induce antiapoptotic factors, such as XIAP, and function like antioxidants in preventing TGF-beta 1-JNK induced-apoptosis [126]. Moreover, NF-κB collaborates with p38 MAP kinase signaling cascade to protect hepatocytes from liver injury induced by TNF-alpha [125]. NRFs also regulates oxidative stress response in the liver. In particular, NRF-1 has been shown to promote cell survival of hepatocytes during development, sustaining the transcription of antioxidant genes and protecting embryonic hepatocytes from TNF-mediated apoptosis [124]. Moreover, NRF-1 has been shown to be induced under prooxidant conditions and to promote the transcription of mitochondrial transcription factor A (Tfam), required for mitochondrial DNA transcription and replication [123]. Hypoxia is another clear example of cell signaling mediated by ROS. Hypoxia is a clinical relevant event both in liver ischemia/reperfusion injury and in hepatocellular carcinoma development. It leads to an increase in production of H_2O_2 from mitochondrial complex III, thus creating a cytosolic signal that stabilizes the hypoxia inducible transcription factors HIF-1 [27, 127]. Moreover, during hypoxia ROS activates AMPK, which in turn phosphorylates Na/K ATPase (in order to promote its endocytosis) and mTOR (in order to decrease protein translation), thus contributing to energy conservation [128, 129]. In addition, hypoxia-induced mitochondrial ROS enhance the DNA binding of NF-κB through a redox-dependent mechanism involving the mitochondrial glutathione (mGSH) pool in cancer cells, including hepatoma cell lines [130, 131]. In this context, mGSH regulates the intensity of ROS diffusion in the cytoplasm, allowing activation of the c-Src kinase, with subsequent phosphorylation of the inhibitory subunit IκB, activation of NF-κB and promotion of cancer cell survival [127, 130]. The liver is one of the organs with the highest content of GSH and mGSH plays a central role in regulating both in antioxidant defense against excessive ROS production and in regulation of ROS signaling in liver physiology and pathology [130]. Alcohol consumption has been shown to sensitize hepatocytes to TNF because of mGSH depletion through impaired transport of GSH to mitochondria [132]. Interestingly, GSH transport impairment and TNF sensitization correlate with free cholesterol accumulation in mitochondrial membranes and seem to be a common pro-inflammatory mechanism in both alcoholic and nonalcoholic steatohepatitis [133]. Similar alterations in mGSH regulation have been reported in liver cirrhosis, in particular in an experimental model of secondary biliary cirrhosis in rats induced by bile-duct ligation [134, 135].

During the past decade, a new family of enzymes, the nicotinamide-adenine-dinucleotide- (NAD-) dependent protein deacetylases named sirtuins, has been described to contribute to extended lifespan many animal models, including mammals [136]. Interestingly of the six mammalian sirtuins, three (SIRT3, 4, and 5) are expressed in the mitochondria where they mediate physiologic adaptation to reduced energy consumption [137]. In the liver, SIRT4 activity was shown to decline during calorie restriction, allowing the consumption of glutamine as a fuel source for glucose synthesis. Moreover, SIRT4 depletion increased fatty acid oxidation [138]. Mitochondrial sirtuins could be also interesting targets in the regulation of ammonia production and disposal. Nakagawa et al. [139] have shown that the sirtuin SIRT5 activates CPS1, which we mentioned before as the first enzyme in the urea cycle. In mice, NAD in liver mitochondria increases during fasting, thereby triggering SIRT5-mediated deacetylation of CPS1 and adaptation to increase in amino acid catabolism. These data indicate SIRT5 also has an emerging role in the metabolic adaptation to fasting, high protein diet and calorie restriction. Finally, recent findings correlate SIRT3 to the production of ROS. In particular, SIRT3$^{-/-}$ cells produce increased levels of ROS and have concomitantly a reduced ATP production [11, 12, 140]. These results suggest that SIRT3-mediated deacetylation of electron transport chain may render OXPHOS more efficient [137]. Moreover, SIRT3 may deacetylate and activate the antioxidant enzyme mitochondrial superoxide dismutase (SOD2) and the isocitrate dehydrogenase 2, which generates NADPH for the glutathione synthesis, in mice [141–143].

The sirtuins' antiaging role and their ability of controlling energy metabolism make them interesting target in cancer and metabolic diseases. Interestingly, in a mouse model of metabolic syndrome-associated liver cancer, overexpression of SIRT1 reduced the susceptibility to liver cancer and improved hepatic protection from both DNA damage and metabolic damage [136]. However, recent studies showed that SIRT1 was upregulated in HCC and it has a role in telomere maintenance [144, 145]. Downregulation of SIRT1 suppressed proliferation of HCC cells and induced cellular senescence or apoptosis [144]. Finally, many recent papers show a possible synergic action of cytosolic and mitochondrial sirtuins in regulating glucose and lipid metabolism in the liver [138, 140, 146–148]. SIRT1 has been shown to regulate hepatic glucose and lipid metabolism by activating AMPK and by inducing gluconeogenic genes via activation of PGC-1 alpha in hepatic cell and mouse liver [146, 147]. Interestingly, SIRT1 did not regulate the PGC-1 alpha effects on mitochondrial biogenesis. The mitochondrial SIRT3 was shown to positively modulate fatty acid oxidation and ATP production, in particular deacetylating the long-chain acyl-CoA dehydrogenase and Complex I of the electron transport chain [140, 148]. Finally, in a recent paper, high-fat diet induced a decrease of hepatic SIRT3, hyperacetylation of mitochondrial proteins and fatty liver in mice [149].

Altogether, the studies reviewed show that mitochondria are much more dynamic organelles than considered traditionally. They are key organelles in the integration and adaptation to external stimuli, such as changing composition of diet (i.e., calorie restriction *versus* high fat diet), hypoxia, cold exposure, or physical exercise [150]. Mitochondrial homeostasis is a highly controlled process balancing organelle biogenesis and degradation (essentially by autophagy/mitophagy) and an alteration of this balance may bring to organelle dysfunction, contributing to the development of liver chronic diseases.

5. Conclusions

The liver is one of the organs richest in mitochondria. Hepatic mitochondria have unique features compared to other organs' mitochondria, since they are the hub that integrates hepatic metabolism of carbohydrates, lipids, and proteins. Thus, correct functioning of hepatic mitochondria is essential not only to prevent liver disease, such as NAFLD, but also to avoid systemic diseases, such as ammonia-induced hepatic encephalopathy. Mitochondria are also essential in hepatocyte survival as mediator of apoptosis and necrosis. Hepatocyte cell death is involved in most liver pathologies, such as alcoholic and nonalcoholic steatohepatitis, viral hepatitis, liver fibrosis, and carcinogenesis. Hepatocytes have developed different mechanisms to keep mitochondrial integrity or to prevent the effects of mitochondrial lesions, in particular regulating organelle biogenesis and degradation. A better knowledge of the mechanisms and pathways involved in mitochondria homeostasis should improve preventive and therapeutic strategies for liver diseases.

Abbreviations

2-AAF: 2-Acetylaminofluorene
DAG: Diacylglycerol
GS: Glutamine synthetase
GSH: Glutathione
HCC: Hepatocellular carcinoma
MIM: Mitochondrial inner membrane
MMP: Mitochondrial membrane permeabilization
MOM: Mitochondrial outer membrane
MRC: Mitochondrial respiratory chain
NAG: N-acetyl-L-glutamate
NASH: Nonalcoholic steatohepatitis
OXPHOS: Oxidative phosphorylation
PTP: Permeability transition pore
ROS: Reactive oxygen species.

References

[1] G. Kroemer, L. Galluzzi, and C. Brenner, "Mitochondrial membrane permeabilization in cell death," *Physiological Reviews*, vol. 87, no. 1, pp. 99–163, 2007.

[2] H. Malhi, M. E. Guicciardi, and G. J. Gores, "Hepatocyte death: a clear and present danger," *Physiological Reviews*, vol. 90, no. 3, pp. 1165–1194, 2010.

[3] K. L. Veltri, M. Espiritu, and G. Singh, "Distinct genomic copy number in mitochondria of different mammalian organs," *Journal of Cellular Physiology*, vol. 143, no. 1, pp. 160–164, 1990.

[4] P. H. Yin, H. C. Lee, G. Y. Chau et al., "Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma," *British Journal of Cancer*, vol. 90, no. 12, pp. 2390–2396, 2004.

[5] A. Chatterjee, S. Dasgupta, and D. Sidransky, "Mitochondrial subversion in cancer," *Cancer Prevention Research*, vol. 4, no. 5, pp. 638–654, 2011.

[6] R. S. Rector, J. P. Thyfault, G. M. Uptergrove et al., "Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model," *Journal of Hepatology*, vol. 52, no. 5, pp. 727–736, 2010.

[7] M. E. Breuer, P. H. G. M. Willems, F. G. M. Russel, W. J. H. Koopman, and J. A. M. Smeitink, "Modeling mitochondrial dysfunctions in the brain: from mice to men," *Journal of Inherited Metabolic Disease*, vol. 35, no. 2, pp. 193–210, 2011.

[8] S. Ong and A. B. Gustafsson, "New roles for mitochondria in cell death in the reperfused myocardium," *Cardiovascular Research*, vol. 94, no. 2, pp. 190–196, 2012.

[9] D. Degli Esposti, M. C. Domart, M. Sebagh et al., "Autophagy is induced by ischemic preconditioning in human livers formerly treated by chemotherapy to limit necrosis," *Autophagy*, vol. 6, no. 1, pp. 172–174, 2010.

[10] Y. M. Yang, C. Y. Han, Y. J. Kim, and S. G. Kim, "AMPK-associated signaling to bridge the gap between fuel metabolism and hepatocyte viability," *World Journal of Gastroenterology*, vol. 16, no. 30, pp. 3731–3742, 2010.

[11] H. S. Kim, K. Patel, K. Muldoon-Jacobs et al., "SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress," *Cancer Cell*, vol. 17, no. 1, pp. 41–52, 2010.

[12] K. H. Kim, Y. S. Kum, Y. Y. Park et al., "The protective effect of bee venom against ethanol-induced hepatic injury via

regulation of the mitochondria-related apoptotic pathway," *Basic and Clinical Pharmacology and Toxicology*, vol. 107, no. 1, pp. 619–624, 2010.

[13] D. R. Green, L. Galluzzi, and G. Kroemer, "Mitochondria and the autophagy-inflammation-cell death axis in organismal aging," *Science*, vol. 333, no. 6046, pp. 1109–1112, 2011.

[14] M. Pérez-Carreras, P. Del Hoyo, M. A. Martín et al., "Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis," *Hepatology*, vol. 38, no. 4, pp. 999–1007, 2003.

[15] E. Bugianesi, A. J. McCullough, and G. Marchesini, "Insulin resistance: a metabolic pathway to chronic liver disease," *Hepatology*, vol. 42, no. 5, pp. 987–1000, 2005.

[16] E. Fabbrini, S. Sullivan, and S. Klein, "Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications," *Hepatology*, vol. 51, no. 2, pp. 679–689, 2010.

[17] L. Boon, W. J. C. Geerts, A. Jonker, W. H. Lamers, and C. J. F. Van Noorden, "High protein diet induces pericentral glutamate dehydrogenase and ornithine aminotransferase to provide sufficient glutamate for pericentral detoxification of ammonia in rat liver lobules," *Histochemistry and Cell Biology*, vol. 111, no. 6, pp. 445–452, 1999.

[18] G. Vial, H. Dubouchaud, and X. M. Leverve, "Liver mitochondria and insulin resistance," *Acta Biochimica Polonica*, vol. 57, no. 4, pp. 389–392, 2010.

[19] G. Serviddio, F. Bellanti, R. Tamborra et al., "Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia-reperfusion injury," *Gut*, vol. 57, no. 7, pp. 957–965, 2008.

[20] R. S. Kaplan and J. A. Mayor, "Structure, function and regulation of the tricarboxylate transport protein from rat liver mitochondria," *Journal of Bioenergetics and Biomembranes*, vol. 25, no. 5, pp. 503–514, 1993.

[21] L. Caldovic and M. Tuchman, "N-acetylglutamate and its changing role through evolution," *Biochemical Journal*, vol. 372, no. 2, pp. 279–290, 2003.

[22] J. M. Matés, J. A. Segura, J. A. Campos-Sandoval et al., "Glutamine homeostasis and mitochondrial dynamics," *International Journal of Biochemistry and Cell Biology*, vol. 41, no. 10, pp. 2051–2061, 2009.

[23] S. W. M. Olde Damink, R. Jalan, and C. H. C. Dejong, "Interorgan ammonia trafficking in liver disease," *Metabolic Brain Disease*, vol. 24, no. 1, pp. 169–181, 2009.

[24] J. Albrecht, "Roles of neuroactive amino acids in ammonia neurotoxicity," *Journal of Neuroscience Research*, vol. 51, no. 2, pp. 133–138, 1998.

[25] V. Felipo, C. Hermenegildo, C. Montoliu, M. Llansola, and M. D. Minana, "Neurotoxicity of ammonia and glutamate: molecular mechanisms and prevention," *NeuroToxicology*, vol. 19, no. 4-5, pp. 675–682, 1998.

[26] R. F. Butterworth, "Pathophysiology of hepatic encephalopathy: the concept of synergism," *Hepatology Research*, vol. 38, no. 1, pp. S116–S121, 2008.

[27] R. B. Hamanaka and N. S. Chandel, "Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes," *Trends in Biochemical Sciences*, vol. 35, no. 9, pp. 505–513, 2010.

[28] T. A. Young, C. C. Cunningham, and S. M. Bailey, "Reactive oxygen species production by the mitochondrial respiratory chain in isolated rat hepatocytes and liver mitochondria: studies using myxothiazol," *Archives of Biochemistry and Biophysics*, vol. 405, no. 1, pp. 65–72, 2002.

[29] Y. Kushnareva, A. N. Murphy, and A. Andreyev, "Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and $NAD(P)^+$ oxidation-reduction state," *Biochemical Journal*, vol. 368, no. 2, pp. 545–553, 2002.

[30] H. Nohl, L. Gille, and K. Staniek, "Intracellular generation of reactive oxygen species by mitochondria," *Biochemical Pharmacology*, vol. 69, no. 5, pp. 719–723, 2005.

[31] D. C. Wallace, W. Fan, and V. Procaccio, "Mitochondrial energetics and therapeutics," *Annual Review of Pathology*, vol. 5, pp. 297–348, 2010.

[32] S. Rexroth, A. Poetsch, M. Rögner et al., "Reactive oxygen species target specific tryptophan site in the mitochondrial ATP synthase," *Biochimica et Biophysica Acta*, vol. 1817, no. 2, pp. 381–387, 2012.

[33] M. O. Dietrich and T. L. Horvath, "The role of mitochondrial uncoupling proteins in lifespan," *Pflugers Archiv European Journal of Physiology*, vol. 459, no. 2, pp. 269–275, 2010.

[34] A. Letai, M. C. Bassik, L. D. Walensky, M. D. Sorcinelli, S. Weiler, and S. J. Korsmeyer, "Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics," *Cancer Cell*, vol. 2, no. 3, pp. 183–192, 2002.

[35] S. Shimizu, M. Narita, and Y. Tsujimoto, "Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC," *Nature*, vol. 399, no. 6735, pp. 483–487, 1999.

[36] M. Zoratti and I. Szabo, "The mitochondrial permeability transition," *Biochimica et Biophysica Acta*, vol. 1241, no. 2, pp. 139–176, 1995.

[37] J. Montero, M. Mari, A. Colell et al., "Cholesterol and peroxidized cardiolipin in mitochondrial membrane properties, permeabilization and cell death," *Biochimica et Biophysica Acta*, vol. 1797, no. 6-7, pp. 1217–1224, 2010.

[38] C. Brenner and S. Grimm, "The permeability transition pore complex in cancer cell death," *Oncogene*, vol. 25, no. 34, pp. 4744–4756, 2006.

[39] A. Rasola and P. Bernardi, "Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis," *Cell Calcium*, vol. 50, no. 3, pp. 222–233, 2011.

[40] J. E. Chipuk and D. R. Green, "Do inducers of apoptosis trigger caspase-indipendent cell death?" *Nature Reviews Molecular Cell Biology*, vol. 6, no. 3, pp. 268–275, 2005.

[41] D. R. Green and G. Kroemer, "The pathophysiology of mitochondrial cell death," *Science*, vol. 305, no. 5684, pp. 626–629, 2004.

[42] T. Takehara, T. Tatsumi, T. Suzuki et al., "Hepatocyte-specific disruption of Bcl-xL leads to continuous hepatocyte apoptosis and liver fibrotic responses," *Gastroenterology*, vol. 127, no. 4, pp. 1189–1197, 2004.

[43] B. Vick, A. Weber, T. Urbanik et al., "Knockout of myeloid cell leukemia-1 induces liver damage and increases apoptosis susceptibility of murine hepatocytes," *Hepatology*, vol. 49, no. 2, pp. 627–636, 2009.

[44] F. Charlotte, A. L'Hermine, N. Martin et al., "Immunohistochemical detection of bcl-2 protein in normal and pathological human liver," *American Journal of Pathology*, vol. 144, no. 3, pp. 460–465, 1994.

[45] M. C. Domart, D. Degli Esposti, M. Sebagh et al., "Concurrent induction of necrosis, apoptosis, and autophagy in ischemic preconditioned human livers formerly treated by chemotherapy," *Journal of Hepatology*, vol. 51, no. 5, pp. 881–889, 2009.

[46] P. R. Galle, W. J. Hofmann, H. Walczak et al., "Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage,"

Journal of Experimental Medicine, vol. 182, no. 5, pp. 1223–1230, 1995.

[47] B. Mundt, F. Kühnel, L. Zender et al., "Involvement of TRAIL and its receptors in viral hepatitis," *The FASEB Journal*, vol. 17, no. 1, pp. 94–96, 2003.

[48] N. K. Mohd-Ismail, L. Deng, S. K. Sukumaran, V. C. Yu, H. Hotta, and Y. J. Tan, "The hepatitis C virus core protein contains a BH3 domain that regulates apoptosis through specific interaction with human Mcl-1," *Journal of Virology*, vol. 83, no. 19, pp. 9993–10006, 2009.

[49] G. C. Farrell, C. Z. Larter, J. Y. Hou et al., "Apoptosis in experimental NASH is associated with p53 activation and TRAIL receptor expression," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 3, pp. 443–452, 2009.

[50] N. Alkhouri, C. Carter-Kent, and A. E. Feldstein, "Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications," *Expert Review of Gastroenterology and Hepatology*, vol. 5, no. 2, pp. 201–212, 2011.

[51] R. S. Hotchkiss, A. Strasser, J. E. McDunn, and P. E. Swanson, "Mechanisms of disease: cell death," *New England Journal of Medicine*, vol. 361, no. 16, pp. 1570–1583, 2009.

[52] M. Selzner, H. A. RüDiger, D. Sindram, J. Madden, and P. A. Clavien, "Mechanisms of ischemic injury are different in the steatotic and normal rat liver," *Hepatology*, vol. 32, no. 6, pp. 1280–1288, 2000.

[53] C. Peralta, R. Bartrons, A. Serafin et al., "Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat," *Hepatology*, vol. 34, no. 6, pp. 1164–1173, 2001.

[54] D. Degli Esposti, M. Sebagh, P. Pham et al., "Ischemic preconditioning induces autophagy and limits necrosis in human recipients of fatty liver grafts, decreasing the incidence of rejection episodes," *Cell Death and Disease*, vol. 2, no. 1, article e111, 2011.

[55] P. Vandenabeele, L. Galluzzi, T. Vanden Berghe, and G. Kroemer, "Molecular mechanisms of necroptosis: an ordered cellular explosion," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 10, pp. 700–714, 2010.

[56] C. Plin, P. S. Haddad, J. P. Tillement, A. Elimadi, and D. Morin, "Protection by cyclosporin A of mitochondrial and cellular functions during a cold preservation-warm reperfusion of rat liver," *European Journal of Pharmacology*, vol. 495, no. 2-3, pp. 111–118, 2004.

[57] J. S. Kim, L. He, T. Qian, and J. J. Lemasters, "Role of the mitochondrial permeability transition in apoptotic and necrotic death after ischemia/reperfusion injury to hepatocytes," *Current Molecular Medicine*, vol. 3, no. 6, pp. 527–535, 2003.

[58] S. Yang, H. Zhu, Y. Li et al., "Mitochondrial adaptations to obesity-related oxidant stress," *Archives of Biochemistry and Biophysics*, vol. 378, no. 2, pp. 259–268, 2000.

[59] N. N. Danial, C. F. Gramm, L. Scorrano et al., "BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis," *Nature*, vol. 424, no. 6951, pp. 952–956, 2003.

[60] C. Arden, S. Baltrusch, and L. Agius, "Glucokinase regulatory protein is associated with mitochondria in hepatocytes," *FEBS Letters*, vol. 580, no. 8, pp. 2065–2070, 2006.

[61] K. Begriche, A. Igoudjil, D. Pessayre, and B. Fromenty, "Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it," *Mitochondrion*, vol. 6, no. 1, pp. 1–38, 2006.

[62] D. Zhang, Z. X. Liu, S. C. Cheol et al., "Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 43, pp. 17075–17080, 2007.

[63] D. Pessayre, "Role of mitochondria in non-alcoholic fatty liver disease," *Journal of Gastroenterology and Hepatology*, vol. 22, no. 1, pp. S20–S27, 2007.

[64] D. Pessayre and B. Fromenty, "NASH: a mitochondrial disease," *Journal of Hepatology*, vol. 42, no. 6, pp. 928–940, 2005.

[65] K. Hensley, Y. Kotake, H. Sang et al., "Dietary choline restriction causes complex I dysfunction and increased H_2O_2 generation in liver mitochondria," *Carcinogenesis*, vol. 21, no. 5, pp. 983–989, 2000.

[66] M. A. Robin, H. K. Anandatheerthavarada, J. K. Fang, M. Cudic, L. Otros, and N. G. Avadhani, "Mitochondrial targeted cytochrome P450 2E1 (P450 MT5) contains an intact N terminus and requires mitochondrial specific electron transfer proteins for activity," *Journal of Biological Chemistry*, vol. 276, no. 27, pp. 24680–24689, 2001.

[67] H. Raza, S. K. Prabu, M. A. Robin, and N. G. Avadhani, "Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: tissue-specific variations and roles in Oxidative stress," *Diabetes*, vol. 53, no. 1, pp. 185–194, 2004.

[68] S. K. Mantena, A. L. King, K. K. Andringa, A. Landar, V. Darley-Usmar, and S. M. Bailey, "Novel interactions of mitochondria and reactive oxygen/nitrogen species in alcohol mediated liver disease," *World Journal of Gastroenterology*, vol. 13, no. 37, pp. 4967–4973, 2007.

[69] N. Sato, T. Kamada, and S. Kawano, "Effect of acute and chronic ethanol consumption on hepatic tissue oxygen tension in rats," *Pharmacology Biochemistry and Behavior*, vol. 18, no. 1, pp. 443–447, 1983.

[70] N. Sato, "Central role of mitochondria in metabolic regulation of liver pathophysiology," *Journal of Gastroenterology and Hepatology*, vol. 22, no. 1, pp. S1–S6, 2007.

[71] S. M. Bailey and C. C. Cunningham, "Contribution of mitochondria to oxidative stress associated with alcoholic liver disease," *Free Radical Biology and Medicine*, vol. 32, no. 1, pp. 11–16, 2002.

[72] A. Venkatraman, A. Landar, A. J. Davis et al., "Modification of the mitochondrial proteome in response to the stress of ethanol-dependent hepatotoxicity," *Journal of Biological Chemistry*, vol. 279, no. 21, pp. 22092–22101, 2004.

[73] A. Cahill, X. Wang, and J. B. Hoek, "Increased oxidative damage to mitochondrial DNA following chronic ethanol consumption," *Biochemical and Biophysical Research Communications*, vol. 235, no. 2, pp. 286–290, 1997.

[74] W. B. Coleman and C. C. Cunningham, "Effects of chronic ethanol consumption on the synthesis of polypeptides encoded by the hepatic mitochondrial genome," *Biochimica et Biophysica Acta*, vol. 1019, no. 2, pp. 142–150, 1990.

[75] T. Wang, R. V. Campbell, M. K. Yi, S. M. Lemon, and S. A. Weinman, "Role of Hepatitis C virus core protein in viral-induced mitochondrial dysfunction," *Journal of Viral Hepatitis*, vol. 17, no. 11, pp. 784–793, 2010.

[76] W. Lim, S. H. Kwon, H. Cho et al., "HBx targeting to mitochondria and ROS generation are necessary but insufficient for HBV-induced cyclooxygenase-2 expression," *Journal of Molecular Medicine*, vol. 88, no. 4, pp. 359–369, 2010.

[77] K. Begriche, J. Massart, M. A. Robin, A. Borgne-Sanchez, and B. Fromenty, "Drug-induced toxicity on mitochondria

and lipid metabolism: mechanistic diversity and deleterious consequences for the liver," *Journal of Hepatology*, vol. 54, no. 4, pp. 773–794, 2011.

[78] M. T. Kuo and N. Savaraj, "Roles of reactive oxygen species in hepatocarcinogenesis and drug resistance gene expression in liver cancers," *Molecular Carcinogenesis*, vol. 45, no. 9, pp. 701–709, 2006.

[79] H. M. Shen, C. Y. Shi, Y. Shen, and C. N. Ong, "Detection of elevated reactive oxygen species level in cultured rat hepatocytes treated with aflatoxin B1," *Free Radical Biology and Medicine*, vol. 21, no. 2, pp. 139–146, 1996.

[80] P. C. Klohn, H. Massalha, and H. G. Neumann, "A metabolite of carcinogenic 2-acetylaminofluorene, 2-nitrosofluorene, induces redox cycling in mitochondria," *Biochimica et Biophysica Acta*, vol. 1229, no. 3, pp. 363–372, 1995.

[81] T. K. Macus, Z. Liu, Y. Wei et al., "Induction of human MDR1 gene expression by 2-acetylaminofluorene is mediated by effectors of the phosphoinositide 3-kinase pathway that activate NF-κB signaling," *Oncogene*, vol. 21, no. 13, pp. 1945–1954, 2002.

[82] Y. S. Bae, S. W. Kang, M. S. Seo et al., "Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation," *Journal of Biological Chemistry*, vol. 272, no. 1, pp. 217–221, 1997.

[83] Y. S. Bae, J. Y. Sung, O. S. Kim et al., "Platelet-derived growth factor-induced H_2O_2 production requires the activation of phosphatidylinositol 3-kinase," *Journal of Biological Chemistry*, vol. 275, no. 14, pp. 10527–10531, 2000.

[84] V. M. Factor, A. Kiss, J. T. Woitach, P. J. Wirth, and S. S. Thorgeirsson, "Disruption of redox homeostasis in the transforming growth factor-α/c- myc transgenic mouse model of accelerated hepatocarcinogenesis," *Journal of Biological Chemistry*, vol. 273, no. 25, pp. 15846–15853, 1998.

[85] A. P. Rolo, J. S. Teodoro, C. Peralta, J. Rosello-Catafau, and C. M. Palmeira, "Prevention of I/R injury in fatty livers by ischemic preconditioning is associated with increased mitochondrial tolerance: the key role of ATPsynthase and mitochondrial permeability transition," *Transplant International*, vol. 22, no. 11, pp. 1081–1090, 2009.

[86] D. Pessayre, B. Fromenty, A. Berson et al., "Central role of mitochondria in drug-induced liver injury," *Drug Metabolism Reviews*, vol. 44, no. 1, pp. 34–87, 2012.

[87] G. Serviddio, F. Bellanti, J. Sastre, G. Vendemiale, and E. Altomare, "Targeting mitochondria: a new promising approach for the treatment of liver diseases," *Current Medicinal Chemistry*, vol. 17, no. 22, pp. 2325–2337, 2010.

[88] C. Handschin, "The biology of PGC-1α and its therapeutic potential," *Trends in Pharmacological Sciences*, vol. 30, no. 6, pp. 322–329, 2009.

[89] R. C. Scarpulla, "Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network," *Biochimica et Biophysica Acta*, vol. 1813, no. 7, pp. 1269–1278, 2011.

[90] J. C. Yoon, P. Puigserver, G. Chen et al., "Control of hepatic gluconeogenesis through the transcriptional coaotivator PGC-1," *Nature*, vol. 413, no. 6852, pp. 131–138, 2001.

[91] P. Puigserver, J. Rhee, J. Donovan et al., "Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1α interaction," *Nature*, vol. 423, no. 6939, pp. 550–555, 2003.

[92] J. Rhee, Y. Inoue, J. C. Yoon et al., "Regulation of hepatic fasting response by PPARγ coactivator-1α (PGC-1): requirement for hepatocyte nuclear factor 4α in gluconeogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 7, pp. 4012–4017, 2003.

[93] C. Handschin, J. Lin, J. Rhee et al., "Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1α," *Cell*, vol. 122, no. 4, pp. 505–515, 2005.

[94] J. M. Huss and D. P. Kelly, "Nuclear receptor signaling and cardiac energetics," *Circulation Research*, vol. 95, no. 6, pp. 568–578, 2004.

[95] S. Song, R. R. Attia, S. Connaughton et al., "Peroxisome proliferator activated receptor α (PPARα) and PPAR gamma coactivator (PGC-1α) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements," *Molecular and Cellular Endocrinology*, vol. 325, no. 1-2, pp. 54–63, 2010.

[96] Y. Lustig, J. L. Ruas, J. L. Estall et al., "Separation of the gluconeogenic and mitochondrial functions of pgc-1α through s6 kinase," *Genes and Development*, vol. 25, no. 12, pp. 1232–1244, 2011.

[97] L. F. Yu, B. Y. Qiu, F. J. Nan, and J. Li, "AMPK activators as novel therapeutics for type 2 diabetes," *Current Topics in Medicinal Chemistry*, vol. 10, no. 4, pp. 397–410, 2010.

[98] S. Jäger, C. Handschin, J. St-Pierre, and B. M. Spiegelman, "AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 29, pp. 12017–12022, 2007.

[99] A. Shlomai, N. Paran, and Y. Shaul, "PGC-1α controls hepatitis B virus through nutritional signals," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 43, pp. 16003–16008, 2006.

[100] L. C. Gomes, G. D. Benedetto, and L. Scorrano, "During autophagy mitochondria elongate, are spared from degradation and sustain cell viability," *Nature Cell Biology*, vol. 13, no. 5, pp. 589–598, 2011.

[101] C. Mitchell, M. A. Robin, A. Mayeuf et al., "Protection against hepatocyte mitochondrial dysfunction delays fibrosis progression in mice," *American Journal of Pathology*, vol. 175, no. 5, pp. 1929–1937, 2009.

[102] H. Yadav, C. Quijano, A. K. Kamaraju et al., "Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling," *Cell Metabolism*, vol. 14, no. 1, pp. 67–79, 2011.

[103] D. W. Hailey, A. S. Rambold, P. Satpute-Krishnan et al., "Mitochondria supply membranes for autophagosome biogenesis during starvation," *Cell*, vol. 141, no. 4, pp. 656–667, 2010.

[104] C. He and D. J. Klionsky, "Regulation mechanisms and signaling pathways of autophagy," *Annual Review of Genetics*, vol. 43, pp. 67–93, 2009.

[105] G. Kroemer, G. Mariño, and B. Levine, "Autophagy and the integrated stress response," *Molecular Cell*, vol. 40, no. 2, pp. 280–293, 2010.

[106] R. J. Youle and D. P. Narendra, "Mechanisms of mitophagy," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 1, pp. 9–14, 2011.

[107] R. A. Gottlieb and R. M. Mentzer, "Autophagy during cardiac stress: joys and frustrations of autophagy," *Annual Review of Physiology*, vol. 72, pp. 45–59, 2009.

[108] D. Chen, F. Gao, B. Li et al., "Parkin mono-ubiquitinates Bcl-2 and regulates autophagy," *Journal of Biological Chemistry*, vol. 285, no. 49, pp. 38214–38223, 2010.

[109] J. Y. Lee, Y. Nagano, J. P. Taylor, K. L. Lim, and T. P. Yao, "Disease-causing mutations in Parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy," *Journal of Cell Biology*, vol. 189, no. 4, pp. 671–679, 2010.

[110] M. d'Amora, C. Angelini, M. Marcoli, C. Cervetto, T. Kitada, and M. Vallarino, "Expression of PINK1 in the brain, eye and ear of mouse during embryonic development," *Journal of Chemical Neuroanatomy*, vol. 41, no. 2, pp. 73–85, 2011.

[111] D. F. Egan, D. B. Shackelford, M. M. Mihaylova et al., "Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy," *Science*, vol. 331, no. 6016, pp. 456–461, 2011.

[112] I. Kim and J. J. Lemasters, "Mitochondrial degradation by autophagy (mitophagy) in GFP-LC3 transgenic hepatocytes during nutrient deprivation," *American Journal of Physiology*, vol. 300, no. 2, pp. C308–C317, 2011.

[113] Y. Cheng, M. Zhou, C. H. Tung, M. Ji, and F. Zhang, "Studies on two types of PTP1B inhibitors for the treatment of type 2 diabetes: hologram QSAR for OBA and BBB analogues," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 11, pp. 3329–3337, 2010.

[114] S. Gottschalk, C. Zwingmann, V.-A. Raymond, M. C. Hohnholt, T. S. Chan, and M. Bilodeau, "Hepatocellular apoptosis in mice is associated with early upregulation of mitochondrial glucose metabolism," *Apoptosis*, vol. 17, no. 2, pp. 143–153, 2012.

[115] C. Poussin, M. Ibberson, D. Hall et al., "Oxidative phosphorylation flexibility in the liver of mice resistant to high-fat diet-induced hepatic steatosis," *Diabetes*, vol. 60, no. 9, pp. 2216–2224, 2011.

[116] J. Albrecht and M. D. Norenberg, "Glutamine: a Trojan horse in ammonia neurotoxicity," *Hepatology*, vol. 44, no. 4, pp. 788–794, 2006.

[117] M. D. Norenberg, K. V. Rama Rao, and A. R. Jayakumar, "Mechanisms of ammonia-induced astrocyte swelling," *Metabolic Brain Disease*, vol. 20, no. 4, pp. 303–318, 2005.

[118] S. Ahmad, C. W. White, L. Y. Chang, B. K. Schneider, and C. B. Allen, "Glutamine protects mitochondrial structure and function in oxygen toxicity," *American Journal of Physiology*, vol. 280, no. 4, pp. L779–L791, 2001.

[119] C. H. Eng and R. T. Abraham, "Glutaminolysis yields a metabolic by-product that stimulates autophagy," *Autophagy*, vol. 6, no. 7, pp. 968–970, 2010.

[120] I. Nissim, O. Horyn, I. Nissim et al., "Down-regulation of Hepatic Urea Synthesis by Oxypurines: xanthine and uric acid inhibit N-acetylglutamate synthase," *Journal of Biological Chemistry*, vol. 286, no. 25, pp. 22055–22068, 2011.

[121] D. Han, F. Antunes, R. Canali, D. Rettori, and E. Cadenas, "Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol," *Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5557–5563, 2003.

[122] H. L. Pahl, "Activators and target genes of Rel/NF-κB transcription factors," *Oncogene*, vol. 18, no. 49, pp. 6853–6866, 1999.

[123] C. A. Piantadosi and H. B. Suliman, "Mitochondrial transcription factor A induction by redox activation of nuclear respiratory factor 1," *Journal of Biological Chemistry*, vol. 281, no. 1, pp. 324–333, 2006.

[124] L. Chen, M. Kwong, R. Lu et al., "Nrf1 is critical for redox balance and survival of liver cells during development," *Molecular and Cellular Biology*, vol. 23, no. 13, pp. 4673–4686, 2003.

[125] T. Luedde and R. F. Schwabe, "NF-κB in the liver-linking injury, fibrosis and hepatocellular carcinoma," *Nature Reviews Gastroenterology and Hepatology*, vol. 8, no. 2, pp. 108–118, 2011.

[126] F. Wang, S. Kaur, L. G. Cavin, and M. Arsura, "Nuclear-factor-κB (NF-κB) and radical oxygen species play contrary roles in transforming growth factor-β1 (TGF-β1)-induced apoptosis in hepatocellular carcinoma (HCC) cells," *Biochemical and Biophysical Research Communications*, vol. 377, no. 4, pp. 1107–1112, 2008.

[127] N. S. Chandel, D. S. McClintock, C. E. Feliciano et al., "Reactive oxygen species generated at mitochondrial Complex III stabilize hypoxia-inducible factor-1α during hypoxia: a mechanism of O_2 sensing," *Journal of Biological Chemistry*, vol. 275, no. 33, pp. 25130–25138, 2000.

[128] L. A. Dada, N. S. Chandel, K. M. Ridge, C. Pedemonte, A. M. Bertorello, and J. I. Sznajder, "Hypoxia-induced endocytosis, of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC-ζ," *Journal of Clinical Investigation*, vol. 111, no. 7, pp. 1057–1064, 2003.

[129] L. Liu, T. P. Cash, R. G. Jones, B. Keith, C. B. Thompson, and M. C. Simon, "Hypoxia-induced energy stress regulates mRNA translation and cell growth," *Molecular Cell*, vol. 21, no. 4, pp. 521–531, 2006.

[130] M. Marí, A. Morales, A. Colell, C. García-Ruiz, and J. C. Fernández-Checa, "Mitochondrial glutathione, a key survival antioxidant," *Antioxidants and Redox Signaling*, vol. 11, no. 11, pp. 2685–2700, 2009.

[131] J. M. Lluis, F. Buricchi, P. Chiarugi, A. Morales, and J. C. Fernandez-Checa, "Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-KB via c-SRC- and oxidant-dependent cell death," *Cancer Research*, vol. 67, no. 15, pp. 7368–7377, 2007.

[132] O. Coll, A. Colell, C. García-Ruiz, N. Kaplowitz, and J. C. Fernández-Checa, "Sensitivity of the 2-oxoglutarate carrier to alcohol intake contributes to mitochondrial glutathione depletion," *Hepatology*, vol. 38, no. 3, pp. 692–702, 2003.

[133] M. Marí, F. Caballero, A. Colell et al., "Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis," *Cell Metabolism*, vol. 4, no. 3, pp. 185–198, 2006.

[134] S. Krahenbuhl, J. Stucki, and J. Reichen, "Reduced activity of the electron transport chain in liver mitochondria isolated from rats with secondary biliary cirrhosis," *Hepatology*, vol. 15, no. 6, pp. 1160–1166, 1992.

[135] S. Krahenbuhl, C. Talos, B. H. Lauterburg, and J. Reichen, "Reduced antioxidative capacity in liver mitochondria from bile duct ligated rats," *Hepatology*, vol. 22, no. 2, pp. 607–612, 1995.

[136] D. Herranz, M. Muñoz-Martin, M. Cañamero et al., "Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer," *Nature Communications*, vol. 1, no. 1, 2010.

[137] L. Guarente, "Sirtuins, aging, and medicine," *New England Journal of Medicine*, vol. 364, no. 23, pp. 2235–2244, 2011.

[138] N. Nasrin, X. Wu, E. Fortier et al., "SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells," *Journal of Biological Chemistry*, vol. 285, no. 42, pp. 31995–32002, 2010.

[139] T. Nakagawa, D. J. Lomb, M. C. Haigis, and L. Guarente, "SIRT5 deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle," *Cell*, vol. 137, no. 3, pp. 560–570, 2009.

[140] B. H. Ahn, H. S. Kim, S. Song et al., "A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 38, pp. 14447–14452, 2008.

[141] X. Qiu, K. Brown, M. D. Hirschey, E. Verdin, and D. Chen, "Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation," *Cell Metabolism*, vol. 12, no. 6, pp. 662–667, 2010.

[142] R. Tao, M. C. Coleman, J. D. Pennington et al., "Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress," *Molecular Cell*, vol. 40, no. 6, pp. 893–904, 2010.

[143] S. Someya, W. Yu, W. C. Hallows et al., "Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under Caloric Restriction," *Cell*, vol. 143, no. 5, pp. 802–812, 2010.

[144] J. Chen, B. Zhang, N. Wong et al., "Sirtuin 1 is upregulated in a subset of hepatocellular carcinomas where it is essential for telomere maintenance and tumor cell growth," *Cancer Research*, vol. 71, no. 12, pp. 4138–4149, 2011.

[145] H. N. Choi, J. S. Bae, U. Jamiyandorj et al., "Expression and role of SIRT1 in hepatocellular carcinoma," *Oncology Reports*, vol. 26, no. 2, pp. 503–510, 2011.

[146] J. T. Rodgers, C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman, and P. Puigserver, "Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1," *Nature*, vol. 434, no. 7029, pp. 113–118, 2005.

[147] X. Hou, S. Xu, K. A. Maitland-Toolan et al., "SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase," *Journal of Biological Chemistry*, vol. 283, no. 29, pp. 20015–20026, 2008.

[148] M. D. Hirschey, T. Shimazu, E. Goetzman et al., "SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation," *Nature*, vol. 464, no. 7285, pp. 121–125, 2010.

[149] A. A. Kendrick, M. Choudhury, S. M. Rahman et al., "Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation," *Biochemical Journal*, vol. 433, no. 3, pp. 505–514, 2011.

[150] S. Michel, A. Wanet, A. De Pauw, G. Rommelaere, T. Arnould, and P. Renard, "Crosstalk between mitochondrial (dys)function and mitochondrial abundance," *Journal of Cellular Physiology*, vol. 227, no. 6, pp. 2297–2310, 2012.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 213403, 13 pages
doi:10.1155/2012/213403

Review Article

Inhibition of the Mitochondrial Permeability Transition for Cytoprotection: Direct *versus* Indirect Mechanisms

Cécile Martel, Le Ha Huynh, Anne Garnier, Renée Ventura-Clapier, and Catherine Brenner

LabEx LERMIT, INSERM U769, Faculté de Pharmacie, Université Paris-Sud, 5 Rue J.-B. Clément, 92290 Châtenay-Malabry, France

Correspondence should be addressed to Catherine Brenner, catherine.brenner-jan@u-psud.fr

Received 22 December 2011; Accepted 7 February 2012

Academic Editor: Etienne Jacotot

Mitochondria are fascinating organelles, which fulfill multiple cellular functions, as diverse as energy production, fatty acid β oxidation, reactive oxygen species (ROS) production and detoxification, and cell death regulation. The coordination of these functions relies on autonomous mitochondrial processes as well as on sustained cross-talk with other organelles and/or the cytosol. Therefore, this implies a tight regulation of mitochondrial functions to ensure cell homeostasis. In many diseases (e.g., cancer, cardiopathies, nonalcoholic fatty liver diseases, and neurodegenerative diseases), mitochondria can receive harmful signals, dysfunction and then, participate to pathogenesis. They can undergo either a decrease of their bioenergetic function or a process called mitochondrial permeability transition (MPT) that can coordinate cell death execution. Many studies present evidence that protection of mitochondria limits disease progression and severity. Here, we will review recent strategies to preserve mitochondrial functions via direct or indirect mechanisms of MPT inhibition. Thus, several mitochondrial proteins may be considered for cytoprotective-targeted therapies.

1. Introduction

Mitochondria are intracellular organelles, whose first discovered function is energy production by oxidative phosphorylation [1]. Depending on the mammalian tissue, mitochondria may have additional functions, such as β oxidation, heat production, reactive oxygen species (ROS) metabolism, and cell death coordination. However, since the emergence of the concept of mitochondrial control of cell death in the 95's (for recent reviews: [2, 3]), it became evident that mitochondria participate to various types of cell death, that are, apoptosis, necrosis, oncosis and mitotic catastrophy via mitochondrial membrane permeabilization (MMP), release of proapoptotic factors contained in the intermembrane space to the cytosol and possibly fission, even if mitochondrial fragmentation is not sufficient *per se* to induce cell death [4, 5]. Mitochondrial dysfunction has been associated with a series of human diseases such as cancer, cardiopathies, nonalcoholic fatty liver diseases, neurodegenerative diseases, and aging. When due to genetic dysfunction, the diseases have been systematically characterized in animal models [6, 7]. Thus, mitochondrial impairment can be linked either to the metabolic function of these organelles, their role in cell death, or both. In addition, in chronic pathologies, such as cardiac volume overload-induced hypertrophy [8], mitochondrial dysfunction precedes cell loss by apoptosis and necrosis, meaning that both dysfunctions can be separated chronologically during the progression of the disease. This is also observed in the pathogenesis of nonalcoholic steatohepatitis, whatever its initial cause, as extensively reviewed [9]. Hepatic mitochondrial dysfunction would lead to apoptosis or necrosis depending on the energy status of the cell [9].

Metabolic impairment manifests by decreased ATP synthesis capacity, enhanced ROS production due to electron leak from the respiratory chain, change in intracellular pH and frequently, by morphological alterations of mitochondrial network. For instance, heart failure which is defined as the inability of the heart to keep up with the demands and to provide adequate blood flow to other organs such as the brain, liver, and kidneys is accompanied

by a decrease in energy production and energy transfer capacity [10]. This leads to a decrease in energy charge of the myocardium that has been described as a prognostic factor in dilated cardiomyopathies [11]. This metabolic impairment also affects the peripheric circulation and was shown to involve decreased mitochondrial biogenesis [10].

MMP corresponds to multiple events that irreversibly lead to cell death [2, 12]. These lethal events are nonexclusive, some of them can occur independently, whereas others are intimately linked. Thus, MMP refers to protein translocation from cytosol to outer membrane (OM), rupture of outer mitochondrial membrane, loss of inner membrane potential ($\Delta\Psi m$), cristae remodeling and release of intermembrane space proteins such as cytochrome c or apoptosis-inducing factor (AIF). For instance, upon various stress, Bax or tBid, which reside in the cytosol, can translocate to mitochondrial membranes, oligomerize with mitochondrial proteins to form large channels allowing cytochrome c release and activation of the intrinsic apoptosis signaling cascade (for review: [2]).

In many pathophysiological models, but not all, MMP also involves the so-called opening of the permeability transition pore complex (PTPC), which mediates a nonselective permeabilization of the IM and OM to molecules of molecular mass (MM) under 1.5 kDa (see below for more details) [2, 12]. Thus, in chemotherapy-treated tumor cell lines and ischemic neuronal cells, Bax can interact with the adenine nucleotide translocase (ANT) and/or the voltage-dependent anion channel (VDAC) to promote MMP and cell death.

Here, we will review direct and indirect mechanisms or means to protect mitochondrial functions via a closure of PTPC and a prevention of mitochondrial permeability transition (MPT). The discussion of MPT regulatory mechanisms will be based on selected articles focusing on heart diseases and cancer.

2. Mitochondrial Membrane Permeability and PTPC

Mitochondrial membrane permeability is strictly controlled in unicellular and multicellular organisms harboring these organelles. The OM is believed to be freely permeable to ions and metabolites via entry *through* protein channels (e.g., voltage-dependent anion channel, VDAC), whereas the inner membrane (IM) is considered as impermeable. Thus, the entry and exit of ions or metabolites trough the IM are mediated by integral proteins such as the members of the mitochondrial carrier family [13]. The prototypic protein of this family is ANT or ADP/ATP carrier, which mediates the stoichiometric exchange of ADP and ATP between the matrix and the intermembrane space [14]. Moreover, osmotic movements of water accompany solutes transport from cytosol to matrix, but the molecular basis of these transports is still largely unknown [15]. When excessive stimulation by endogenous signals (excessive ROS, calcium (Ca^{2+}) overload, protease activation, lipid accumulation etc) or activation of harmful signaling pathways (e.g., kinases/phosphatases, proteases, Bax/-Bid-mediated pathways etc.) occur, mitochondria undergo the MPT, a phenomenon that consists in a sudden increase in IM permeability to small molecules. MPT is a phenomenon first studied in isolated beef heart mitochondria in response to Ca^{2+} overload [16]. Thus, the response of isolated mitochondria to doses of Ca^{2+} is a nonspecific increase of the permeability of the IM, resulting in entry of water and solutes, loss of $\Delta\Psi m$, matrix swelling, and simultaneous uncoupling of oxidative phosphorylation (Figure 1). Of note, the doses of Ca^{2+} depend largely on the tissue origin of mitochondria and the amount of Ca^{2+} present in the buffers. Ultimately, MPT is accompanied by matrix swelling and OM ruptures as shown by transmission electron microscopy [17–19]. This phenomenon can be blocked by Ca^{2+} chelation, ATP, Mg^{2+}, and cyclosporin A (CsA) *in vitro* as well as *in vivo* [20–22].

MPT can be followed experimentally by the loss of absorbance of a suspension of isolated mitochondria by spectrophotometry and by the loss of $\Delta\Psi m$ using suitable fluorescent probes (e.g. tetramethylrhodamine methyl ester (TMRM), rhodamine 123 (Rhod123), 5, 5′, 6, 6′-tetrachloro-1, 1′, 3, 3′-tetraethylbenzimidazol-carbocyanine iodide (JC-1)) [19, 23]. The main interests of the use of isolated organelles are to monitor mitochondrial responses that are directly induced by compounds independently of other cellular compartments and the possibility to automate the measure in the perspective of pharmacological studies [19, 23].

One major pitfall is that, whereas isolation procedures are believed to be nondestructive for liver and cell lines mitochondria [24, 25], mitochondrial responses of isolated mitochondria from skeletal muscle and heart that may rely on the cell architecture are (obviously) lost [26].

Another pitfall is the cross-contamination of the mitochondrial fraction with other cellular compartments and purity of preparations must be checked carefully. MPT can also be measured by imaging with the fluorescent probe calcein in the presence of cobalt in living cell as various as hepatocytes, astrocytes and cardiomyocytes [27–31]. The principle is that calcein (molecular weight, 620 Da) can diffuse into the whole cell, whereas cobalt, a fluorescence quencher, cannot enter into the mitochondrial matrix and diffuses into the rest of the cell. Thus, in physiological conditions mitochondria appear fluorescent and following MPT, the quenching of calcein by cobalt triggers a decrease in fluorescence. For instance, HeLa cells treated by thapsigargin, a SERCA pump inhibitor or A23187, a Ca^{2+} ionophore [32], undergo MPT as shown by a significant decrease in calcein fluorescence due to IM permeabilization and cobalt quenching [30]. MPT has also been monitored in whole heart by 2-deoxy[^{3}H]glucose entrapment technique [33].

Of note, the full demonstration that the process is mediated by PTPC opening requires its inhibition by pretreatment of cells or isolated mitochondria by CsA, the well-known cyclophilin D (CypD) ligand [20, 34]. Moreover, silencing of CypD by siRNA to prevent the induction of MPT is becoming mandatory *in cellulo*, since the genetic demonstration that CypD is critical for MPT and cell death [35].

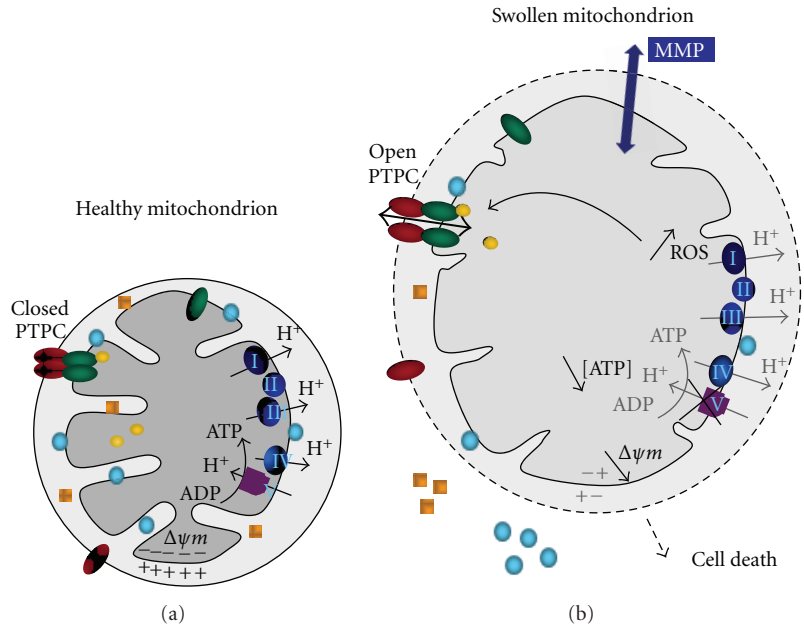


Figure 1: Scheme of mitochondrial alterations following mitochondrial membrane permeabilization (MMP). In this model, in response to the opening of the permeability transition pore (PTPC; green and red ellipses, corresponding to ANT and VDAC resp.), swollen mitochondria exhibit an increase in volume, a more translucide matrix with less cristae and a permeabilized outer membrane. Cytochrome c and apoptosis-inducing factor (AIF) (blue circles and yellow squares), normally confined into the intermembrane space, are released trough ruptures in the outer membrane. The transmembrane inner potential ($\Delta\Psi m$) is dissipated in response to the arrest of the function of the respiratory complexes (I to V), which contributes to an inhibition of ATP biosynthesis. Altogether, these alterations are lethal, irreversible and lead to cell death.

3. Direct Mechanisms of MPT Inhibition

PTPC is defined as a voltage-dependent polyprotein complex, which in certain conditions might form a nonselective channel at contact sites between both mitochondrial membranes [36, 37]. By definition, mitochondria contain all the proteins necessary for MPT induction and then MPT does not necessitate any neosynthesis. Since the initial PTPC identification by electrophysiology, the molecular identity of this pore and its regulators is still controversial [38–40]. ANT, VDAC and CypD, the three former PTPC candidates, have their own functions, irrespective of their association within PTPC or other putative polyprotein complexes such as the ATP synthasome [41] and Bcl-2 family member oligomers [3, 12]. Thus, some of the unknown members of PTPC may have their own role in metabolism (e.g., kinase, peptidyl-prolyl isomerase, deshydrogenase), transport (e.g., mitochondrial carrier, channel) or structure (e.g., dynamic machinery, cytoskeleton, AKAP proteins). This means that lethal MPT needs a stimulation to occur and in pathophysiological conditions, this is mainly achieved by Ca^{2+} and ROS. Whatever its composition, the PTPC is a widespread phenomenon occurring in many diseases. Although it has been the subject of intense research and therapeutic developments in cancer with the search for PTPC inducers [42], it has emerged only recently as a promising target for cytoprotection in various diseases such as neurodegenerative, cardiovascular and metabolic diseases. PTPC can be modulated directly by a large panel of pharmacological agents, by post-translational modifications and by cooperation with other proteins that may have a major impact on the cell life as discussed below.

3.1. Pharmacological Inhibition of PTPC. Using isolated mitochondria from various sources, an impressive body of literature reports that many molecules or compounds modulate the PTPC in response to Ca^{2+}, ROS, or a disease. Thus, some compounds can activate MPT, whereas a more limited number of them can prevent the opening of PTPC. Some compounds have known mitochondrial targets such as ANT, VDAC, CypD, and translocator protein 18 kDa (Table 1). To summarize, the most investigated inhibitor of PTPC is CsA, which modulates CypD, as discussed below.

One example with future therapeutic applications is cardiac ischaemia/reperfusion injury. During an acute myocardial infarction (AMI), tissue injury occurring after reperfusion represents a significant amount of the whole, irreversible damage. Ischaemia and reperfusion cause a wide array of functional and structural alterations of mitochondria. Some of these responses are directly under the control of the highly conserved transcriptional complex HIF-1 and result from a modulation in expression of genes involved in glycolysis, glucose metabolism, mitochondrial function, cell survival, apoptosis, and resistance to oxidative stress [43].

PTPC opening plays a crucial role in this specific component of myocardial infarction. Strong support for this concept has recently been provided by the reduced infarct size observed in mice lacking CypD [35]. Thus targeting

Table 1: List of mitochondrial permeability transition (MPT) inhibitors. CypD, Cyclophilin D; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; UQ, ubiquinone.

MPT inhibitor	Target	Model	References
Cyclosporin A	CypD binding with ANT	*In vivo*, isolated mitochondria, cells	[44, 45]
Sanglifehrin A	CypD	*In vivo*, isolated mitochondria, cells	[46, 47]
Bongkrekic acid	ANT	Isolated mitochondria, cells	[48–50]
ADP/ATP	ANT	Isolated mitochondria, *in vitro*	[51, 52]
NADH/NAD+	VDAC	Isolated mitochondria, *in vitro*	[53–55]
DIDS	VDAC	Isolated mitochondria, *in vitro*, cells	[52–58]
glutamate	VDAC	Isolated mitochondria, *in vitro*	[59–61]
Ro 68–3400	ANT or PiC, not VDAC1	Isolated mitochondria, *in vitro*	[61–63]
UQ(0)	ANT or PiC	Isolated mitochondria, *in vitro*	[64, 65]
S15176	unknown, in IM	*In vivo*, isolated mitochondria	[66, 67]
Sildenafil	unknown	*In vivo*, isolated mitochondria	[68]
Debio-025	CypD	*In vivo,* isolated mitochondria	[69]
TRO19622	VDAC, translocator protein 18 kDa	*In vivo,* isolated mitochondria	[70]
Carbon monoxide	ANT, unknown	Isolated mitochondria, cells	[71]
Antamanide	CypD	Isolated mitochondria, cells	[72]

PTPC appears a relevant strategy to reduce ischaemic damages at reperfusion. A large body of evidence has shown that it is possible to reduce infarct size and to protect the heart after an infarct by a postconditioning or pharmacological strategy. Brief episodes of myocardial ischemia-reperfusion employed during reperfusion after a prolonged ischaemic insult may attenuate the total ischaemia-reperfusion injury. Recently, CsA has been shown to dramatically reduce infarct size in many animal species and in human. Recent proof-of-concept clinical trials support the idea that targeting MPT by either coronary intervention postconditioning or CsA can reduce infarct size and improve the recovery of contractile function after reperfusion [21, 73]. Such a strategy was also applied to ischaemia-reperfusion damages in other tissues and cells like vascular endothelial cells [74], hepatocytes [75, 76], and neurons [77–79]. Moreover, some attempts to target CsA to the mitochondrial compartment by conjugation to the lipophilic triphenylphosphonium cation proved to be promising in cytoprotection from glucose and oxygen deprivation in neurons [80], in cardiomyocytes [81], and in other various organs [82].

3.2. Role of Mitochondrial Kinases to Prevent PTPC Opening. Several protective signal pathways involving multiple kinases have been shown to converge on mitochondria and the PTPC [83] to promote cell survival (Table 2). For instance, in cardiomyocytes, pools of kinases such as Akt, protein kinase C-ε (PKCε), extracellular-regulated kinases (ERK), glycogen synthase kinase-3 beta (GSK-3β), and hexokinases (HK) I and II, are localized in or on mitochondria in addition to the cytosol. These mitochondria-associated protein kinases may integrate cytosolic stimuli and in turn, enhance tolerance of myocytes to injury.

Moreover, systematic proteomic approaches revealed that some of these kinases might form hetero-oligomers with putative components of the PTPC. Briefly, GSK-3β and HKs are directly responsible for inhibition of opening of the PTPC and, thus, for myocyte protection from necrosis [96]. As a result, postconditioning, which leads to GSK-3β inhibition, allows the myocardial salvage from reperfusion injury by modulating MPT [86]. In the context of anticancer chemotherapy, β-adrenergic receptors (β-ARs) modulate anthracycline response through crosstalk with multiple signaling pathways. β2-ARs are cardioprotective during exposure to oxidative stress induced by doxorubicin (DOX). DOX cardiotoxicity is mediated in part through a Ca^{2+}-dependent triggering of MPT as clearly shown by a 41% reduction of DOX-induced mortality by CsA [97]. β2-ARs activate prosurvival kinases and attenuate mitochondrial dysfunction caused by oxidative stress. Accordingly, the invalidation of β2-ARs enhances cardiotoxicity via negative regulation of survival kinases and enhancement of intracellular Ca^{2+}, thus predisposing the mitochondria to opening of the PTPC [97].

Moreover, in cancer cell lines, activation of mitochondrial ERK protects cancer cells from death through inhibition of the MPT [88]. ERK inhibition enhanced GSK-3β-dependent phosphorylation of the pore regulator CypD, whereas GSK-3β inhibition protected from PTPC opening.

By different molecular mechanisms, some kinases such as creatine kinase (CK) and HK have also cytoprotective effects and prevent PTPC opening. Depending on the tissue, which supports their expression, these kinases may be cytoprotective via a role in energy transfer to metabolites such as creatine and glucose (Table 2).

3.3. Stabilization of Mitochondrial Membrane Permeability by Bcl-2 Family Members. The Bcl-2 family is composed of more than 25 proteins implicated in the control of life-or-death decision [98]. This protein family has been particularly studied in cancer, which led to the classification of Bcl-2 and

Table 2: List of kinases contributing to a closure of PTP via phosphorylation mechanisms or protein-protein interaction. HK, hexokinase, CK, creatine kinase, PKG, protein kinase G, PKA, protein kinase A, PKC, protein kinase C, ERK, extracellular signal-regulated kinase, GSK3, glucose-regulated kinase 3, PI3K, phosphoinositol3 kinase, and Akt/PKB, protein kinase B.

Kinase	Effect	Target/pathway	Model	References
Akt/PKB, PI3K	Indirect	GSK3 via PI3K or eNos/PKG pathways	Cells	[84, 85]
GSK3	Direct	VDAC, ANT, CypD	Isolated mitochondria, cells, *in vivo*, *in vitro*	[8, 37, 86, 87]
ERK	Indiret	GSK3 via PI3K pathway	Cells	[85, 88]
PKA	Direct	VDAC	Isolated mitochondria	[89]
PKC epsilon	Direct	VDAC	Isolated mitochondria, *in vivo*	[90]
PKG	Direct	Unknown	Isolated mitochondria, *in vivo*	[91, 92]
CK	Local regulation of ATP/creatine pools	Energetic metabolism	CK-expressing tissues	[93, 94]
HK	Local regulation of glucose/ATP pools	Energetic metabolism	Isolated mitochondria, cells, *in vitro*	[57, 74, 87, 95]

Bax as oncogenes and tumor-suppressors, respectively. Some members (e.g., Bax/Bad proteins, BH3-only proteins) favor apoptosis, whereas other members, such as Bcl-2 and Bcl-XL, prevent apoptosis. Moreover, it has been shown that the effects of Bcl-2 family proteins on mitochondria in cancer cells are linked to clinical responses to chemotherapy [99].

The cytoprotective mechanisms of Bcl-2 family members are multiple and include direct mitochondrial effects [3, 12]. Thus, Bcl-2 contributes to the stabilization of the mitochondrial membrane permeability, inhibition of $\Delta\Psi m$ loss and cytochrome *c* release and, at least in tumor cells, to the stimulation of oxidative phosphorylation [100–103]. Thus, direct protein-protein interactions between Bcl-2 family members, but also with several constitutive mitochondrial proteins such as ANT (IM), VDAC (OM), or FoF1-ATP synthase (IM) have been evidenced [104]. Bcl-2 cooperates directly with ANT, to prevent PTPC opening and to inhibit cell death [101, 105, 106]. In addition, Bcl-2 blocks the Ca^{2+}-induced channel function of ANT and favors ADP/ATP translocase function, which positively impacts the intracellular levels of ATP [107]. Similarly, Bcl-2 and Bcl-XL directly interact with VDAC modulating its channel function [108]. Bcl-XL would bind also to the mitochondrial F0F1-ATP synthase and regulate metabolic efficiency in neurons [104]. Accordingly, recombinant proteins of some members of the Bcl-2 family directly modulate PTPC in isolated mitochondria from various sources, that is, liver and cancer cells [25, 109]. Some specific regions of Bcl-2 (e.g., BH3, BH4 domains) are responsible for these effects and as expected, peptides corresponding to these regions proved to modulate apoptosis *in cellulo* or in isolated mitochondria, again indicating a direct targeting of mitochondria [25]. Finally, *in vivo*, the BH4 domain of Bcl-XL exerts antiapoptotic effects and attenuates ischaemia/reperfusion injury through anti-apoptotic mechanism in rat hearts [110, 111].

4. Indirect Mechanisms, Which Lead to an Increased Resistance of PTPC Opening

By definition, several indirect mechanisms may lead to blockade of PTPC opening via modulation of $\Delta\Psi m$, mitochondrial mass regulation, redox state, fusion/fission processes and calcium retention capacity. This may be due to modification in protein expression, in posttranslational modifications and in their interactome, which consequently affect signaling pathways. Below, we will analyze three indirect mechanisms of PTPC protection that have recently been elucidated.

4.1. Anti-Oxidant Protection. Mitochondria are major sites of ROS production, which may contribute to the development of various diseases including cardiovascular diseases and aging. Several studies have thus described the effects of antioxidant administration in the context of cardiac and liver pathologies in mice [112]. It is widely admitted that natural antioxidants such as resveratrol and curcumin have beneficial effects against ischaemia/reperfusion damages to mitochondria and cells in rat liver or heart [113, 114]. These effects are complex since resveratrol has been proposed to have multiple intracellular targets such as AMPK, SIRT1 and Nrf2, which can influence the transcriptome to increase the anti-oxidant defense (e.g., catalase, GPx, and GCLC), and other genes such eNOS and PGC1α, which favor an increase in mitochondrial mass and bioenergetics and decrease in apoptosis and inflammation (for review: [115]).

Resveratrol treatment exerts beneficial protective effects on survival, endothelium-dependent relaxation, and cardiac contractility and mitochondrial function, suggesting that resveratrol or metabolic activators could be a relevant therapy in hypertension-induced heart failure [116]. Similarly, in the heart, curcumin another polyphenol with antioxidant

properties showed cardioprotective effects in catecholamine induced cardiotoxicity through prevention of mitochondrial damage, PTPC opening [117], and ventricular dysfunction [118] and in protecting rat myocardium against ischaemic insult by decreasing oxidative stress [119].

Another promising example of compound is MitoQ10, an ubiquinone derivative, which is a mitochondria-targeted antioxidant [120]. It has proven to be useful for protecting endothelial function and attenuating cardiac hypertrophy in stroke-prone hypertensive rats [121]. Moreover, MitoQ10 potently inhibits cocain-induced cardiac damage via a restoration of oxygen consumption and a stabilization of ROS levels, specifically in interfibrillar mitochondria [122]. However, when used on isolated cardiac mitochondria, MitoQ10 can be enhanced in a dose-dependent-manner MPT in the presence of the prooxidant tert-butyl hydoperoxide (t-BHP) and suboptimal doses of Ca^{2+} (Figure 2), although it acts as an antioxidant on rat liver mitochondria [123]. This underscores the duality of anti- and prooxidant compounds, whose effects can depend either on the dose, the redox state of the cell, the tissue, and/or the mode of administration. This probably explains, at least in part, the failure of anti-oxidants to protect efficiently the heart function in clinical trials, as recently reviewed in [124].

4.2. Estrogens Protection. Sex and gender influence the onset and the progression of many human diseases, notably age-related diseases. Thus, estrogens, mainly 17β-estradiol, may have pleiotropic effects depending on the tissue [125]. For instance, certain cardiovascular diseases, such as myocardial hypertrophy and heart failure, differ clearly in their clinical manifestation and prognosis between women and men [126]. As a consequence, hormonal mechanisms underlying sex and gender differences are currently under intense investigation.

Animal and cellular models have been particularly instrumental to better understand estrogen protection at the level of mitochondria [127]. For instance, in cerebral circulation, estrogens mediate an enhancement of vasodilator capacity, suppression of vascular inflammation and increase of mitochondrial efficiency [125, 128, 129]. This effect is, at least in part, due to an increase in mitochondrial biogenesis via gene expression modulation [129] and a decrease in superoxide production [125]. Accordingly, chronic estrogen treatment increases mitochondrial capacity for oxidative phosphorylation while decreasing production of ROS. In breast and lung cancer cells, long-term estradiol treatment activates transcription of NRF-1 and increases mitochondrial biogenesis [130].

Moreover, mitochondrial effects on PTPC might be mediated indirectly by estrogen receptors, α and β, present in nucleus, plasma membrane, endoplasmic reticulum and even mitochondria [131]. In the context of ischaemia-reperfusion injury, it is widely admitted that estrogens protects from myocardial damage via an inhibition of PTPC function. Notably, estradiol may activate the signaling cascade which involves Akt, NO synthase, guanylyl cyclase and protein kinase G, which results in blockade of MPT-induced release of cytochrome c from mitochondria, respiratory inhibition and caspase activation [131]. As a result, estrogens effects are multifactorial, mostly indirect. Even if some estrogen-like molecules can be effective on isolated mitochondria, a precise target of estrogen within PTPC is still unknown [132]. Thus, intriguingly, estrogens may prevent Ca^{2+}-induced cytochrome *c* release in isolated heart mitochondria, but not mitochondrial swelling [133].

Estrogens also protect from chemotherapy-induced cardiomyopathy in ovariectomized rats. Again, effects on the anti-oxidant cellular defenses have been proposed as one of the target mechanism of estrogen [134].

4.3. Exercise Protection. Exercise training has proven to be beneficial in chronic diseases including heart failure, obesity, diabetes or metabolic syndrome. Because endurance training improves symptoms and quality of life and decreases mortality rate and hospitalization, it is increasingly recognized as a beneficial practice for these patients. Adaptation to endurance training mainly involves energetic remodeling in skeletal and cardiac muscles [135]. The mechanisms involved in the beneficial effects of exercise training are far from being understood. Skeletal muscles adapt to repeated prolonged exercise by marked quantitative and qualitative changes in mitochondria. Endurance training promotes an increase in mitochondrial volume density and mitochondrial proteins by activating mitochondrial biogenesis [136]. Exercise training decreases apoptotic processes, and protects mitochondrial function from oxidative stress and other cardiac insults [137, 138]. Exercise training results in a reduced sensitivity to PTPC opening in heart mitochondria and confers mitochondrial protection. Moreover, even acute exercise protects against cardiac mitochondrial dysfunction, preserving mitochondrial phosphorylation capacity and attenuating DOX-induced decreased tolerance to PTPC opening [139]. Proposed mechanisms to explain the cardioprotective effects of exercise are mediated, at least partially, by redox changes and include the induction of myocardial heat shock proteins, improved cardiac antioxidant capacity and/or elevation of other cardioprotective molecules [137].

5. Conclusion and Open Questions

In the last decade, direct and indirect approaches to protect mitochondrial functions via PTPC modulation have been explored. However, it is still too early to decipher the most efficient strategies in term of cytoprotection. Nevertheless, recent studies and research advances have propelled mitochondria on the scene front of new therapeutic strategies. However, a contradiction emerges between the need to kill tumor cells in cancer therapy and to protect other cells from injuries. Even more worrying is the fact that many anticancer therapies have mitochondrial toxicity that becomes dramatic when highly oxidative nondividing cells like cardiomyocytes are concerned. Indeed, mitochondria are the main target when cardiotoxicity of anticancer drugs is concerned [44, 140]. Thus one challenging issue of cytoprotection directed

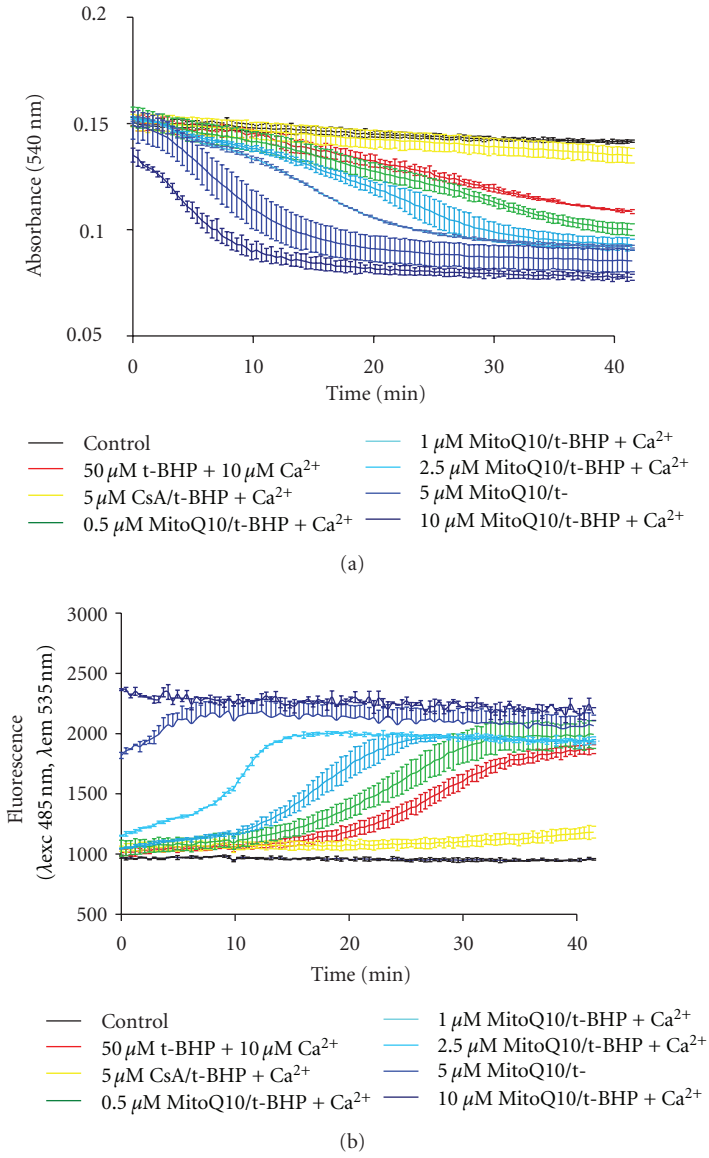


Figure 2: MitoQ10 stimulates MPT in isolated cardiac mitochondria. (a) MitoQ10 increases the mitochondrial swelling induced by an oxidant stress. Mitochondria (25 μg of proteins) have been pretreated by the indicated doses of MitoQ10 and treated by 50 μM t- BHP + 10 μM Ca^{2+}. Absorbance at 540 nm has been registered for 60 min at 37°C. (b) MitoQ10 increases the mitochondrial depolarization induced by an oxidant stress. Mitochondria (25 μg of proteins) have been loaded with 2 μM Rhodamine 123, pretreated by the indicated doses of MitoQ10, and treated by 50 μM t-BHP + 10 μM Ca^{2+}. Fluorescence has been registered for 60 min at 37°C.

to mitochondria would be to uncover new molecules or treatments that would selectively target cancer cells without affecting cardiac mitochondria. This should stimulate new studies devoted to increase our basic knowledge of the mechanisms and the tissue specificity of PTPC opening and mitochondrial function. At the same time, this will open the possibility to search for new drugs with tissue-specific effects on mitochondria. Finally, another challenge that basic and clinical research will face in the future is the notion of sex and gender influence that might be decisive for the treatment of many severe diseases.

Abbreviations

ANT: Adenine nucleotide translocase
β-Ars: β-adrenergic receptors
Ca^{2+}: Calcium
CK: Creatine kinase
CsA: Cyclosporin A
CypD: Cyclophilin D
$\Delta\Psi m$: Mitochondrial inner membrane potential
DOX: Doxorubicin
ERK: Extracellular-signal-regulated kinase

GSK-3β: Glycogen synthase kinase-3 beta
HK: Hexokinase
IM: Inner membrane
JC-1: 5, 5′, 6, 6′ -tetrachloro-1, 1′, 3, 3′ -tetraethylbenzimidazol-carbocyanine iodine
MMP: Mitochondrial membrane permeabilization
MPT: Mitochondrial permeability transition
OM: Outer membrane
PTPC: permeability transition pore complex
Rhod123: Rhodamine 123
ROS: Reactive oxygen species
TMRM: Tetramethylrhodamine methyl ester
VDAC: Voltage-dependent anion channel.

Acknowledgments

C. Brenner and R. V. Clapier are senior scientists at the Centre National de la Recherche Scientifique. A. Garnier, C. Brenner and R. V. Clapier are supported by LabEx LERMIT. C. Brenner is supported by ANR (ANR-08PCVI 0008-01). C. Martel received a fellowship from Association pour la Recherche sur le Cancer. L. H. Huynh received a fellowship from INSERM.

References

[1] A. L. Lehninger, "The transfer of energy in oxidative phosphorylation," *Bulletin de la Société de Chimie Biologique*, vol. 46, pp. 1555–1575, 1964.
[2] G. Kroemer, L. Galluzzi, and C. Brenner, "Mitochondrial membrane permeabilization in cell death," *Physiological Reviews*, vol. 87, no. 1, pp. 99–163, 2007.
[3] J.-C. Martinou and R. Youle, "Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics," *Developmental Cell*, vol. 21, no. 1, pp. 92–101, 2011.
[4] G. Majno and I. Joris, "Apoptosis, oncosis, and necrosis: an overview of cell death," *American Journal of Pathology*, vol. 146, no. 1, pp. 3–15, 1995.
[5] K. N. Papanicolaou, G. A. Ngoh, E. R. Dabkowski et al., "Cardiomyocyte deletion of mitofusin-1 leads to mitochondrial fragmentation and improves tolerance to ROS-induced mitochondrial dysfunction and cell death," *American Journal of Physiology*, vol. 302, no. 1, pp. H167–H179, 2012.
[6] D. C. Wallace, "Mitochondrial diseases in man and mouse," *Science*, vol. 283, no. 5407, pp. 1482–1488, 1999.
[7] A. Ramachandran, S. Jha, and D. J. Lefer, "Review paper: pathophysiology of myocardial reperfusion injury: the role of genetically engineered mouse models," *Veterinary Pathology*, vol. 45, no. 5, pp. 698–706, 2008.
[8] J. Matas, N. Tien Sing Young, C. Bourcier-Lucas et al., "Increased expression and intramitochondrial translocation of cyclophilin-D associates with increased vulnerability of the permeability transition pore to stress-induced opening during compensated ventricular hypertrophy," *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 3, pp. 420–430, 2009.
[9] K. Begriche, A. Igoudjil, D. Pessayre, and B. Fromenty, "Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it," *Mitochondrion*, vol. 6, no. 1, pp. 1–38, 2006.
[10] R. Ventura-Clapier, A. Garnier, V. Veksler, and F. Joubert, "Bioenergetics of the failing heart," *Biochimica et Biophysica Acta*, vol. 1813, no. 7, pp. 1360–1372, 2011.
[11] S. Neubauer, "The failing heart-An engine out of fuel," *New England Journal of Medicine*, vol. 356, no. 11, pp. 1140–1151, 2007.
[12] I. R. Indran, G. Tufo, S. Pervaiz, and C. Brenner, "Recent advances in apoptosis, mitochondria and drug resistance in cancer cells," *Biochimica et Biophysica Acta*, vol. 1807, no. 6, pp. 735–745, 2011.
[13] F. Palmieri, "The mitochondrial transporter family (SLC25): physiological and pathological implications," *Pflugers Archiv European Journal of Physiology*, vol. 447, no. 5, pp. 689–709, 2004.
[14] M. Klingenberg, "The ADP and ATP transport in mitochondria and its carrier," *Biochimica et Biophysica Acta*, vol. 1778, no. 10, pp. 1978–2021, 2008.
[15] P. Gena, E. Fanelli, C. Brenner, M. Svelto, and G. Calamita, "News and views on mitochondrial water transport," *Frontiers in Bioscience*, vol. 14, pp. 4189–4198, 2009.
[16] D. R. Hunter, R. A. Haworth, and J. H. Southard, "Relationship between configuration, function, and permeability in calcium treated mitochondria," *Journal of Biological Chemistry*, vol. 251, no. 16, pp. 5069–5077, 1976.
[17] M. G. Vander Heiden, N. S. Chandel, E. K. Williamson, P. T. Schumacker, and C. B. Thompson, "Bcl-x(L) regulates the membrane potential and volume homeostasis of mitochondria," *Cell*, vol. 91, no. 5, pp. 627–637, 1997.
[18] E. Jacotot, L. Ravagnan, M. Loeffler et al., "The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore," *Journal of Experimental Medicine*, vol. 191, no. 1, pp. 33–45, 2000.
[19] A. S. Belzacq-Casagrande, C. Martel, C. Pertuiset, A. Borgne-Sanchez, E. Jacotot, and C. Brenner, "Pharmacological screening and enzymatic assays for apoptosis," *Frontiers in Bioscience*, vol. 14, pp. 3550–3562, 2009.
[20] A. R. Halestrap, C. R. Connern, E. J. Griffiths, and P. M. Kerr, "Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury," *Molecular and Cellular Biochemistry*, vol. 174, no. 1-2, pp. 167–172, 1997.
[21] C. Piot, P. Croisille, P. Staat et al., "Effect of cyclosporine on reperfusion injury in acute myocardial infarction," *New England Journal of Medicine*, vol. 359, no. 5, pp. 473–481, 2008.
[22] A. L. Nieminen, T. G. Petrie, J. J. Lemasters, and W. R. Selman, "Cyclosporin A delays mitochondrial depolarization induced by N-methyl-D-aspartate in cortical neurons: evidence of the mitochondrial permeability transition," *Neuroscience*, vol. 75, no. 4, pp. 993–997, 1996.
[23] J. R. Blattner, L. He, and J. J. Lemasters, "Screening assays for the mitochondrial permeability transition using a fluorescence multiwell plate reader," *Analytical Biochemistry*, vol. 295, no. 2, pp. 220–226, 2001.
[24] C. Frezza, S. Cipolat, and L. Scorrano, "Organelle isolation: functional mitochondria from mouse liver, muscle and cultured filroblasts," *Nature Protocols*, vol. 2, no. 2, pp. 287–295, 2007.
[25] N. Buron, M. Porceddu, M. Brabant et al., "Use of human cancer cell lines mitochondria to explore the mechanisms of BH3 peptides and ABT-737-induced mitochondrial membrane permeabilization," *PloS One*, vol. 5, no. 3, article e9924, 2010.

[26] M. Picard, T. Taivassalo, G. Gouspillou, and R. T. Hepple, "Mitochondria: isolation, structure and function," *Journal of Physiology*, vol. 589, no. 18, pp. 4413–4421, 2011.

[27] V. Petronilli, G. Miotto, M. Canton et al., "Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence," *Biophysical Journal*, vol. 76, no. 2, pp. 725–734, 1999.

[28] G. Zahrebelski, A. L. Nieminen, K. Al-Ghoul, T. Qian, B. Herman, and J. J. Lemasters, "Progression of subcellular changes during chemical hypoxia to cultured rat hepatocytes: a laser scanning confocal microscopic study," *Hepatology*, vol. 21, no. 5, pp. 1361–1372, 1995.

[29] A. L. Nieminen, A. K. Saylor, S. A. Tesfai, B. Herman, and J. J. Lemasters, "Contribution of the mitochondrial permeability transition to lethal injury after exposure of hepatocytes to t-butylhydroperoxide," *Biochemical Journal*, vol. 307, no. 1, pp. 99–106, 1995.

[30] E. N. Dedkova and L. A. Blatter, "Measuring mitochondrial function in intact cardiac myocytes," *Journal of Molecular and Cellular Cardiology*, vol. 52, no. 1, pp. 48–61, 2012.

[31] K. V. Rama Rao, A. R. Jayakumar, and M. D. Norenberg, "Ammonia neurotoxicity: role of the mitochondrial permeability transition," *Metabolic Brain Disease*, vol. 18, no. 2, pp. 113–127, 2003.

[32] L. Galluzzi, N. Zamzami, T. De La Motte Rouge, C. Lemaire, C. Brenner, and G. Kroemer, "Methods for the assessment of mitochondrial membrane permeabilization in apoptosis," *Apoptosis*, vol. 12, no. 5, pp. 803–813, 2007.

[33] E. J. Griffiths and A. P. Halestrap, "Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion," *Biochemical Journal*, vol. 307, no. 1, pp. 93–98, 1995.

[34] A. P. Halestrap and C. Brenner, "The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death," *Current Medicinal Chemistry*, vol. 10, no. 16, pp. 1507–1525, 2003.

[35] C. P. Baines, R. A. Kaiser, N. H. Purcell et al., "Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death," *Nature*, vol. 434, no. 7033, pp. 658–662, 2005.

[36] M. Crompton, "Mitochondrial intermembrane junctional complexes and their role in cell death," *Journal of Physiology*, vol. 529, no. 1, pp. 11–21, 2000.

[37] D. Brdiczka, G. Beutner, A. Rück, M. Dolder, and T. Wallimann, "The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition," *BioFactors*, vol. 8, no. 3-4, pp. 235–242, 1998.

[38] A. P. Halestrap, "What is the mitochondrial permeability transition pore?" *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 6, pp. 821–831, 2009.

[39] F. Ricchelli, J. Šileikyte, and P. Bernardi, "Shedding light on the mitochondrial permeability transition," *Biochimica et Biophysica Acta*, vol. 1807, no. 5, pp. 482–490, 2011.

[40] I. Szabo and M. Zoratti, "The mitochondrial megachannel is the permeability transition pore," *Journal of Bioenergetics and Biomembranes*, vol. 24, no. 1, pp. 111–117, 1992.

[41] Y. H. Ko, M. Delannoy, J. Hullihen, W. Chiu, and P. L. Pedersen, "Mitochondrial ATP synthasome: cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP," *Journal of Biological Chemistry*, vol. 278, no. 14, pp. 12305–12309, 2003.

[42] E. Jacotot, A. Deniaud, A. Borgne-Sanchez et al., "Therapeutic peptides: targeting the mitochondrion to modulate apoptosis," *Biochimica et Biophysica Acta*, vol. 1757, no. 9-10, pp. 1312–1323, 2006.

[43] G. Loor and P. T. Schumacker, "Role of hypoxia-inducible factor in cell survival during myocardial ischemia-reperfusion," *Cell Death and Differentiation*, vol. 15, no. 4, pp. 686–690, 2008.

[44] M. Tokarska-Schlattner, M. Zaugg, C. Zuppinger, T. Wallimann, and U. Schlattner, "New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics," *Journal of Molecular and Cellular Cardiology*, vol. 41, no. 3, pp. 389–405, 2006.

[45] K. M. Broekemeier, M. E. Dempsey, and D. R. Pfeiffer, "Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria," *Journal of Biological Chemistry*, vol. 264, no. 14, pp. 7826–7830, 1989.

[46] E. J. Griffiths and A. P. Halestrap, "Protection by cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts," *Journal of Molecular and Cellular Cardiology*, vol. 25, no. 12, pp. 1461–1469, 1993.

[47] S. J. Clarke, G. P. McStay, and A. P. Halestrap, "Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A," *Journal of Biological Chemistry*, vol. 277, no. 38, pp. 34793–34799, 2002.

[48] D. J. Hausenloy, M. R. Duchen, and D. M. Yellon, "Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury," *Cardiovascular Research*, vol. 60, no. 3, pp. 617–625, 2003.

[49] P. J. Henderson and H. A. Lardy, "Bongkrekic acid. An inhibitor of the adenine nucleotide translocase of mitochondria," *Journal of Biological Chemistry*, vol. 245, no. 6, pp. 1319–1326, 1970.

[50] M. Narita, S. Shimizu, T. Ito et al., "Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 25, pp. 14681–14686, 1998.

[51] N. Zamzami, C. E. Hamel, C. Maisse et al., "Bid acts on the permeability transition pore complex to induce apoptosis," *Oncogene*, vol. 19, no. 54, pp. 6342–6350, 2000.

[52] S. A. Novgorodov, T. I. Gudz, Y. M. Milgrom, and G. P. Brierley, "The permeability transition in heart mitochondria is regulated synergistically by ADP and cyclosporin A," *Journal of Biological Chemistry*, vol. 267, no. 23, pp. 16274–16282, 1992.

[53] R. A. Haworth and D. R. Hunter, "Control of the mitochondrial permeability transition pore by high- affinity ADP binding at the ADP/ATP translocase in permeabilized mitochondria," *Journal of Bioenergetics and Biomembranes*, vol. 32, no. 1, pp. 91–96, 2000.

[54] A. C. Lee, M. Zizi, and M. Colombini, "β-NADH decreases the permeability of the mitochondrial outer membrane to ADP by a factor of 6," *Journal of Biological Chemistry*, vol. 269, no. 49, pp. 30974–30980, 1994.

[55] A. Deniaud, C. Rossi, A. Berquand et al., "Voltage-dependent anion channel transports calcium ions through biomimetic membranes," *Langmuir*, vol. 23, no. 7, pp. 3898–3905, 2007.

[56] A. Deniaud, O. Sharaf El Dein, E. Maillier et al., "Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis," *Oncogene*, vol. 27, no. 3, pp. 285–299, 2008.

[57] F. P. Thinnes, H. Florke, H. Winkelbach et al., "Channel active mammalian porin, purified from crude membrane fractions of human B lymphocytes or bovine skeletal muscle, reversibly binds the stilbene-disulfonate group of the chloride channel blocker DIDS," *Biological Chemistry Hoppe-Seyler*, vol. 375, no. 5, pp. 315–322, 1994.

[58] V. Shoshan-Barmatz, N. Keinan, S. Abu-Hamad, D. Tyomkin, and L. Aram, "Apoptosis is regulated by the VDAC1 N-terminal region and by VDAC oligomerization: release of cytochrome c, AIF and Smac/Diablo," *Biochimica et Biophysica Acta*, vol. 1797, no. 6-7, pp. 1281–1291, 2010.

[59] M. P. Rigobello, F. Turcato, and A. Bindoli, "Inhibition of rat liver mitochondrial permeability transition by respiratory substrates," *Archives of Biochemistry and Biophysics*, vol. 319, no. 1, pp. 225–230, 1995.

[60] D. Gincel, S. Silberberg, and V. Shoshan-Barmatz, "Modulation of the voltagedependent anion channel (VDAC) by glutamate," *Journal of Bioenergetics and Biomembranes*, vol. 32, pp. 571–583, 2000.

[61] D. Gincel and V. Shoshan-Barmatz, "Glutamate interacts with VDAC and modulates opening of the mitochondrial permeability transition pore," *Journal of Bioenergetics and Biomembranes*, vol. 36, no. 2, pp. 179–186, 2004.

[62] A. M. Cesura, E. Pinard, R. Schubenel et al., "The voltage-dependent anion channel is the target for a new class of inhibitors of the mitochondrial permeability transition pore," *Journal of Biological Chemistry*, vol. 278, no. 50, pp. 49812–49818, 2003.

[63] A. Krauskopf, O. Eriksson, W. J. Craigen, M. A. Forte, and P. Bernardi, "Properties of the permeability transition in VDAC1$^{-/-}$ mitochondria," *Biochimica et Biophysica Acta*, vol. 1757, no. 5-6, pp. 590–595, 2006.

[64] A. W. C. Leung, P. Varanyuwatana, and A. P. Halestrap, "The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition," *Journal of Biological Chemistry*, vol. 283, no. 39, pp. 26312–26323, 2008.

[65] E. Fontaine, F. Ichas, and P. Bernardi, "A ubiquinone-binding site regulates the mitochondrial permeability transition pore," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25734–25740, 1998.

[66] A. Elimadi, R. Sapena, A. Settaf, H. Le Louet, J. P. Tillement, and D. Morin, "Attenuation of liver normothermic ischemia—reperfusion injury by preservation of mitochondrial functions with S-15176, a potent trimetazidine derivative," *Biochemical Pharmacology*, vol. 62, no. 4, pp. 509–516, 2001.

[67] A. Elimadi, V. Jullien, J. P. Tillement, and D. Morin, "S-15176 inhibits mitochondrial permeability transition via a mechanism independent of its antioxidant properties," *European Journal of Pharmacology*, vol. 468, no. 2, pp. 93–101, 2003.

[68] A. Ascah, M. Khairallah, F. Daussin et al., "Stress-induced opening of the permeability transition pore in the dystrophin-deficient heart is attenuated by acute treatment with sildenafil," *American Journal of Physiology*, vol. 300, no. 1, pp. H144–H153, 2011.

[69] D. P. Millay, M. A. Sargent, H. Osinska et al., "Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis attenuates muscular dystrophy," *Nature Medicine*, vol. 14, no. 4, pp. 442–447, 2008.

[70] T. Bordet, B. Buisson, M. Michaud et al., "Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis," *Journal of Pharmacology and Experimental Therapeutics*, vol. 322, no. 2, pp. 709–720, 2007.

[71] C. S. F. Queiroga, A. S. Almeida, C. Martel, C. Brenner, P. M. Alves, and H. L. A. Vieira, "Glutathionylation of adenine nucleotide translocase induced by carbon monoxide prevents mitochondrial membrane permeabilization and apoptosis," *Journal of Biological Chemistry*, vol. 285, no. 22, pp. 17077–17088, 2010.

[72] L. Azzolin, N. Antolini, A. Calderan et al., "Antamanide, a derivative of amanita phalloides, is a novel inhibitor of the mitochondrial permeability transition pore," *PLoS One*, vol. 6, no. 1, article e16280, 2011.

[73] N. Mewton, P. Croisille, G. Gahide et al., "Effect of cyclosporine on left ventricular remodeling after reperfused myocardial infarction," *Journal of the American College of Cardiology*, vol. 55, no. 12, pp. 1200–1205, 2010.

[74] M. Okorie, D. Bhavsar, D. Ridout et al., "Postconditioning protects against human endothelial ischaemia-reperfusion injury via subtype-specific KATP channel activation and is mimicked by inhibition of the mitochondrial permeability transition pore," *European Heart Journal*, pp. 1266–1274, 2011.

[75] J. S. Kim, L. He, T. Qian, and J. J. Lemasters, "Role of the mitochondrial permeability transition in apoptotic and necrotic death after ischemia/reperfusion injury to hepatocytes," *Current Molecular Medicine*, vol. 3, no. 6, pp. 527–535, 2003.

[76] T. Qian, A. L. Nieminen, B. Herman, and J. J. Lemasters, "Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes," *American Journal of Physiology*, vol. 273, no. 6, pp. C1783–C1792, 1997.

[77] H. Friberg, M. Ferrand-Drake, F. Bengtsson, A. P. Halestrap, and T. Wieloch, "Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death," *Journal of Neuroscience*, vol. 18, no. 14, pp. 5151–5159, 1998.

[78] L. Khaspekov, H. Friberg, A. Halestrap, I. Viktorov, and T. Wieloch, "Cyclosporin A and its nonimmunosuppressive analogue N-Me-Val-4-cyclosporin A mitigate glucose/oxygen deprivation-induced damage to rat cultured hippocampal neurons," *European Journal of Neuroscience*, vol. 11, no. 9, pp. 3194–3198, 1999.

[79] S. Matsumoto, H. Friberg, M. Ferrand-Drake, and T. Wieloch, "Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion," *Journal of Cerebral Blood Flow and Metabolism*, vol. 19, no. 7, pp. 736–741, 1999.

[80] S. Malouitre, H. Dube, D. Selwood, and M. Crompton, "Mitochondrial targeting of cyclosporin A enables selective inhibition of cyclophilin-D and enhanced cytoprotection after glucose and oxygen deprivation," *Biochemical Journal*, vol. 425, no. 1, pp. 137–148, 2010.

[81] H. Dube, D. Selwood, S. Malouitre, M. Capano, M. I. Simone, and M. Crompton, "A mitochondrial-targeted cyclosporin A with high binding affinity for cyclophilin D yields improved cytoprotection of cardiomyocytes," *Biochemical Journal*, vol. 441, no. 3, pp. 901–907, 2012.

[82] C. M. Porteous, A. Logan, C. Evans et al., "Rapid uptake of

lipophilic triphenylphosphonium cations by mitochondria in vivo following intravenous injection: implications for mitochondria-specific therapies and probes," *Biochimica et Biophysica Acta*, vol. 1800, no. 9, pp. 1009–1017, 2010.

[83] T. Miura, M. Tanno, and T. Sato, "Mitochondrial kinase signalling pathways in myocardial protection from ischaemia/reperfusion-induced necrosis," *Cardiovascular Research*, vol. 88, no. 1, pp. 7–15, 2010.

[84] J. Feng, E. Lucchinetti, P. Ahuja, T. Pasch, J. C. Perriard, and M. Zaugg, "Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3β," *Anesthesiology*, vol. 103, no. 5, pp. 987–995, 2005.

[85] S. Javadov, V. Rajapurohitam, A. Kilić, A. Zeidan, A. Choi, and M. Karmazyn, "Anti-hypertrophic effect of NHE-1 inhibition involves GSK-3β-dependent attenuation of mitochondrial dysfunction," *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 6, pp. 998–1007, 2009.

[86] L. Gomez, M. Paillard, H. Thibault, G. Derumeaux, and M. Ovize, "Inhibition of GSK3β by postconditioning is required to prevent opening of the mitochondrial permeability transition pore during reperfusion," *Circulation*, vol. 117, no. 21, pp. 2761–2768, 2008.

[87] J. G. Pastorino, J. B. Hoek, and N. Shulga, "Activation of glycogen synthase kinase 3β disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity," *Cancer Research*, vol. 65, no. 22, pp. 10545–10554, 2005.

[88] A. Rasola, M. Sciacovelli, F. Chiara, B. Pantic, W. S. Brusilow, and P. Bernardi, "Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 2, pp. 726–731, 2010.

[89] P. Pediaditakis, J. S. Kim, L. He, X. Zhang, L. M. Graves, and J. J. Lemasters, "Inhibition of the mitochondrial permeability transition by protein kinase A in rat liver mitochondria and hepatocytes," *Biochemical Journal*, vol. 431, no. 3, pp. 411–421, 2010.

[90] C. P. Baines, C. X. Song, Y. T. Zheng et al., "Protein kinase Cε interacts with and inhibits the permeability transition pore in cardiac mitochondria," *Circulation Research*, vol. 92, no. 8, pp. 873–880, 2003.

[91] K. Takuma, P. Phuagphong, E. Lee, K. Mori, A. Baba, and T. Matsuda, "Anti-apoptotic effect of cGMP in cultured astrocytes: inhibition by cGMP-dependent protein kinase of mitochondrial permeable transition pore," *Journal of Biological Chemistry*, vol. 276, no. 51, pp. 48093–48099, 2001.

[92] V. Borutaite, R. Morkuniene, O. Arandarcikaite, A. Jekabsone, J. Barauskaite, and G. C. Brown, "Nitric oxide protects the heart from ischemia-induced apoptosis and mitochondrial damage via protein kinase G mediated blockage of permeability transition and cytochrome c release," *Journal of Biomedical Science*, vol. 16, no. 1, article 70, 2009.

[93] M. Wyss, J. Smeitink, R. A. Wevers, and T. Wallimann, "Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism," *Biochimica et Biophysica Acta*, vol. 1102, no. 2, pp. 119–166, 1992.

[94] E. O'Gorman, G. Beutner, M. Dolder, A. P. Koretsky, D. Brdiczka, and T. Wallimann, "The role of creatine kinase in inhibition of mitochondrial permeability transition," *FEBS Letters*, vol. 414, no. 2, pp. 253–257, 1997.

[95] G. Beutner, A. Rück, B. Riede, and D. Brdiczka, "Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition by the kinases," *Biochimica et Biophysica Acta*, vol. 1368, no. 1, pp. 7–18, 1998.

[96] T. Miura, M. Nishihara, and T. Miki, "Drug development targeting the glycogen synthase kinase-3β (GSK-3β)-mediated signal transduction pathway: role of GSK-3β in myocardial protection against ischemia/reperfusion injury," *Journal of Pharmacological Sciences*, vol. 109, no. 2, pp. 162–167, 2009.

[97] G. Fajardo, M. Zhao, G. Berry, L.-J. Wong, D. Mochly-Rosen, and D. Bernstein, "β2-adrenergic receptors mediate cardioprotection through crosstalk with mitochondrial cell death pathways," *Journal of Molecular and Cellular Cardiology*, vol. 51, no. 5, pp. 781–789, 2011.

[98] J. M. Adams and S. Cory, "Life-or-death decisions by the Bcl-2 protein family," *Trends in Biochemical Sciences*, vol. 26, no. 1, pp. 61–66, 2001.

[99] T. N. Chonghaile, K. A. Sarosiek, T.-T. Vo et al., "Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy," *Science*, vol. 334, no. 6059, pp. 1129–1133, 2011.

[100] F. Llambi, T. Moldoveanu, S. Tait et al., "A unified model of mammalian BCL-2 protein family interactions at the mitochondria," *Molecular Cell*, vol. 44, no. 4, pp. 517–531, 2011.

[101] I. Marzo, C. Brenner, N. Zamzami et al., "The permeability transition pore complex: a target for apoptosis regulation by caspases and Bcl-2-related proteins," *Journal of Experimental Medicine*, vol. 187, no. 8, pp. 1261–1271, 1998.

[102] S. Shimizu, M. Narita, and Y. Tsujimoto, "Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC," *Nature*, vol. 399, no. 6735, pp. 483–487, 1999.

[103] Z. X. Chen and S. Pervaiz, "Bcl-2 induces pro-oxidant state by engaging mitochondrial respiration in tumor cells," *Cell Death and Differentiation*, vol. 14, no. 9, pp. 1617–1627, 2007.

[104] K. N. Alavian, H. Li, L. Collis et al., "Bcl-x_L regulates metabolic efficiency of neurons through interaction with the mitochondrial F 1 F O ATP synthase," *Nature Cell Biology*, vol. 13, no. 10, pp. 1224–1233, 2011.

[105] I. Marzo, C. Brenner, N. Zamzami et al., "Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis," *Science*, vol. 281, no. 5385, pp. 2027–2031, 1998.

[106] C. Brenner, H. Cadiou, H. L. A. Vieira et al., "Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator," *Oncogene*, vol. 19, no. 3, pp. 329–336, 2000.

[107] A. S. Belzacq, H. L. A. Vieira, F. Verrier et al., "Bcl-2 and Bax modulate adenine nucleotide translocase activity," *Cancer Research*, vol. 63, no. 2, pp. 541–546, 2003.

[108] S. Shimizu, A. Konishi, T. Kodama, and Y. Tsujimoto, "BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 7, pp. 3100–3105, 2000.

[109] H. L. A. Vieira, P. Boya, I. Cohen et al., "Cell permeable BH3-peptides overcome the cytoprotective effect of Bcl-2 and Bcl-XL," *Oncogene*, vol. 21, no. 13, pp. 1963–1977, 2002.

[110] R. Sugioka, S. Shimizu, T. Funatsu et al., "BH4-domain peptide from Bcl-xL exerts anti-apoptotic activity in vivo,"

Oncogene, vol. 22, no. 52, pp. 8432–8440, 2003.

[111] M. Ono, Y. Sawa, M. Ryugo et al., "BH4 peptide derivative from Bcl-xL attenuates ischemia/reperfusion injury thorough anti-apoptotic mechanism in rat hearts," *European Journal of Cardio-thoracic Surgery*, vol. 27, no. 1, pp. 117–121, 2005.

[112] D. Morin, R. Assaly, S. Paradis, and A. Berdeaux, "Inhibition of mitochondrial membrane permeability as a putative pharmacological target for cardioprotection," *Current Medicinal Chemistry*, vol. 16, no. 33, pp. 4382–4398, 2009.

[113] C. Plin, J. P. Tillement, A. Berdeaux, and D. Morin, "Resveratrol protects against cold ischemia-warm reoxygenation-induced damages to mitochondria and cells in rat liver," *European Journal of Pharmacology*, vol. 528, no. 1–3, pp. 162–168, 2005.

[114] H. Ligeret, S. Barthelemy, R. Zini, J. P. Tillement, S. Labidalle, and D. Morin, "Effects of curcumin and curcumin derivatives on mitochondrial permeability transition pore," *Free Radical Biology and Medicine*, vol. 36, no. 7, pp. 919–929, 2004.

[115] Z. Ungvari, W. E. Sonntag, R. De Cabo, J. A. Baur, and A. Csiszar, "Mitochondrial protection by resveratrol," *Exercise and Sport Sciences Reviews*, vol. 39, no. 3, pp. 128–132, 2011.

[116] S. Rimbaud, M. Ruiz, J. Piquereau et al., "Resveratrol improves survival, hemodynamics and energetics in a rat model of hypertension leading to heart failure," *PLoS One*, vol. 6, no. 10, article e26391, 2011.

[117] M. Izem-Meziane, B. Djerdjouri, S. Rimbaud et al., "Catecholamine-induced cardiac mitochondrial dysfunction and mPTP opening: protective effect of curcumin," *American Journal of Physiology*, vol. 302, no. 3, pp. H665–H674, 2012.

[118] V. Tanwar, J. Sachdeva, M. Golechha, S. Kumari, and D. S. Arya, "Curcumin protects rat myocardium against isoproterenol-induced ischemic injury: attenuation of ventricular dysfunction through increased expression of hsp27 alongwith strengthening antioxidant defense system," *Journal of Cardiovascular Pharmacology*, vol. 55, no. 4, pp. 377–384, 2010.

[119] P. Manikandan, M. Sumitra, S. Aishwarya, B. M. Manohar, B. Lokanadam, and R. Puvanakrishnan, "Curcumin modulates free radical quenching in myocardial ischaemia in rats," *The International Journal of Biochemistry & Cell Biology*, vol. 36, no. 10, pp. 1967–1980, 2004.

[120] G. F. Kelso, C. M. Porteous, C. V. Coulter et al., "Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties," *Journal of Biological Chemistry*, vol. 276, no. 7, pp. 4588–4596, 2001.

[121] D. Graham, N. N. Huynh, C. A. Hamilton et al., "Mitochondria-targeted antioxidant mitoq10 improves endothelial function and attenuates cardiac hypertrophy," *Hypertension*, vol. 54, no. 2, pp. 322–328, 2009.

[122] A. Vergeade, P. Mulder, C. Vendeville-Dehaudt et al., "Mitochondrial impairment contributes to cocaine-induced cardiac dysfunction: prevention by the targeted antioxidant MitoQ," *Free Radical Biology and Medicine*, vol. 49, no. 5, pp. 748–756, 2010.

[123] L. Plecitá-Hlavatá, J. Ježek, and P. Ježek, "Pro-oxidant mitochondrial matrix-targeted ubiquinone MitoQ10 acts as anti-oxidant at retarded electron transport or proton pumping within Complex I," *International Journal of Biochemistry and Cell Biology*, vol. 41, no. 8-9, pp. 1697–1707, 2009.

[124] J. M. Núñez-Córdoba and M. A. Martínez-González, "Antioxidant vitamins and cardiovascular disease," *Current Topics in Medicinal Chemistry*, vol. 11, no. 14, pp. 1861–1869, 2011.

[125] J. Q. Chen, P. R. Cammarata, C. P. Baines, and J. D. Yager, "Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications," *Biochimica et Biophysica Acta*, vol. 1793, no. 10, pp. 1540–1570, 2009.

[126] V. Regitz-Zagrosek, S. Oertelt-Prigione, U. Seeland, and R. Hetzer, "Sex and gender differences in myocardial hypertrophy and heart failure," *Wiener Medizinische Wochenschrift*, vol. 161, pp. 109–116, 2011.

[127] A. H. E. M. Maas, Y. T. Van Der Schouw, V. Regitz-Zagrosek et al., "Red alert for womens heart: the urgent need for more research and knowledge on cardiovascular disease in women," *European Heart Journal*, vol. 32, no. 11, pp. 1362–1368, 2011.

[128] S. P. Duckles and D. N. Krause, "Mechanisms of cerebrovascular protection: oestrogen, inflammation and mitochondria," *Acta Physiologica*, vol. 203, no. 1, pp. 149–154, 2011.

[129] C. M. Klinge, "Estrogenic control of mitochondrial function and biogenesis," *Journal of Cellular Biochemistry*, vol. 105, no. 6, pp. 1342–1351, 2008.

[130] K. A. Mattingly, M. M. Ivanova, K. A. Riggs, N. S. Wickramasinghe, M. J. Barch, and C. M. Klinge, "Estradiol stimulates transcription of nuclear respiratory factor-1 and increases mitochondrial biogenesis," *Molecular Endocrinology*, vol. 22, no. 3, pp. 609–622, 2008.

[131] R. Morkuniene, O. Arandarcikaite, L. Ivanoviene, and V. Borutaite, "Estradiol-induced protection against ischemia-induced heart mitochondrial damage and caspase activation is mediated by protein kinase G," *Biochimica et Biophysica Acta*, vol. 1797, no. 6-7, pp. 1012–1017, 2010.

[132] C. Borrás, J. Gambini, R. López-Grueso, F. V. Pallardó, and J. Viña, "Direct antioxidant and protective effect of estradiol on isolated mitochondria," *Biochimica et Biophysica Acta*, vol. 1802, no. 1, pp. 205–211, 2010.

[133] R. Morkuniene, A. Jekabsone, and V. Borutaite, "Estrogens prevent calcium-induced release of cytochrome c from heart mitochondria," *FEBS Letters*, vol. 521, no. 1-3, pp. 53–56, 2002.

[134] J. R. Muñoz-Castañeda, P. Montilla, M. C. Muñoz, I. Bujalance, J. Muntané, and I. Túnez, "Effect of 17-β-estradiol administration during adriamycin-induced cardiomyopathy in ovariectomized rat," *European Journal of Pharmacology*, vol. 523, no. 1–3, pp. 86–92, 2005.

[135] R. Ventura-Clapier, B. Mettauer, and X. Bigard, "Beneficial effects of endurance training on cardiac and skeletal muscle energy metabolism in heart failure," *Cardiovascular Research*, vol. 73, no. 1, pp. 10–18, 2007.

[136] A. Garnier, D. Fortin, J. Zoll et al., "Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle," *FASEB Journal*, vol. 19, no. 1, pp. 43–52, 2005.

[137] A. Ascensão, R. Ferreira, and J. Magalhães, "Exercise-induced cardioprotection—biochemical, morphological and functional evidence in whole tissue and isolated mitochondria," *International Journal of Cardiology*, vol. 117, no. 1, pp. 16–30, 2007.

[138] M. Ciminelli, A. Ascah, K. Bourduas, and Y. Burelle, "Short term training attenuates opening of the mitochondrial permeability transition pore without affecting myocardial function following ischemia-reperfusion," *Molecular and Cellular Biochemistry*, vol. 291, no. 1-2, pp. 39–47, 2006.

[139] M. Marcil, K. Bourduas, A. Ascah, and Y. Burelle, "Exercise training induces respiratory substrate-specific decrease in Ca^{2+}-induced permeability transition pore opening in heart

mitochondria," *American Journal of Physiology*, vol. 290, no. 4, pp. H1549–H1557, 2006.

[140] A. Ascensão, J. Lumini-Oliveira, N. G. Machado et al., "Acute exercise protects against calcium-induced cardiac mitochondrial permeability transition pore opening in doxorubicin-treated rats," *Clinical Science*, vol. 120, no. 1, pp. 37–49, 2011.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 395974, 16 pages
doi:10.1155/2012/395974

Review Article

Implication of Mitochondrial Cytoprotection in Human Islet Isolation and Transplantation

Yong Wang, Joshua E. Mendoza-Elias, Meirigeng Qi, Tricia A. Harvat, Sang Joon Ahn, Dongyoung Lee, Diana Gutierrez, Hyojin Jeon, Daniel Paushter, and José Oberholzer

Department of Transplant/Surgery, University of Illinois at Chicago, Chicago, IL 60612, USA

Correspondence should be addressed to José Oberholzer, jober@uic.edu

Received 1 October 2011; Accepted 30 January 2012

Academic Editor: Catherine Brenner

Islet transplantation is a promising therapy for type 1 diabetes mellitus; however, success rates in achieving both short- and long-term insulin independence are not consistent, due in part to inconsistent islet quality and quantity caused by the complex nature and multistep process of islet isolation and transplantation. Since the introduction of the Edmonton Protocol in 2000, more attention has been placed on preserving mitochondrial function as increasing evidences suggest that impaired mitochondrial integrity can adversely affect clinical outcomes. Some recent studies have demonstrated that it is possible to achieve islet cytoprotection by maintaining mitochondrial function and subsequently to improve islet transplantation outcomes. However, the benefits of mitoprotection in many cases are controversial and the underlying mechanisms are unclear. This article summarizes the recent progress associated with mitochondrial cytoprotection in each step of the islet isolation and transplantation process, as well as islet potency and viability assays based on the measurement of mitochondrial integrity. In addition, we briefly discuss immunosuppression side effects on islet graft function and how transplant site selection affects islet engraftment and clinical outcomes.

1. Introduction

Since the introduction of the Edmonton Protocol in 2000 [1], human islet transplantation has emerged as a promising therapy for type 1 diabetes mellitus (TIDM) and is the only minimally invasive therapy able to achieve glycemic control without exogenous insulin. As a cell therapy, islet transplantation is a multistep process involving pancreas organ procurement and preservation, tissue digestion and dissociation, islet purification, cell culture, islet transplantation via the hepatic portal vein of the recipient, and maintenance of the graft by nonsteroidal immunosuppressant regiments. Successful islet transplantation is dictated by the cumulative success of the aforementioned steps. To date, islet transplantation has been shown to have variable success in both short- and long-term insulin independence [2–5] and much of this variability is associated with factors from both the organ donor and recipient. In order to maximize the success rate of achieving insulin independence, considerable efforts have been focused on the following aspects: (i) development of a suitable organ preservation solution; (ii) standardization of good manufacturing principles (GMP) in islet isolation; (iii) development of an accurate potency test that can predict islet graft function prior to transplantation (as outlined by requirements for an FDA Biologics License); (iv) development of a new immunosuppression regimen with less islet graft toxicity; (v) improvement of islet graft survival via *ex vivo* and *in vivo* manipulation; (vi) selection of a more ideal transplant site.

While we often focus on getting more islets from each islet isolation, one of the top priorities that should not be neglected is the attainment of better quality islets with preserved β-cell function and viability. Located in the cytoplasm, the main function of the mitochondria is to provide energy for the cell in the form of ATP. Cells use this energy to perform the work necessary for cell survival and function. Moreover, mitochondria are also involved in other cell processes such as cell growth, division, and apoptosis. Pancreatic islets consist of a cluster of 1000–2000 cells and ranging in size between 50–400 μm in diameter.

Composing 1-2% of the pancreas mass, each islet consists of at least five different cell types: α-cells (15–20% of islet mass) producing glucagon; β-cells (65–80% of islet mass) producing insulin and amylin; δ-cells (3–10% of islet mass) producing somatostatin; PP cells (3–5% of islet mass) producing pancreatic polypeptide; ε-cells (<1% of islet mass) producing grhelin. Specifically for β-cells, mitochondria play a key role in glucose-stimulated insulin secretion, not only by providing energy in the form of ATP to support insulin secretion, but also by synthesizing anapleurtoic metabolites that can act, both intra- and extramitochondrially, as factors that couple glucose sensing to insulin granule exocytosis. ATP on its own, and possibly modulated by these anapleurotic coupling factors, triggers closure of the ATP-sensitive potassium channel, resulting in membrane depolarization that increases intracellular calcium to cause insulin secretion [6–9].

Recently, the roles of mitochondria in cytoprotective applications involving ischemia-reperfusion (I/R) injury have been acknowledged and continue to be actively investigated using chemical and physical means during both islet isolation and on posttransplanted islet grafts. However, no systematic review has been done in this respect. In this review, we will examine current and past research from the last ten years on the role of mitochondria and mitoprotective strategies that have been applied in each phase of the islet isolation process.

2. Mitochondria and Mitoprotection in Pancreas Organ Preservation and Storage

While initial results of human islet transplantation using the Edmonton Protocol are promising, the need for a large quantity of islets to achieve insulin independence from multiple organs per patient creates an obstacle to the application of human islet transplant as a routine clinical procedure. Pancreas preservation and storage is the first step in the islet isolation process and has a critical impact on islet yield and quality. During the isolation process, only a fraction of the islets in a whole pancreas can be successfully isolated with good function and viability; this is especially true for organs with a prolonged cold ischemia time.

Ischemia injury has been shown to significantly decrease the quantity and quality of islets that can be isolated from a pancreas [10–12]. Hypoxia and ischemia, during the process of pancreas procurement and storage, triggers a cascade of cell signaling pathways that compromise islet viability and function [13–15]. University of Wisconsin (UW) and Histidine-Tryptophan-Ketoglutarate (HTK) solutions are the two most commonly used organ preservation solutions intended for islet isolation. Both are designed to protect the organ from ischemia-related cell injury. As a gold standard, UW solution is the first solution thoughtfully designed for use in organ transplantation and the first intracellular-like preservation medium. UW solution contains metabolically inert substances (lactobionate, raffinose, and hydroxyethyl starch) as a glucose substitute that maintains native osmotic pressure, thereby preventing cellular edema. In addition, UW solution also adds free radical scavengers along with steroids and insulin. In contrast, the composition of HTK is similar to that of the extracellular fluid and is developed based on the principle of deactivating and suspending of cellular organ function by withdrawal of extracellular sodium and calcium. Together with intensive buffering of the extracellular space by means of histidine/histidine hydrochloride, the period during which organs will tolerate prolonged interruption of oxygenated blood may be increased. However, both solutions do not prevent the deleterious effects of hypoxia and ischemia *per se*, especially for prolonged cold storage of the pancreas [16–18].

Enormous attempts have been made to reduce ischemia injuries through the oxygenation of preservation solution. Perfluorochemicals (PFCs) are one of the chemicals that have been developed so far with the most significant impact on islet isolation. The PFCs are cyclic or aliphatic hydrocarbon molecules in which all the hydrogen atoms have been replaced with fluorine. PFCs are a good solvent for gases and approximately 40 mL of oxygen can be dissolved in 100 mL of 90–95% PFC solutions. This makes the compound attractive as a vehicle to deliver oxygen and other gases *in vivo* and *in vitro*. PFCs were first induced by Kuroda et al. for whole pancreas preservation in 1988 [19–21] as a two-layer storage method (TLM) in combination with UW solution and later adopted in pancreas preservation for islet isolation. Since then, more than 50 research papers have been published in both human and animal models and the impacts of PFCs on islet isolation outcomes have been reviewed [22–27]. In the following section, we will briefly address the major findings from these studies and discuss the possible reasons for the observed discrepancy in success and failure for islet isolation using PFCs and summarize these observations in Table 1, along with additional oxygen carriers used for islet isolation.

In their early studies, Matsumoto and Kuroda showed that when PFCs were used within the TLM protocol, they demonstrated significant increases in islet yield, function, and viability. In addition, PFCs extended preservation times [28–30] when compared against UW solution alone. The TLM was then immediately and widely adopted early by several renowned islet programs, which demonstrated similar results [10, 31–33] and later adopted by others [34, 35]. One study demonstrated that islets isolated using the TLM had an improved energetic profile with higher ATP content and ATP/ADP ratio, as wells as 40% less peroxidative damage, indicating the mitochondria as a likely target in a potential mechanism [36]. In 2006, Ramachandran et al. analyzed the expression of pro- and antiapoptotic genes in the islets isolated using the TLM, which showed a significant increase in the expression of inhibitor of apoptosis (IAP) and survival; accompanied by decreases in the expression of BAD, BAX, and caspases (caspase-2, -8, and -9). The improved islet yield by PFCs was linked to the inhibition of apoptosis mediated by an undescribed mitochondrial pathway [37]. However, more recently, several large-scale studies and a retrospective meta-analysis indicated that the beneficial effect of the TLM on islet isolation and clinical transplantation outcomes are minimal as PFCs may only improve the preservation of marginal organs [38–42]. Many contribute this finding to

Table 1: Oxygen carriers used in pancreas organ preservation and islet isolation.

Oxygen carriers	Benefits	Limitation	References
PFC	(i) Prevention of ischemia injury (ii) Improvement of islet yield and quality (iii) Inhibition of apoptosis by mitochondrial protection via oxygen loading (iv) Preservation of islet cell energetic status (v) Low islet oxidative stress (vi) Better *in vivo* islet graft function	(i) Inconsistent results despite several large-scale human trials (ii) May only be useful for marginal donors with prolonged ischemia (iii) Limited oxygen penetration into pancreas (iv) Poor water solubility (v) Indeterminate biosafety	[22–42]
PFD	(i) Preservation of islet ATP levels	(i) Only tested in animal model (ii) Impairment of insulin secretion	[43]
poly SFH-P	(i) Improvement of islet yield and quality (ii) Preservation of mitochondrial integrity (iii) No increase in oxidative stress (iv) Better *in vivo* islet graft function (v) High oxygen tension with oxygen saturation curve similar to RBCs	(i) Only in rodent model (ii) Warm-ischemia model used may not be applicable in a cold-ischemia model (iii) Product discontinued	[44]

the poor capability of PFCs to promote oxygen penetration into the whole pancreas as PFCs cannot be flushed into the pancreatic vascular system either with or without UW solution due to its poor water solubility. In 2009, Matsumoto et al. reanalyzed their previous data and contributed the observed discrepancy to the lack of experience of organ procurement teams [27] since a standardized protocol was not widely accepted across all centers.

To date, there have been few other oxygen carriers that have been tested for their effects on pancreas preservation intended for islet isolation, mainly due to their low oxygen carrier capability, poor water solubility, and poor biocompatibility. In the pig pancreas model, oxygenated perfluorodecalin (PFD) was used at various temperatures showing that pig pancreas oxygenation at 20°C increases islet ATP generation; however, the islets were found to have compromised insulin secretion in response to glucose stimulation [43]. In a human islet study, F6H8S5, a newly developed oxygen carrier composed of F6H8 (perfluorohexyloctane) and silicone oil polydimethylsiloxane 5, was compared to PFD, showing comparable results linking the optimization of ATP production during the cold storage period [44].

In a warm-ischemia rat model, oxygenated poly SFH-P (polymerized and stroma-free hemoglobin-pyridoxalated) was applied to the pancreas prior to enzyme digestion, showing that the islets isolated from a poly SFH-P treated pancreas had improved β-cell viability and mitochondrial potential changes in response to glucose stimulation. In addition, O_2 delivery by the poly SFH-P did not increase glutathione (GSH) levels (an indicator for oxidative stress) or malondialdehyde (MDA) levels (an indicator for oxidative injury) [45]. These results supported the notion that there was a mitoprotective effect associated with the Poly SFH-P treatment. However, it is worth noting that other groups did not further confirm this study either in rodent or human islet isolation process due to discontinuation of the product.

There have been multiple attempts to supplement UW solution with other mitoprotective agents such as trophic factors [46, 47], mitochondria-targeted antioxidant mitoquinone [48], carvedilol (a non-selective β blocker/α-1 blocker) [49], epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1) [50, 51]. However, the benefits of these chemicals have not been verified by systematic studies or used in pancreas organs intended for islet isolation.

Furthermore, a specific problem linked to pancreas organ preservation is the leakage of zymogens from the exocrine pancreas. Within the pancreas, serine proteases exist in the form of an inactive proenzymes and are secreted from the pancreas as trypsinogen, chymotrypsinogen, and proelastase. The activation pancreatic proteases during isolation has been observed and found to be caused in part by crude impurities in collagenase preparations [52]. The resulting cell death during the isolation process releases immunogenic intracellular proteases that prompt the generation of free radicals in the mitochondria and trigger activation of macrophages to produce proinflammatory cytokines. In addition, Heiser et al. reported that there was a strong increase in trypsin activity levels throughout the digestion phase and that these increased levels correlated with poor islet yields and function [53]. It has also been shown in several studies that the addition of protease inhibitors, such as Pefabloc and α_1-antitrypsin (A1AT) in UW and HTK solutions, as well as used as in the TLM, can increase islet yield and function by effectively inhibiting this uncontrolled protease activity [32, 54–56]. However, a recent large-scale study showed that Pefabloc actually did not improve the overall islet yields. In addition, in one study, it was found in that pancreas preserved in the TLM may provide even better transplant quality islets in the absence of Pefabloc [57].

In summary, although some promising studies have shown the beneficial mitoprotective effects for islets by oxygenation and chemical addition to organ preservation solutions, the involved underlying mechanisms and their overall impacts remain controversial and grounds for further study. In addition, since the introduction of the Edmonton Protocol, islet yield from one pancreas remains low with an average of 300,000–400,000 IEq depending on center expertise. Organ preservation solutions such as UW or HTK are still considered the most effective solution for preventing ischemic injury until other alternatives are available that

specifically address the needs unique to pancreata intended for islet transplant.

3. Mitochondria and Mitoprotection in Islet Isolation and Culture

Since reperfusion injury is one of the primary factors associated with islet cell death during islet isolation, there have been extensive investigations dedicated to minimizing the effects of reperfusion injury in order to improve islet isolation outcomes. Reperfusion-related cell damage is due in part to the inflammatory response of islet tissues mediated by the release of inflammatory factors including interleukins and reactive oxygen species (ROS). Although the mechanism is not fully characterized, both inflammatory factors and ROS intertwine as either initiator or effector in an inflammatory cascade during islet isolation that may cause β-cell dysfunction and death. During reperfusion and reoxygenation, significantly increased levels of ROS can degrade cell and capillary membranes that cause additional release of free radicals and act indirectly to initiate redox-signaling pathways leading to apoptosis. In addition, reoxygenation can restore ATP levels that may cause an increase in calcium uptake by the mitochondria, resulting in massive calcium overload and destruction of the mitochondria.

Oxidative stress has been shown to play a pivotal role in cell injury during the islet isolation and transplantation processes [106–108]; therefore, blocking oxidative stress should have a beneficial impact on transplantation outcomes. To date, several antioxidants have been used to protect islet cells from oxidative injury during the isolation and culture period. Herein, we will briefly discuss these findings.

Bottino et al. demonstrated that activations of nuclear factor-kappaB (NF-κB) and poly (ADP-ribose) polymerase (PARP), two of the major pathways responsible for cellular responses to stress, occur in islet cells during the isolation procedure and precedes cellular dysfunction and demise including the disruption of mitochondrial membrane potentials (MMPs); mitochondrial permeability transition pore (MPT) formation; and an intracellular increase of accumulating ROS. NF-κB-dependent reactions, such as the production and release of interleukins (IL-6 and IL-8) and macrophage chemoattractant protein-1 (MCP-1), were observed days after the isolation procedure. Proinflammatory responses were even more pronounced when islets were cultured under specific conditions which mimicked isolation stress and correlated well with higher islet cell loss and impaired secretory function [58]. In addition, Bottino et al. showed that early interventions aimed at reducing β-cell oxidative stress through the use of the catalytic antioxidant probe AEOL10150 (manganese [III] 5,10,15,20-tetrakis [1,3,-diethyl-2imidazoyl] manganese-porphyrin pentachloride [TDE-2,5-IP]) can effectively reduce DNA binding of NF-κB and the subsequent release of cytokines, chemokines, and PARP activation in islet cells; thus resulting in higher survival and better insulin release when compared to controls [58]. A similar study conducted by the same authors demonstrated that the application of two SOD mimetics (AEOL10113 and AEOL10150) can also protect human islets from oxidative stress, showing a significantly higher viable islet mass and better *in vivo* islet graft function using a marginal islet mass transplant model [59].

GSH (glutamate-glycine-cysteine) is one of the most important nonenzymatic antioxidants available; however, it is not cell permeable. Cells can synthesize GSH from its precursors, such as glutamine. The importance of glutamine for cell survival and proliferation *in vitro* was first described by Eagle et al. in 1956 [60]. The cytoprotective and anti-apoptotic effects of glutamine have been demonstrated in intestinal epithelial cells by Evans et al. [61] and also in pancreatic islets showing that GSH increases β-cell insulin secretion [62–64]. In both human and rodent models, intraductal delivery of glutamine into the pancreas prior to digestion can increase GSH levels, reduce MDA levels, and reduce the number of apoptotic cells. In addition to an improved islet yield, the percentage of nude mice rendered normoglycemic with glutamine-treated islets was higher than the controls and time to reach normoglycemia was decreased from average of 7.3 ± 3 days to 1.83 ± 0.4 days [65, 66].

Although most antioxidant compounds have a wide extracellular and intracellular distribution, they often fail to accumulate within the mitochondria and require conjugation with lipophilic cations for mitochondrial targeting. Their ability to enter the mitochondria and accumulate within the matrix also depends on the inner mitochondrial membrane potential (MMP) and its proton gradient; a feature which may change depending on the metabolic status of cells. This requirement may limit their capacity to permeate depolarized cells. Moreover, the accumulation of these antioxidant cations within the mitochondrial matrix can lead to dissipation of inner MMP, an event that often leads to cell death. As a result, these agents exhibit narrow therapeutic dose ranges. To combat this, genetic strategies are being explored to bolster the antioxidant defense of islets. *Ex vivo* transfer of the manganese superoxide dismutase (MnSOD) gene to mouse islets has extended islet graft function in autoimmune diabetic mice. It has been observed that the islets from mice that overexpress GSH peroxidase and the two isoforms of SOD improve blood glucose control in a marginal islet mass model.

A point of concern to its clinical application is that it has been demonstrated that viral delivery systems can pose potential oncogenic risks, compromise islet function, and increase immunogenicity; the latter being especially concerning given the pathophysiological setting of T1DM. Recently, two research groups have demonstrated that polyvalent gold nanoparticles densely functionalized with covalently immobilized DNA oligonucleotides (AuNP-DNA) have a high penetrative capacity for islet cells which can reach the islet cores of both mouse and human islets with no evidence of toxicity; demonstrating that islet function is preserved well both *in vitro* and *in vivo* as compared to control. One study showed that AuNP-treated islets have normal mitochondrial response kinetics when stimulated by glucose, in conjunction with preserved calcium influx and insulin secretion when compared to control [109]. The other study indicated that AuNP-DNA conjugated with antisense eGFP reduced eGFP

expression in MIP-eGFP islets (mouse insulin promoter controlled eGFP) [110]. AuNP and/or AuNP-conjugates may represent a new generation of nucleic acid-based therapeutic platforms aimed at improving islet engraftment, survival, and long-term *in vivo* function. It is expected that more investigations into the use of nanoparticles for cargo delivery to islets will follow to confirm this observation while improving this functional delivery system.

In addition to using physical, chemical, and viral platforms for delivery of antioxidative cargo, molecules that have good penetrative ability have also been investigated. SS-31 (D-Arg-2′,6′-dimethyltyrosine-Lys-Phe-NH_2), a novel water-soluble antioxidant peptide, is one of a few molecules tested that have demonstrated the ability to penetrate into the core of islets and localize in the mitochondria [67]. In addition, SS-31 preserves MMPs, reduces islet cell apoptosis, increases islet cell yield, and improves posttransplantation function as demonstrated by a prompt and sustained normoglycemia; whereas untreated islet graft recipients remained diabetic. One study has demonstrated that SS-31 can inhibit, with minimal cellular toxicity, mitochondrial swelling, oxidative cell death, and I/R injury of cardiac and neuronal cells [68]. However, to date there are no published reports of the effect of SS-31 in transplanted human islets.

The effector mechanisms by which cytokines induce β-cell death may also encompass both nitric oxide (NO) dependent and independent pathways [69]. In rodent islets, interleukin-1 beta (IL-1β) alone can induce iNOS expression and the resultant NO production impairs β-cell function by oxidation of mitochondrial aconitase (m-con) which leads to diminished glucose oxidation and ATP production [70]. Steer et al. demonstrate that this NO-mediated pathway of β-cell death is primarily necrosis mediated by the release of the high-mobility group box 1 protein (HMGB1) [71]. In contrast, human islets appear to be more resistant to cytokine induced NO production and the primary mechanism of dysfunction may be the result of mitochondrial ROS production and activation of proapoptotic caspase enzymes [72]. A variety of specific and nonspecific inhibitors of reactive nitrogen and oxygen species have been evaluated for their effects on islets in culture and *in vivo* posttransplant. Inhibition of iNOS by NG-monomethyl-L-arginine (NMMA) or aminoguanidine (Pimagidine) in culture can block NO production in both rodent and human islets [73]. Treatment with N-acetyl cysteine or glutathione peroxidase can enhance the antioxidant capacity of islet β-cells [74, 75].

When human pancreatic islets are treated with a cytokine combination of IL-1β, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IGN-γ) for 72 h, a significant increase in the amount of cell death was observed by TUNEL (terminal deoxynucleotidyl transferase dUTP end labeling) and cell death detection [68]. Furthermore, this study demonstrated that this cell death was associated with apoptosis and mitochondrial swelling. A possible link has been suggested between peripheral benzodiazepine receptors (PBR), also known as translocator protein (TSPO), which implicates a specific mitochondrial permeability transition pore and tissue damage associated with the production of inflammatory mediators.

Various vitamins (D3, E, Riboflavin, and C) have also been used as antioxidants *in vitro* and *in vivo* and show protective benefits including increased insulin secretion, higher islet cell viability, increased insulin gene expression, and reduced lipid peroxidation. Since these results have been reviewed previously [76], in this review we will not further discuss the findings.

Recently, it has been hypothesized that a better strategy could be to block the initiation of the inflammatory pathways triggered by the interaction of IL-1β with its cellular receptor (interleukin-1 receptor, IL-1R). The effectiveness of the IL-1R antagonist (Kineret, (Anakinra)) in blocking the proapoptotic and necrotic effects of exogenous IL-1β, TNF-α, and IFN-γ on cultured rat islets has been investigated. Anakinra is a U.S. FDA approved drug used as an anti-inflammatory therapy in clinical trials of human subjects with rheumatoid arthritis [77] and type 2 diabetes mellitus (T2DM) [78]. It is a human recombinant, nonglycosylated form of the human interleukin-1 receptor antagonist (IL-1ra) consisting of 153 amino acids with a molecular weight of 17.3 kDa. The biologic activity of Anakinra derives from competitive inhibition of IL-1β binding to the interleukin-1 type I receptor (IL-1RI). In several animal models and *in vitro* human islet studies, blocking IL-1β receptor (IL-1R) has shown an effective inhibition of activation of IL-1β-dependent inflammatory pathways [79], enhancement of islet engraftment [80] and islet graft function, and attenuation of amyloid polypeptide-induced proinflammatory cytokine release [81]. All of these results support its feasible application to human islets *in vivo* as a posttransplant therapeutic regiment.

Two studies conducted by one group have demonstrated the application of either pan-caspase inhibitor (N-benzyloxycabonalyl-Val-Ala-Asp-fluoromethyl ketone [Z-VAD-FMK]) or more selective caspase inhibitor (EP1013 [zVD-FMK]) during postisolation culture and *in vivo* treatment can reduce islet loss following prolonged culture and reverse diabetes following implantation of a marginal human islet mass (80–90% reduction) into mice [82, 83]. Several similar studies have also demonstrated the benefits of caspase inhibitors for islet transplant [84, 111].

Some studies have demonstrated a significant beneficial effect of recombinant human prolactin (rhPRL) on β-cell proliferation, islet secretion, cytoprotection, islet engraftment, and function of the transplanted islets by revascularization and β-cell survival [85–87]; however the underlying mechanism is unclear. One recent study has shown that cytoprotection may involve an increase in BCL2/BAX ratio and inhibition of several caspases such as 8, 9, and 3 [88].

The signal transduction pathway of c-Jun NH_2-terminal kinase (JNK), a member of the stress-activated group of mitogen-activated protein kinases (MAPKs), is preferentially activated in response to islet isolation process, oxidative stress, and cytokine toxicity [89]. It has been reported that an IL-1β storm caused by brain death significantly activates JNK and reduces islet yield, viability, and function both *in vitro* and *in vivo* after transplantation [90]. It has also been shown that islet injection into the hepatic portal vein system leads to a strong activation of JNK [91]. The application of JNK

inhibitor during islet culture and transplantation can prevent islet loss and *in vivo* graft function [91, 92]. In addition, a JNK inhibitor has also been also added to pancreas preservation solution showing a strong inhibition of JNK which prevented apoptosis immediately after isolation [93].

Previously, we discussed that the activation of trypsin and other endogenous pancreatic zymogens contribute to decreased islet yields via the uncontrolled proteolysis of collagenase and the prolongation of digestion times. Attempts to use Pefabloc during the isolation process instead of organ preservation phase have been made, showing that the Pefabloc can inhibit serine protease activity throughout the tissue digestion process; but, with controversial results on islet yield and function [94–96]. In addition, two research papers recently published by the same group show that when human islets were cultured or perifused with Pefabloc postisolation, insulin secretion of human islets in response to glucose stimulation was compromised, suggesting that the Pefabloc is not suitable for human islet isolation [97, 98].

Summarized in Table 2, are the major antioxidative and anti-inflammatory chemicals used in pancreas preservation, islet isolation, and islet culture.

4. Evaluation of Mitochondrial Integrity for Human Islet Function and Viability Assays

The U.S. Food and Drug Administration (FDA) defines the preparation of the islet product as a biological drug; therefore, islet products for transplant need to be prepared under FDA-approved guidance before islet transplant can be approved as a clinical therapy. Despite standardization and application of current good manufacturing practices (cGMPs) in the islet isolation process, lot-to-lot variability still cannot be avoided. To reduce the risk of transplanting low-quality islets, appropriate product release tests are needed. While tests for sterility, identity, and purity are well established, so far no reliable assessment of islet potency is available prior to transplant. This continues to be one of the key variables associated with clinical transplant outcomes.

The existing standard assays used to assess islet function and viability include static glucose-stimulated insulin stimulation (GSIS) for potency, and inclusive and exclusive dyes to stain membrane integrity for viability. Both of these techniques have low predictive values and do not correlate well with clinical transplant outcomes [112–116]. Presently, clinicians determine the suitability of a given islet preparation mainly based on both subjective measurements (e.g., morphology) and islet cell mass (IEq, a volumetric quantification of islet mass). The underlying reasons for the poor predictive value of GSIS are less clear; however, there are several possible explanations. Static GSIS only measures "bulk" insulin release from an islet preparation under extreme conditions (16.7 mM and 60 min glucose exposure), completely ignoring the dynamic physiological nature of β-cell insulin secretory kinetics in response to glucose. In addition, the "bulk" insulin data does not provide any useful information of the key stimulus-coupling factors that are necessary for controlling and regulating both insulin secretion and viability *in vivo*. For example, mild stress during pancreas preservation and islet isolation process can lead to temporary insulin degranulation and leakage caused by cell membrane damage, even though the islets are still viable. Therefore, a low GSIS may not indicate irreversible loss of function, as the temporarily impaired islet cells may recover when transplanted into a recipient. Likewise, a high GSIS result may be caused by insulin degranulation via cell membrane damage.

Islet size is another factor that can cause variable GSIS results. Unlike *in situ*, the isolated islet's sensing of ambient glucose changes is totally dependent on the passive diffusion of glucose [99, 114]. Since smaller islets have a larger surface area, a better GSIS would be expected [100, 117] in a population of smaller islets. In clinical practice, IEq is used as a determining factor in deciding the suitability of a given islet preparation when human islet potency is less defined or hard to quantify. It is a general consensus that, in order to reverse diabetes in type 1 diabetes recipients, at least 5,000 IEq per kilogram of recipient body weight are needed for one transplant. Paradoxically, larger size islets contribute more IEq but release less insulin which is a likely explanation of why islet graft success does not correlate strongly with transplanted islet mass.

On the contrary, the *in vivo* potency assay which evaluates islets by transplanting human islets into immunodeficient nude mice correlates extremely well with clinical transplant outcomes and is currently the gold standard for evaluating islet potency. However, this *in vivo* analysis takes several weeks to complete and, therefore, only provides a retrospective indication of islet function that renders this assay impractical as a pretransplant assessment in the time critical clinical setting [101, 103, 118, 119].

To address this problem, a variety of *in vitro* tests have been investigated and developed in order to assess islet potency and viability prior to islet release for transplant, including: measuring the oxygen consumption rate (OCR) [104, 114, 115, 120, 121]; the quantification of reactive oxygen species (ROS) [116]; and ADP/ATP ratios [105, 122]. In addition to being retrospectively useful and less predictive, these assays have multiple limitations. To begin with, most OCR and ROS assays are conducted statically as a single parameter. Additionally, OCR and ROS assays are less β-cell specific, as an islet has at least five different cell types and β-cells only make up 65–80% of a human islet cell population. Without a weighted analysis of ROS for each individual cell group, this information is confounded; and even then the measurements may be artefactual as islets are one physiologic and metabolic functional unit. Recently, more and more evidence has demonstrated that chemical and/or ion communication among β-cells or between β-cells and α-cells is important for the regulation of insulin secretion [123, 124]. In addition, gap junctional complexes between adjacent islet cells are also needed to facilitate intraislet cell-cell communications and coordination for hormonal output [102]. Therefore, others often challenge the accuracy of ADP/ATP assay since the assay requires islet dissociation. A comprehensive review on these assays has been recently published [113]. Herein, we will briefly

Table 2: Major anti-oxidative and anti-inflammatory chemicals used in pancreas preservation, islet isolation, and islet culture.

Chemicals	Benefits	Limitation	References
AEOL10150 AEOL10113	(i) Antioxidative (SOS mimics) used in both isolation stage and culture (ii) Reduction of NF-κB binding of DNA (iii) $\downarrow$IL-6,8; $\downarrow$MCP-1 (iv) Inhibition of the release of cytokines and chemokines and PARP activation (v) Protection of islets from oxidative stress	(i) No *in vivo* islet graft function available (ii) Has not been demonstrated by other groups	[58, 59]
Glutamine	(i) Benefits in both animal and human models during islet isolation (ii) Increasing of GSH levels (iii) Reduction of malondialdehyde and apoptotic cells (iv) Improvement of *in vivo* islet graft function	(i) Needs to be demonstrated by large-scale or multicenter studies (ii) Short-term effect due to instability and short half-life of glutamine (iii) Relationship with inflammatory cascade needs to be tested	[60–66]
SS-31	(i) Water-soluble antioxidative peptide with high islet penetration (ii) Specific mitochondrial targeting (iii) Preservation of mitochondrial polarization and reduced apoptosis (iv) Improvement of islet *in vivo* function	(i) Needs to be demonstrated by large-scale or multicenter studies (ii) Only tested in animal model	[67, 68]
NMMA Aminoguanidine N-acetyl cysteine Glutathione peroxidase	(i) Blockage of NO production via inhibition of iNOS in rodent and human islets (ii) Enhancement of islet antioxidant capability	(i) Only demonstrated *in vitro* (ii) The benefits for islet transplant need to be shown	[69–75]
Vitamins (D3, E, Riboflavin, C)	(i) Increased insulin secretion (ii) Higher islet viability (iii) Increased insulin gene expression (iv) Decreased lipid peroxidation	(i) Application in human islet isolation is limited (ii) No documented benefits on human islet receipts	[76]
Anakinra	(i) IL-1R antagonist via competitive inhibition (ii) $\downarrow$TNF-α; $\downarrow$IL-1β; $\downarrow$IFN-γ (iii) FDA approved as anti-inflammatory agent	(i) Not demonstrated in human islet isolation and transplant	[77–81]
Pan-caspase (ZVAD-FMK) and selective caspase inhibitor (zVD-FMK)	(i) Reduced islet loss during culture (ii) Improve islet graft function (iii) Reduced islet cell apoptosis	(i) No demonstrated benefits in human islet receipts	[82–84]
Prolactin	(i) Increased β-cell proliferation (ii) Increased insulin secretion (iii) Cytoprotection (iv) Improved islet engraftment (v) Increased islet revascularization (vi) Increased BCL2/BAX ratio (vii) Inhibition of caspase 8, 9 and 3	(i) Less understanding of mechanism (ii) Needs to be demonstrated by large-scale or multicenter studies	[85–88]
JNK inhibitor	(i) Increased islet yield (ii) Improved islet viability (iii) Improved *in vivo* graft function	(i) Limited information on human patients (ii) Large-scale study need to confirm	[89–93]
Pefabloc	(i) Efficient inhibition of serine protease activity (ii) Has been applied in all phases of isolation (iii) No interference with collagenase activity	(i) Controversial results on islet yield (ii) Inhibited insulin secretion *in vitro*	[94–98]

discuss the assays measuring mitochondrial integrity for islet potency and viability. In Table 3, we summarized the strengths and limitations of these assays.

In the search for a more reliable and β-cell specific potency assay, the measurement of mitochondrial integrity has been emerging as an alternative assay in the form of a single parameter or combined with other parameters for islet viability and potency.

Zinc is highly concentrated in β-cells but much less concentrated in other islet cell types. Zinc plays an important role in the packaging insulin granules as it is firmly established as an integral coordinating atom of the insulin crystal as a 2-Zn-insulin hexamer. Recently, Newport Green (NP), a less-toxic zinc-sensitive fluorescent probe, has been used for the identification and purification of human β-cells [125]. In 2005, Itchii et al. reported a novel combined analytical method in which β-cells were first specifically identified using NP labeling and sorted by laser scanning cytometry (LSC). NP^+ positive cells were then evaluated for viability using TMRE (tetramethylrhodamine ethyl ester),

Table 3: Advantages and disadvantages of mitochondria-based islet potency and viability assays.

Assays	Advantages	Disadvantages	References
Newport Green + TMRE	(i) Low toxicity of Newport Green Dye (ii) β-cell specific (iii) Correlates with *in vivo* islet function	(i) Islet dissociation needed (ii) Nonstimulated static assay (iii) Complex instrumentation and setup required (iv) Difficult to quantify MMP (v) Not real time	[99, 100]
FluoZin-3 + TMRE	(i) β-cell specific (ii) FluoZin-3 has higher affinity (K_D= 15 nM) for Zinc and higher quantum yield	(i) No correlation has been demonstrated with *in vitro* and *in vivo* islet function (ii) Nonstimulated static assay (iii) Complex equipment requirement and setup (iv) Difficult to quantify MMP (v) Not real time	[101, 102]
JC-1 + ROS	(i) Multiparametric assay (ii) Strong correlation of MMP with ROS (iii) Dynamic ROS assay (iv) Correlate with *in vivo* function	(i) Non β-cell specific (ii) Non-stimulated static assay of MMP (iii) Procedure complexity (iv) Intact islet assay for ROS but need islet dissociation for MMP (v) Not real time	[88, 103]
Multiparametric microfluidic assay (Rh123 + Fura-2AM + insulin kinetics)	(i) Multiparametric assay of key stimulus-secretion coupling factors (MMP: Rh123, Ca^{2+}: Fura-2AM; Insulin: ELISA) (ii) Dynamic response to stimulator (iii) Intact islets (iv) Real time (v) High throughput	(i) Large-scale evaluation needed (ii) Moderate spatiotemporal resolution of the measured parameters	[104, 105]

a mitochondrial membrane potential fluorescence probe [126]. The data presented in the study strongly suggest that analysis of β-cell viability by TMRE is of critical value for the prediction of transplant outcomes in the immunodeficient mouse model. Furthermore, the study finds that DNA-binding viability exclusion dyes do not always correlate with *in vivo* graft function; a strong argument suggesting the inadequacy of inclusion/exclusion dyes as a predictive assay of long-term islet viability. These findings are further confirmed by other studies [127]. In addition, TMRE used in conjunction with FluoZin-3, another zinc-specific probe but with a higher affinity (K_D = 15 nM) and higher quantum yield, can also be used to assess β-cell viability using flow cytometry. The functionality of β-cells is determined by the retention of the MMP indicator in conjunction with TMRE in FluoZin-3^+ β-cells [128, 129]. In other studies, the MMP measured by the mitochondrial JC-1 fluorescence probe are used in parallel to correlate changes in luminol-measured ROS, showing a decrease in the percentage of cells with normal mitochondrial polarity with a low responsive index in both glucose- and rotenone-stimulated islets. Regression analysis shows the rotenone stimulation index is significantly correlated with the percentage of islet cells with polarized mitochondria [116, 130].

While these results are promising, the aforementioned assessments of mitochondrial integrity measure a static value of β-cell energetic status with only retrospective values since these assays may take days to conduct and cannot predict islet graft function at the time of transplant. In addition, as an enzymatic dissociation of the islets is involved, the assays' methodology is often questioned for introduction of artefacts, such as selective damage or and loss of to the β-cell population. Furthermore, most of these assays require more sophisticated laboratory setups, such as FACS, confocal microscope, and laser scanning cytometry. However, these findings strongly suggest that a testing method that has the capability of measuring dynamic changes in mitochondrial potential kinetics in whole islets in response to insulin secretagogues (such as glucose), in parallel with other assays that measure key insulin stimulus-secretion coupling factors, would be useful in determining islet function prior to transplantation. In order to achieve this type of a test, it is necessary to use microfluidic technology.

Microfluidic technology has been used for a wide range of analytical applications and recently these techniques have been adopted to develop versions of islet perfusion apparatuses, integrated with either single or multiple analysis tools for islet and β-cell studies [131–133]. Microfluidic technology has a number of advantages over conventional techniques in that smaller amounts of reagent and bioanalyte are required, it is simple to create and maintain an experimental microenvironment, and microfluidic assessment allows for the easy integration of analytical tools such as optical and electrical assays. In addition, the fabrication techniques are favorable for portability and economic mass production of highly elaborate devices. Recently, a microfluidic-based perfusion setup has been developed for the simultaneous imaging of mitochondrial potentials changes and calcium influx as well as insulin release kinetics of whole islets in response to glucose stimulation [134, 135]. This mitochondria-based islet assay is associated with the following features: (i) multiparametric assay of key insulin

stimulus-secretion coupling factors including intracellular calcium signaling, mitochondrial potentials changes, and insulin secretion kinetics; (ii) real-time whole islet assay with no islet fixing or dissociation is needed; (iii) β-cell specific since islets are evaluated in response to stimulation by insulin stimulators; (iv) sufficient spatiotemporal resolution of all measured parameters. Although the system shows promising results, a large-scale evaluation of human islet products is needed in conjunction with data correlating results to *in vivo* graft function in both animal and human models. This work is currently under way following a clinical trial and is expected to validate a mathematical model for an islet functional index (IFI) score.

In spite of various assays available, a conclusive potency test still needs to be developed and verified for universal acceptance by the islet transplant community. Ideally, this potency test needs to be real-time, β-cell-specific, sensitive, reliable, robust, and have practical applicability.

5. Impact of Mitochondria on Selection of Immunosuppression Regiment and Transplant Site

Besides the infusion of a sufficient number of high-quality islets and careful recipient selection of patients with normal kidney function and low insulin requirements, another important factor for the success of the Edmonton Protocol is the development of a new steroid-free immunosuppressive therapy in combination of sirolimus (rapamycin, an mTOR inhibitor) and low dose of tacrolimus (FK506, a calcineurin inhibitor), and induction with daclizumab (an anti-IL2-receptor monoclonal antibody).

It has been suggested in the past that posttransplant islet graft failures can be caused by several reasons including initial failure of islet engraftment, inflammatory response at the transplant site, alloreactive (rejection) or autoimmune response, and immunosuppressive drug-induced β-cell toxicity [136]. Although their long-term side effects are still not fully known, immediate side effects of immunosuppressive drugs include mouth sores, gastrointestinal disturbance (stomach upset and diarrhea), high cholesterol levels, hypertension, anemia, fatigue, decreased white blood cell counts, decreased kidney function, and increased susceptibility to bacterial and viral infections. Taking immunosuppressive drugs also increases the risk of tumors and cancer. Several studies have systemically reviewed each of these aspects; this review will only briefly address a few of the findings of the effects of immunosuppressants in relation to islet mitochondrial-based cytoprotection.

Although extensive studies have investigated the effects of sirolimus and tacrolimus on islets *in vitro* and *in vivo*, as well as in the islet transplant patients, conflicting results have been reported. Sirolimus inhibits the mTOR (the mammalian target of rapamycin) pathway by directly binding mTOR Complex 1 (mTORC1). It has been shown that the mTOR signaling pathway integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation, and survival. It has also been demonstrated that mitochondrial metabolism and biogenesis are both regulated by mTORC1. Through the inhibition of mTORC1, sirolimus lowers MMP, oxygen consumption, and ATP levels. In addition, it reduces mitochondrial DNA copy number and many genes encoding proteins involved in oxidative metabolism [137, 138]. The mTOR signaling pathway also prevents islet cells from initiating authophagy [139]. Therefore, the inhibition of mTORC1 by sirolimus causes downregulation of T-lymphocytes while promoting authophagy and increasing the probability of initiating apoptosis. This has been demonstrated in a mouse model, whereby treatment with sirolimus induced an increase in membrane-bound light chain 3 (LC3-II), an early marker of authophagy, as well as a decrease in cell viability. In this experiment, sirolimus-mediated activation of authophagy was shown to be rescued by treatment with 3-methyladenine (3-MA), an inhibitor of authophagy [140].

Since sirolimus monotherapy is not sufficient to suppress islet graft rejection, it is often used with other immunosuppressants. Tacrolimus is one of the most commonly used immunosuppressant agents often used in combination with sirolimus. Tacrolimus immunosuppression blocks antigen-stimulated expression of genes such as IL-2 in T-cells, which is required for T-cell proliferation. It is generally assumed that the mechanisms involved in tacrolimus toxicity are similar to those of cyclosporine (CsA) by inhibition of mitochondrial ATP production and disruption of mitochondrial ion channels. However, some researchers have suggested that in an I/R model, tacrolimus protects neural tissues from adverse conditions such as overaccumulation of calcium, oxidative stress, cytochrome c release, and BAD phosphorylation [141, 142].

In clinical practice, sirolimus has been shown to increase basal and stimulated insulin secretion when maintained within a plasma-drug concentration target with reduced β-cell apoptosis [143]. When cultured with human islets, sirolimus decreases TNF-α, IL-1β, MCP-1, and macrophage inflammatory protein-1beta (MIP-1β) from impure islet preparations [144]. In the patients receiving sirolimus as preconditioning for islet transplant, an increase in fasting C-peptide levels and a decreased insulin requirement were observed [145]. In an *in vivo* rodent model, islet graft function was preserved in association with a pretransplant reduction in chemokines CCL2 and a dampened chemokine response posttransplant [146]. However, conflicting results have also been reported. Sirolimus has been found to impair metabolism secretion coupling by suppressing carbohydrate metabolism resulting in lower ATP levels, a slower glucose oxidation rate, and inhibited alpha-ketoglutarate dehydrogenase (AKGDH) [147]. In addition, sirolimus has been associated with a reduction in the amount of vascular endothelial grow factor (VEGF) that is released by the islets; a reduction in islet viability by blocking the VEGF-mediated survival pathways [148], as well as reduction in glucose-induced insulin secretion *in vivo* [149].

The detrimental effects of tacrolimus on β-cell function have been well documented including a reduction of insulin granules; decrease in insulin release; inhibition of insulin transcription as determined by RT-qPCR of insulin

mRNA levels; reduction of glucokinase (I-GLK, hexokinase-4) activity using cell lines, rodent islets, and human islets [150–154]. One study has shown that tacrolimus reduces mitochondrial density and oxygen consumption at a pharmacologically relevant concentration without changes in either the rate of ATP production nor apoptosis. Microarray data indicate that tacrolimus modifies the pathways involving ATP metabolism, membrane trafficking, and cytoskeleton remodeling, indicating that tacrolimus causes mitochondrial dysfunction at the level of gene transcription and translation that causes a reduction in mitochondrial contents and respiration [155]. In 2009, one study compared the effect on sirolimus, tacrolimus, and mycophenolate mofetil (MMF) on glucose-induced insulin secretion in human islets and found out that all three of the drugs tested increased caspase-3 cleavage and caspase-3 activity. Tacrolimus has been shown to have acute and direct effects on insulin exocytosis, whereas MMF does not but still has impaired insulin secretion suggesting indirect effects on insulin exocytosis. Interestingly, exenatide (exendin-4), a GLP-1 agonist, can ameliorate impairments in insulin secretion caused by sirolimus, tacrolimus, and MMF [156].

In an *in vivo* rat model, sirolimus has been used with cyclosporine, showing that the combined treatment increases blood glucose and $HbA1_c$ levels, as well as the HOMA-R [fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5] index. When CsA was withdrawn, *in vivo* islet graft function was compromised when the patient was maintained with sirolimus [157]. One clinical case study reported that a patient who presented with sirolimus toxicity intolerance was converted to MMF with low-dose tacrolimus and maintained with a monthly infusion of daclizumab, showed good long-term islet graft function and improved renal function [158].

While a body of evidence seems to support the notion that most immunosuppressants currently used for islet transplant have at least some level of toxicity on islets and affect islet graft function, some conflicting results have been reported. Many contribute these incongruent observations to the selection of experimental models: *in vivo* versus *in vitro,* short-term versus long-term, cell line versus primary cells, animal versus human, and dosage difference.

In addition to immunosuppressant toxicity, the microenvironment of the islet engraftment site within the hepatic portal vein system has also been suggested as one of the main contributing factors associated with the loss of islet mass and function posttransplant. Within moments of islet implantation, a cascade of nonspecific inflammatory events known as instant blood mediated inflammatory reaction (IBMIR) are initiated which include activation of blood-mediated coagulation pathways [159] and release of proinflammatory cytokines (PIC) primarily from macrophage, Kupffer cells, and endothelial cells. It has also been suggested that cytokines produced by the immune system posttransplant can infiltrate pancreatic islets and serve as mediators of β-cell destruction [136]; as well as higher levels of immusuppressants that are directly toxic or damaging to the transplanted islets. The physical and chemical microenvironments of the hepatic portal system are also considered suboptimal for islet engraftment and survival: low oxygen tension and a high concentration of waste and nutrients may cause islet glucotoxicity and lipotoxicity. When islets are exposed to chronic hyperglycemic and hyperlipidemic environments, their overall function can be compromised including loss of glucose sensitivity, β-cell exhaustion, and glucotoxicity/lipotoxicity [160, 161]. Glucotoxicity and lipotoxicity are closely interrelated and complementary for their deleterious effects on β-cell function. Glucotoxicity involves several transcription factors and is in part mediated by chronic mitochondrial reactive species generation and accumulation. Lipotoxicity is probably mediated by the accumulation of a cytosolic signal derived from the fatty acid esterification pathway. Very few studies have been published that investigate islet grafts in the hepatic portal system due to the lack of good *in vitro* and *in vivo* models that can closely mimic this islet graft microenvironment. New alternative sites are currently being investigated including intramuscular, bone marrow, and the peritoneal cavity that could potentially decrease the stress to islets. While this topic is important for improving islet transplantation outcomes it has been previously reviewed in several other papers in greater depth [162, 163].

6. Summary

Recently, improvements in islet isolation techniques and the selection of optimal immunosuppression regiments have made human islet transplantation a viable clinical avenue in the treatment for T1DM patients. Inconsistent success rates are observed between short-term and long-term graft survival among centers and this could be related to the use of islet products of less than optimal quality and quantity. Mitochondrial functions are potentially compromised in each step of islet isolation and transplant process and subsequently can negatively influence islet engraftment and survival. The evidence presented in this review supports the following hypotheses: (i) mitochondrial dysfunction is a mediator for the onset and progression of islet I/R injury; (ii) potential therapeutics targeting mitochondrial processes, including energy metabolism, cytokine-induced inflammatory reaction, and free radical generation, will be promising in preserving islet function and viability. However, to confirm the role of mitochondrial cytoprotection in the isolated islets for transplant, it will be necessary to demonstrate these results in appropriate animal models and in a large cohort study of islet isolation by multiple centers to correlate clinical transplant outcomes. In addition, it is important to correlate a more sensitive and reliable islet viability and potency assay based on the evaluation of mitochondrial integrity in conjunction with other parameters to prevent the transplantation of a low quality islet preparations. Future efforts are also needed to better understand the underlying mechanisms of immunosuppressant-mediated islet toxicity and to discover a new generation of immunosuppressants that are less damaging to islets. Lastly, it is important to improve the islet transplantation process by decreasing islet loss potentially by finding a more suitable transplantation site in order to achieve consistent transplantation outcomes.

Acknowledgment

The authors sincerely thank the UIC-COM (University of Illinois at Chicago College of Medicine) human islet team for providing technical assistance and the Chicago Diabetes Project (CDP, http://www.chicagodiabetesproject.org).

References

[1] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.

[2] R. Alejandro, F. B. Barton, B. J. Hering, and S. Wease, "2008 Update from the collaborative islet transplant r," *Transplantation*, vol. 86, no. 12, pp. 1783–1788, 2008.

[3] M. D. Bellin, R. Kandaswamy, J. Parkey et al., "Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes," *American Journal of Transplantation*, vol. 8, no. 11, pp. 2463–2470, 2008.

[4] A. Gangemi, P. Salehi, B. Hatipoglu et al., "Islet transplantation for brittle type 1 diabetes: the UIC protocol," *American Journal of Transplantation*, vol. 8, no. 6, pp. 1250–1261, 2008.

[5] E. A. Ryan, B. W. Paty, P. A. Senior et al., "Five-year follow-up after clinical islet transplantation," *Diabetes*, vol. 54, no. 7, pp. 2060–2069, 2005.

[6] A. Wiederkehr and C. B. Wollheim, "Minireview: implication of mitochondria in insulin secretion and action," *Endocrinology*, vol. 147, no. 6, pp. 2643–2649, 2006.

[7] J. C. Henquin, "Triggering and amplifying pathways of regulation of insulin secretion by glucose," *Diabetes*, vol. 49, no. 11, pp. 1751–1760, 2000.

[8] N. Sekine, C. Fasolato, W. F. Pralong, J. M. Theler, and C. B. Wollheim, "Glucose-induced insulin secretion in INS-1 cells depends on factors present in fetal calf serum and rat islet-conditioned medium," *Diabetes*, vol. 46, no. 9, pp. 1424–1433, 1997.

[9] P. Rorsman, "The pancreatic beta-cell as a fuel sensor: an electrophysiologist's viewpoint," *Diabetologia*, vol. 40, no. 5, pp. 487–495, 1997.

[10] J. R. T. Lakey, T. Tsujimura, A. M. J. Shapiro, and Y. Kuroda, "Preservation of the human pancreas before islet isolation using a two-layer (UW solution-perfluorochemical) cold storage method," *Transplantation*, vol. 74, no. 12, pp. 1809–1811, 2002.

[11] T. Tsujimura, Y. Kuroda, J. G. Avila et al., "Influence of pancreas preservation on human islet isolation outcomes: impact of the two-layer method," *Transplantation*, vol. 78, no. 1, pp. 96–100, 2004.

[12] T. Tsujimura, Y. Kuroda, J. G. Avila et al., "Resuscitation of the ischemically damaged human pancreas by the two-layer method prior to islet isolation," *Transplantation Proceedings*, vol. 35, no. 7, pp. 2461–2462, 2003.

[13] D. E. Eckhoff, C. A. Smyth, C. Eckstein et al., "Suppression of the c-Jun N-terminal kinase pathway by 17β-estradiol can preserve human islet functional mass from proinflammatory cytokine-induced destruction," *Surgery*, vol. 134, no. 2, pp. 169–179, 2003.

[14] S. Abdelli, J. Ansite, R. Roduit et al., "Intracellular stress signaling pathways activated during human islet preparation and following acute cytokine exposure," *Diabetes*, vol. 53, no. 11, pp. 2815–2823, 2004.

[15] S. Paraskevas, R. Aikin, D. Maysinger et al., "Activation and expression of ERK, JNK, and p38 MAP-kinases in isolated islets of Langerhans: implications for cultured islet survival," *FEBS Letters*, vol. 455, no. 3, pp. 203–208, 1999.

[16] S. Potdar, S. Malek, B. Eghtesad et al., "Initial experience using histidine-tryptophan-ketoglutarate solution in clinical pancreas transplantation," *Clinical Transplantation*, vol. 18, no. 6, pp. 661–665, 2004.

[17] J. A. Fridell, A. Agarwal, M. L. Milgrom, W. C. Goggins, P. Murdock, and M. D. Pescovitz, "Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution for organ preservation in clinical pancreas transplantation," *Transplantation*, vol. 77, no. 8, pp. 1304–1306, 2004.

[18] R. Canelo, N. S. Hakim, and B. Ringe, "Experience with hystidine tryptophan ketoglutarate versus University Wisconsin preservation solutions in transplantation," *International Surgery*, vol. 88, no. 3, pp. 145–151, 2003.

[19] Y. Kuroda, Y. Fujino, A. Morita, Y. Tanioka, Y. Ku, and Y. Saitoh, "Oxygenation of the human pancreas during preservation by a two-layer (University of Wisconsin solution/perfluorochemical) cold-storage method," *Transplantation*, vol. 54, no. 3, pp. 561–562, 1992.

[20] Y. Kuroda, T. Kawamura, Y. Suzuki, H. Fujiwara, K. Yamamoto, and Y. Saitoh, "A new, simple method for cold storage of the pancreas using perfluorochemical," *Transplantation*, vol. 46, no. 3, pp. 457–460, 1988.

[21] Y. Fujino, Y. Kuroda, Y. Suzuki et al., "Preservation of canine pancreas for 96 hours by a modified two-layer (UW solution/perfluorochemical) cold storage method," *Transplantation*, vol. 51, no. 5, pp. 1133–1135, 1991.

[22] S. Merani and A. M. J. Shapiro, "Current status of pancreatic islet transplantation," *Clinical Science*, vol. 110, no. 6, pp. 611–625, 2006.

[23] S. Matsumoto and Y. Kuroda, "Perfluorocarbon for organ preservation before transplantation," *Transplantation*, vol. 74, no. 12, pp. 1804–1809, 2002.

[24] B. J. Hering, I. Matsumoto, T. Sawada et al., "Impact of two-layer pancreas preservation on islet isolation and transplantation," *Transplantation*, vol. 74, no. 12, pp. 1813–1816, 2002.

[25] R. M. Baertschiger, T. Berney, and P. Morel, "Organ preservation in pancreas and islet transplantation," *Current Opinion in Organ Transplantation*, vol. 13, no. 1, pp. 59–66, 2008.

[26] Y. Iwanaga, D. E. R. Sutherland, J. V. Harmon, and K. K. Papas, "Pancreas preservation for pancreas and islet transplantation," *Current Opinion in Organ Transplantation*, vol. 13, no. 2, pp. 135–141, 2008.

[27] H. Noguchi, M. F. Levy, N. Kobayashi, and S. Matsumoto, "Pancreas preservation by the two-layer method: does it have a beneficial effect compared with simple preservation in University of Wisconsin solution?" *Cell Transplantation*, vol. 18, no. 5-6, pp. 497–503, 2009.

[28] S. Matsumoto, S. A. Qualley, S. Goel et al., "Effect of the two-layer (University of Wisconsin solution-perfluorochemical plus O2) method of pancreas preservation on human islet isolation, as assessed by the edmonton isolation protocol," *Transplantation*, vol. 74, no. 10, pp. 1414–1419, 2002.

[29] S. Matsumoto, T. H. Rigley, S. A. Qualley, Y. Kuroda, J. A. Reems, and R. B. Stevens, "Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata," *Cell Transplantation*, vol. 11, no. 8, pp. 769–777, 2002.

[30] K. Hiraoka, Y. Kuroda, Y. Suzuki et al., "Outcomes in clinical pancreas transplantation with the two-layer cold storage method versus simple storage in University of Wisconsin solution," *Transplantation Proceedings*, vol. 34, no. 7, pp. 2688–2689, 2002.

[31] C. Ricordi, C. Fraker, J. Szust et al., "Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution," *Transplantation*, vol. 75, no. 9, pp. 1524–1527, 2003.

[32] T. Tsujimura, Y. Kuroda, T. Kin et al., "Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method," *Transplantation*, vol. 74, no. 12, pp. 1687–1691, 2002.

[33] B. J. Hering, R. Kandaswamy, J. V. Harmon et al., "Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody," *American Journal of Transplantation*, vol. 4, no. 3, pp. 390–401, 2004.

[34] G. Zhang, S. Matsumoto, H. Newman, D. M. Strong, R. P. Robertson, and J. A. Reems, "Improve islet yields and quality when clinical grade pancreata are preserved by the two-layer method," *Cell and Tissue Banking*, vol. 7, no. 3, pp. 195–201, 2006.

[35] P. Witkowski, Z. Liu, Q. Guo et al., "Two-layer method in short-term pancreas preservation for successful islet isolation," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3398–3401, 2005.

[36] P. Salehi, M. Mirbolooki, T. Kin et al., "Ameliorating injury during preservation and isolation of human islets using the two-layer method with perfluorocarbon and UW solution," *Cell Transplantation*, vol. 15, no. 2, pp. 187–194, 2006.

[37] S. Ramachandran, N. M. Desai, T. A. Goers et al., "Improved islet yields from pancreas preserved in perflurocarbon is via inhibition of apoptosis mediated by mitochondrial pathway," *American Journal of Transplantation*, vol. 6, no. 7, pp. 1696–1703, 2006.

[38] K. K. Papas, B. J. Hering, L. Gunther, M. J. Rappel, C. K. Colton, and E. S. Avgoustiniatos, "Pancreas oxygenation is limited during preservation with the two-layer method," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3501–3504, 2005.

[39] T. Kin, M. Mirbolooki, P. Salehi et al., "Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon," *Transplantation*, vol. 82, no. 10, pp. 1286–1290, 2006.

[40] J. Caballero-Corbalán, T. Eich, T. Lundgren et al., "No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation," *Transplantation*, vol. 84, no. 7, pp. 864–869, 2007.

[41] R. Nano, B. Clissi, R. Melzi et al., "Islet isolation for allotransplantation: variables associated with successful islet yield and graft function," *Diabetologia*, vol. 48, no. 5, pp. 906–912, 2005.

[42] H. Qin, S. Matsumoto, G. B. Klintmalm, and E. B. de vol, "A meta-analysis for comparison of the two-layer and university of Wisconsin pancreas preservation methods in islet transplantation," *Cell Transplantation*, vol. 20, no. 7, pp. 1127–1137, 2011.

[43] M. Iken, H. Brandhorst, O. Korsgren, and D. Brandhorst, "Pig pancreas oxygenation at 20∘C increases islet ATP generation but deteriorates islet function," *Cell Transplantation*, vol. 18, no. 7, pp. 745–751, 2009.

[44] H. Brandhorst, S. Asif, K. Andersson et al., "A new oxygen carrier for improved long-term storage of human pancreata before islet isolation," *Transplantation*, vol. 89, no. 2, pp. 155–160, 2010.

[45] J. G. Avila, Y. Wang, B. Barbaro et al., "Improved outcomes in islet isolation and transplantation by the use of a novel hemoglobin-based O2 carrier," *American Journal of Transplantation*, vol. 6, no. 12, pp. 2861–2870, 2006.

[46] Y. S. Kwon, J. D. Foley, C. J. Murphy, and J. F. McAnulty, "The effect of trophic factor supplementation on cold ischemia-induced early apoptotic changes," *Transplantation*, vol. 83, no. 1, pp. 91–94, 2007.

[47] T. Desurmont, S. Giraud, J. Cau et al., "Trophic factor and FR167653 supplementation during cold storage rescue chronic renal injury," *Journal of Urology*, vol. 185, no. 3, pp. 1139–1146, 2011.

[48] T. Mitchell, D. Rotaru, H. Saba, R. A. J. Smith, M. P. Murphy, and L. A. MacMillan-Crow, "The mitochondria-targeted antioxidant mitoquinone protects against cold storage injury of renal tubular cells and rat kidneys," *Journal of Pharmacology and Experimental Therapeutics*, vol. 336, no. 3, pp. 682–692, 2011.

[49] I. B. Mosbah, J. Roselló-Catafau, I. Alfany-Fernandez et al., "Addition of carvedilol to University Wisconsin solution improves rat steatotic and nonsteatotic liver preservation," *Liver Transplantation*, vol. 16, no. 2, pp. 163–171, 2010.

[50] M. A. Zaouali, I. Ben Mosbah, S. Padrissa-Alts et al., "Relevance of epidermal growth factor to improve steatotic liver preservation in IGL-1 solution," *Transplantation Proceedings*, vol. 42, no. 8, pp. 3070–3075, 2010.

[51] M. A. Zaouali, S. Padrissa-Altés, I. B. Mosbah et al., "Improved rat steatotic and nonsteatotic liver preservation by the addition of epidermal growth factor and insulin-like growth factor-I to University of Wisconsin solution," *Liver Transplantation*, vol. 16, no. 9, pp. 1098–1111, 2010.

[52] S. A. White, H. Djaballah, D. P. Hughes et al., "A preliminary study of the activation of endogenous pancreatic exocrine enzymes during automated porcine islet isolation," *Cell Transplantation*, vol. 8, no. 3, pp. 265–276, 1999.

[53] A. Heiser, K. Ulrichs, and W. Muller-Ruchholtz, "Isolation of porcine pancreatic islets: low trypsin activity during the isolation procedure guarantees reproducible high islet yields," *Journal of Clinical Laboratory Analysis*, vol. 8, no. 6, pp. 407–411, 1994.

[54] S. Matsumoto, O. Lawrence, T. H. Rigley, J. R. T. Lakey, R. B. Stevens, and D. M. Strong, "University of Wisconsin solution with trypsin inhibitor Pefabloc improves survival of viable human and primate impure islets during storage," *Cell and Tissue Banking*, vol. 2, no. 1, pp. 15–21, 2001.

[55] J. R. T. Lakey, L. M. H. Helms, T. Kin et al., "Serine-protease inhibition during islet isolation increases islet yield from human pancreases with prolonged ischemia," *Transplantation*, vol. 72, no. 4, pp. 565–570, 2001.

[56] S. Matsumoto, T. H. Rigley, J. A. Reems, Y. Kuroda, and R. B. Stevens, "Improved islet yields from Macaca nemestrina and marginal human pancreata after two-layer method preservation and endogenous trypsin inhibition," *American Journal of Transplantation*, vol. 3, no. 1, pp. 53–63, 2003.

[57] I. H. Al-Abdullah, K. Bentsi-Barnes, L. Valiente et al., "Testing combinations of protease inhibitor and preservation solution to improve islet quality and yield," *Transplantation Proceedings*, vol. 40, no. 2, pp. 390–392, 2008.

[58] R. Bottino, A. N. Balamurugan, H. Tse et al., "Response of human islets to isolation stress and the effect of antioxidant treatment," *Diabetes*, vol. 53, no. 10, pp. 2559–2568, 2004.

[59] R. Bottino, A. N. Balamurugan, S. Bertera, M. Pietropaolo, M. Trucco, and J. D. Piganelli, "Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimic compound," *Diabetes*, vol. 51, no. 8, pp. 2561–2567, 2002.

[60] H. Eagle, V. I. Oyama, M. Levy, C. L. Horton, and R. Fleischman, "The growth response of mammalian cells in tissue culture to L-glutamine and L-glutamic acid," *Journal of Biological Chemistry*, vol. 218, no. 2, pp. 607–616, 1956.

[61] M. E. Evans, D. P. Jones, and T. R. Ziegler, "Glutamine inhibits cytokine-induced apoptosis in human colonic epithelial cells via the pyrimidine pathway," *American Journal of Physiology*, vol. 289, no. 3, pp. G388–G396, 2005.

[62] P. Newsholme, J. Procopio, M. M. Ramos Lima, T. C. Pithon-Curi, and R. Curi, "Glutamine and glutamate—their central role in cell metabolism and function," *Cell Biochemistry and Function*, vol. 21, no. 1, pp. 1–9, 2003.

[63] C. Li, C. Buettger, J. Kwagh et al., "A signaling role of glutamine in insulin secretion," *Journal of Biological Chemistry*, vol. 279, no. 14, pp. 13393–13401, 2004.

[64] S. Carobbio, H. Ishihara, S. Fernandez-Pascual, C. Bartley, R. Martin-Del-Rio, and P. Maechler, "Insulin secretion profiles are modified by overexpression of glutamate dehydrogenase in pancreatic islets," *Diabetologia*, vol. 47, no. 2, pp. 266–276, 2004.

[65] J. G. Avila, T. Tsujimura, J. Oberholzer et al., "Improvement of pancreatic islet isolation outcomes using glutamine perfusion during isolation procedure," *Cell Transplantation*, vol. 12, no. 8, pp. 877–881, 2004.

[66] J. Avila, B. Barbaro, A. Gangemi et al., "Intra-ductal glutamine administration reduces oxidative injury during human pancreatic islet isolation," *American Journal of Transplantation*, vol. 5, no. 12, pp. 2830–2837, 2005.

[67] D. A. Thomas, C. Stauffer, K. Zhao et al., "Mitochondrial targeting with antioxidant peptide SS-31 prevents mitochondrial depolarization, reduces islet cell apoptosis, increases islet cell yield, and improves posttransplantation function," *Journal of the American Society of Nephrology*, vol. 18, no. 1, pp. 213–222, 2007.

[68] K. Zhao, G. Luo, S. Giannelli, and H. H. Szeto, "Mitochondria-targeted peptide prevents mitochondrial depolarization and apoptosis induced by tert-butyl hydroperoxide in neuronal cell lines," *Biochemical Pharmacology*, vol. 70, no. 12, pp. 1796–1806, 2005.

[69] D. L. Eizirik and M. I. Darville, "β-cell apoptosis and defense mechanisms: lessons from type 1 diabetes," *Diabetes*, vol. 50, no. 1, pp. S64–S69, 2001.

[70] A. L. Scarim, M. Arnush, J. R. Hill et al., "Evidence for the presence of type IL-1 receptors on β-cells of islets of Langerhans," *Biochimica et Biophysica Acta*, vol. 1361, no. 3, pp. 313–320, 1997.

[71] S. A. Steer, A. L. Scarim, K. T. Chambers, and J. A. Corbett, "Interleukin-1 stimulates β-cell necrosis and release of the immunological adjuvant HMGB1," *PLoS Medicine*, vol. 3, no. 2, article e17, 2006.

[72] M. Cnop, N. Welsh, J. -C. Jonas, A. Jörns, S. Lenzen, and D. L. Eizirik, "Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities," *Diabetes*, vol. 54, supplement 2, pp. S97–S107, 2005.

[73] J. A. Corbett, J. L. Wang, T. P. Misko, W. Zhao, W. F. Hickey, and M. L. McDaniel, "Nitric oxide mediates IL-1β-induced islet dysfunction and destruction: prevention by dexamethasone," *Autoimmunity*, vol. 15, no. 2, pp. 145–153, 1993.

[74] H. Kaneto, Y. Kajimoto, Y. Fujitani et al., "Oxidative stress induces p21 expression in pancreatic islet cells: possible implication in beta-cell dysfunction," *Diabetologia*, vol. 42, no. 9, pp. 1093–1097, 1999.

[75] Y. Tanaka, P. O. T. Tran, J. Harmon, and R. P. Robertson, "A role for glutathione peroxidase in protecting pancreatic β cells against oxidative stress in a model of glucose toxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 19, pp. 12363–12368, 2002.

[76] S. S.M.S. Monfared, B. Larijani, and M. Abdollahi, "Islet transplantation and antioxidant management: a comprehensive review," *World Journal of Gastroenterology*, vol. 15, no. 10, pp. 1153–1161, 2009.

[77] C. Botsios, P. Sfriso, P. A. Ostuni, S. Todesco, and L. Punzi, "Efficacy of the IL-1 receptor antagonist, anakinra, for the treatment of diffuse anterior scleritis in rheumatoid arthritis. Report of two cases [8]," *Rheumatology*, vol. 46, no. 6, pp. 1042–1043, 2007.

[78] C. M. Larsen, M. Faulenbach, A. Vaag et al., "Interleukin-1-receptor antagonist in type 2 diabetes mellitus," *New England Journal of Medicine*, vol. 356, no. 15, pp. 1517–1526, 2007.

[79] A. Schwarznau, M. S. Hanson, J. M. Sperger et al., "IL-1β receptor blockade protects islets against pro-inflammatory cytokine induced necrosis and apoptosis," *Journal of Cellular Physiology*, vol. 220, no. 2, pp. 341–347, 2009.

[80] B. R. Hsu, S. H. Fu, S. Hsu, and S. T. Chen, "Interleukin-1 receptor antagonist enhances islet engraftment without impacting serum levels of nitrite or osteopontin," *Transplantation Proceedings*, vol. 41, no. 5, pp. 1781–1785, 2009.

[81] C. Westwell-Roper, D. L. Dai, G. Soukhatcheva et al., "IL-1 blockade attenuates islet amyloid polypeptide-induced proinflammatory cytokine release and pancreatic islet graft dysfunction," *Journal of Immunology*, vol. 187, no. 5, pp. 2755–2765, 2011.

[82] J. A. Emamaullee, L. Stanton, C. Schur, and A. M. J. Shapiro, "Caspase inhibitor therapy enhances marginal mass islet graft survival and preserves long-term function in islet transplantation," *Diabetes*, vol. 56, no. 5, pp. 1289–1298, 2007.

[83] J. A. Emamaullee, J. Davis, R. Pawlick et al., "The caspase selective inhibitor EP1013 augments human islet graft function and longevity in marginal mass islet transplantation in mice," *Diabetes*, vol. 57, no. 6, pp. 1556–1566, 2008.

[84] M. Montolio, N. Téllez, M. Biarnés, J. Soler, and E. Montanya, "Short-term culture with the caspase inhibitor z-VAD.fmk reduces beta cell apoptosis in transplanted islets and improves the metabolic outcome of the graft," *Cell Transplantation*, vol. 14, no. 1, pp. 59–65, 2005.

[85] M. Johansson, J. Olerud, L. Jansson, and P. O. Carlsson, "Prolactin treatment improves engraftment and function of transplanted pancreatic islets," *Endocrinology*, vol. 150, no. 4, pp. 1646–1653, 2009.

[86] T. Yamamoto, A. Mita, C. Ricordi et al., "Prolactin supplementation to culture medium improves β-cell survival," *Transplantation*, vol. 89, no. 11, pp. 1328–1335, 2010.

[87] L. Labriola, G. B. Ferreira, W. R. Montor et al., "Prolactin-induced changes in protein expression in human pancreatic

islets," *Molecular and Cellular Endocrinology*, vol. 264, no. 1-2, pp. 16–27, 2007.

[88] L. F. Terra, M. H. Garay-Malpartida, R. A. Wailemann, M. C. Sogayar, and L. Labriola, "Recombinant human prolactin promotes human beta cell survival via inhibition of extrinsic and intrinsic apoptosis pathways," *Diabetologia*, vol. 54, no. 6, pp. 1388–1397, 2011.

[89] H. Kaneto, Y. Nakatani, T. Miyatsuka et al., "Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide," *Nature Medicine*, vol. 10, no. 10, pp. 1128–1132, 2004.

[90] J. L. Contreras, C. Eckstein, C. A. Smyth et al., "Brain Death Significantly Reduces Isolated Pancreatic Islet Yields and Functionality In Vitro and In Vivo after Transplantation in Rats," *Diabetes*, vol. 52, no. 12, pp. 2935–2942, 2003.

[91] H. Noguchi, Y. Nakai, M. Ueda et al., "Activation of c-Jun NH2-terminal kinase (JNK) pathway during islet transplantation and prevention of islet graft loss by intraportal injection of JNK inhibitor," *Diabetologia*, vol. 50, no. 3, pp. 612–619, 2007.

[92] H. Noguchi, Y. Nakai, S. Matsumoto et al., "Cell permeable peptide of JNK inhibitor prevents islet apoptosis immediately after isolation and improves islet graft function," *American Journal of Transplantation*, vol. 5, no. 8, pp. 1848–1855, 2005.

[93] H. Noguchi, S. Matsumoto, N. Onaca et al., "Ductal injection of JNK inhibitors before pancreas preservation prevents islet apoptosis and improves islet graft function," *Human Gene Therapy*, vol. 20, no. 1, pp. 73–85, 2009.

[94] N. L. Rose, M. M. Palcic, L. M. H. Helms, and J. R. T. Lakey, "Evaluation of pefabloc as a serine protease inhibitor during human-islet isolation," *Transplantation*, vol. 75, no. 4, pp. 462–466, 2003.

[95] W. Wang, L. Upshaw, G. Zhang, M. D. Strong, and J. A. Reems, "Adjustment of digestion enzyme composition improves islet isolation outcome from marginal grade human donor pancreata," *Cell and Tissue Banking*, vol. 8, no. 3, pp. 187–194, 2007.

[96] N. L. Rose, M. M. Palcic, A. M. J. Shapiro, and J. R. T. Lakey, "An evaluation of the activation of endogenous pancreatic enzymes during human islet isolations," *Transplantation Proceedings*, vol. 35, no. 7, pp. 2455–2457, 2003.

[97] C. Umeadi, K. Bentsi-Barnes, F. Kandeel, and I. H. Al-Abdullah, "Endogenous pancreatic protease activity and methods for impeding their function," *Transplantation Proceedings*, vol. 40, no. 2, pp. 355–357, 2008.

[98] C. C. Nduaguibe, K. Bentsi-Barnes, Y. Mullen, F. Kandeel, and I. H. Al-Abdullah, "Serine protease inhibitors suppress pancreatic endogenous proteases and modulate bacterial neutral proteases," *Islets*, vol. 2, no. 3, pp. 200–206, 2010.

[99] K. E. Dionne, C. K. Colton, and M. L. Yarmush, "Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans," *Diabetes*, vol. 42, no. 1, pp. 12–21, 1993.

[100] R. Lehmann, R. A. Zuellig, P. Kugelmeier et al., "Superiority of small islets in human islet transplantation," *Diabetes*, vol. 56, no. 3, pp. 594–603, 2007.

[101] C. Ricordi, J. R. T. Lakey, and B. J. Hering, "Challenges toward standardization of islet isolation technology," *Transplantation Proceedings*, vol. 33, no. 1-2, p. 1709, 2001.

[102] C. P. F. Carvalho, H. C. L. Barbosa, A. Britan et al., "Beta cell coupling and connexin expression change during the functional maturation of rat pancreatic islets," *Diabetologia*, vol. 53, no. 7, pp. 1428–1437, 2010.

[103] N. J. M. London, S. M. Thirdborough, S. M. Swift, P. R. F. Bell, and R. F. L. James, "The diabetic "human reconstituted" severe combined immunodeficient (SCID-hu) mouse: a model for isogeneic, allogeneic, and xenogeneic human islet transplantation," *Transplantation Proceedings*, vol. 23, no. 1, p. 749, 1991.

[104] W. Wang, L. Upshaw, D. M. Strong, R. P. Robertson, and J. Reems, "Increased oxygen consumption rates in response to high glucose detected by a novel oxygen biosensor system in non-human primate and human islets," *Journal of Endocrinology*, vol. 185, no. 3, pp. 445–455, 2005.

[105] M. Goto, J. Holgersson, M. Kumagai-Braesch, and O. Korsgren, "The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets," *American Journal of Transplantation*, vol. 6, no. 10, pp. 2483–2487, 2006.

[106] I. Miwa, N. Ichimura, M. Sugiura, Y. Hamada, and S. Taniguchi, "Inhibition of glucose-induced insulin secretion by 4-hydroxy-2-nonenal and other lipid peroxidation products," *Endocrinology*, vol. 141, no. 8, pp. 2767–2772, 2000.

[107] A. M. Hennige, N. Lembert, M. A. Wahl, and H. P. T. Ammon, "Oxidative stress increases potassium efflux from pancreatic islets by depletion of intracellular calcium stores," *Free Radical Research*, vol. 33, no. 5, pp. 507–516, 2000.

[108] S. P. Wolff, "Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications," *British Medical Bulletin*, vol. 49, no. 3, pp. 642–652, 1993.

[109] R. A. Vega, Y. Wang, T. Harvat et al., "Modified gold nanoparticle vectors: a biocompatible intracellular delivery system for pancreatic islet cell transplantation," *Surgery*, vol. 148, no. 4, pp. 858–866, 2010.

[110] J. S. Rink, K. M. McMahon, X. Chen, C. A. Mirkin, C. S. Thaxton, and D. B. Kaufman, "Transfection of pancreatic islets using polyvalent DNA-functionalized gold nanoparticles," *Surgery*, vol. 148, no. 2, pp. 335–345, 2010.

[111] M. Nakano, I. Matsumoto, T. Sawada et al., "Caspase-3 inhibitor prevents apoptosis of human islets immediately after isolation and improves islet graft function," *Pancreas*, vol. 29, no. 2, pp. 104–109, 2004.

[112] C. N. Street, J. R. T. Lakey, A. M. J. Shapiro et al., "Islet graft assessment in the Edmonton Protocol: implications for predicting long-term clinical outcome," *Diabetes*, vol. 53, no. 12, pp. 3107–3114, 2004.

[113] K. K. Papas, T. M. Suszynski, and C. K. Colton, "Islet assessment for transplantation," *Current Opinion in Organ Transplantation*, vol. 14, no. 6, pp. 674–682, 2009.

[114] K. K. Papas, C. K. Colton, R. A. Nelson et al., "Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice," *American Journal of Transplantation*, vol. 7, no. 3, pp. 707–713, 2007.

[115] I. R. Sweet, M. Gilbert, S. Scott et al., "Glucose-stimulated increment in oxygen consumption rate as a standardized test of human islet quality," *American Journal of Transplantation*, vol. 8, no. 1, pp. 183–192, 2008.

[116] B. Armann, M. S. Hanson, E. Hatch, A. Steffen, and L. A. Fernandez, "Quantification of basal and stimulated ROS levels as predictors of islet potency and function," *American Journal of Transplantation*, vol. 7, no. 1, pp. 38–47, 2007.

[117] K. H. Nam, W. Yong, T. Harvat et al., "Size-based separation and collection of mouse pancreatic islets for functional analysis," *Biomedical Microdevices*, vol. 12, no. 5, pp. 865–874, 2010.

[118] F. Bertuzzi and C. Ricordi, "Prediction of clinical outcome in islet allotransplantation," *Diabetes Care*, vol. 30, no. 2, pp. 410–417, 2007.

[119] C. Ricordi, D. W. Scharp, and P. E. Lacy, "Reversal of diabetes in nude mice after transplantation of fresh and 7-day-cultured (24∘C) human pancreatic islets," *Transplantation*, vol. 45, no. 5, pp. 994–996, 1988.

[120] K. K. Papas, A. Pisania, H. Wu, G. C. Weir, and C. K. Colton, "A stirred microchamber for oxygen consumption rate measurements with pancreatic islets," *Biotechnology and Bioengineering*, vol. 98, no. 5, pp. 1071–1082, 2007.

[121] I. R. Sweet and M. Gilbert, "Contribution of calcium influx in mediating glucose-stimulated oxygen consumption in pancreatic islets," *Diabetes*, vol. 55, no. 12, pp. 3509–3519, 2006.

[122] J. H. Kim, S. G. Park, H. N. Lee et al., "ATP measurement predicts porcine islet transplantation outcome in nude mice," *Transplantation*, vol. 87, no. 2, pp. 166–169, 2009.

[123] Y. Lee, M.-Y. Wang, X. Q. Du, M. J. Charron, and R. H. Unger, "Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice," *Diabetes*, vol. 60, no. 2, pp. 391–397, 2011.

[124] A. F. Spigelman, X. Dai, and P. E. MacDonald, "Voltage-dependent K+ channels are positive regulators of alpha cell action potential generation and glucagon secretion in mice and humans," *Diabetologia*, vol. 53, no. 9, pp. 1917–1926, 2010.

[125] B. Lukowiak, B. Vandewalle, R. Riachy et al., "Identification and purification of functional human β-cells by a new specific zinc-fluorescent probe," *Journal of Histochemistry and Cytochemistry*, vol. 49, no. 4, pp. 519–527, 2001.

[126] H. Ichii, L. Inverardi, A. Pileggi et al., "A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations," *American Journal of Transplantation*, vol. 5, no. 7, pp. 1635–1645, 2005.

[127] I. Iglesias, K. Bentsi-Barnes, C. Umeadi, L. Brown, F. Kandeel, and I. H. Al-Abdullah, "Comprehensive analysis of human pancreatic islets using flow and laser scanning cytometry," *Transplantation Proceedings*, vol. 40, no. 2, pp. 351–354, 2008.

[128] S. Jayaraman, "Assessment of beta cell viability," *Current Protocols in Cytometry*, supplement 55, article 6.27, 2011.

[129] S. Jayaraman, "A novel method for the detection of viable human pancreatic beta cells by flow cytometry using fluorophores that selectively detect labile zinc, mitochondrial membrane potential and protein thiols," *Cytometry Part A*, vol. 73, no. 7, pp. 615–625, 2008.

[130] M. S. Hanson, E. E. Park, M. L. Sears et al., "A simplified approach to human islet quality assessment," *Transplantation*, vol. 89, no. 10, pp. 1178–1188, 2010.

[131] M. G. Roper, J. G. Shackman, G. M. Dahlgren, and R. T. Kennedy, "Microfluidic chip for continuous monitoring of hormone secretion from live cells using an electrophoresis-based immunoassay," *Analytical Chemistry*, vol. 75, no. 18, pp. 4711–4717, 2003.

[132] J. V. Rocheleau, G. M. Walker, S. S. Head, O. P. McGuinness, and D. W. Piston, "Microfluidic glucose stimulation reveals limited coordination of intracellular $Ca2^{+}$ activity oscillations in pancreatic islets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 35, pp. 12899–12903, 2004.

[133] D. Chen, W. Du, Y. Liu et al., "The chemistrode: a droplet-based microfluidic device for stimulation and recording with high temporal, spatial, and chemical resolution," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 44, pp. 16843–16848, 2008.

[134] J. S. Mohammed, Y. Wang, T. A. Harvat, J. Oberholzer, and D. T. Eddington, "Microfluidic device for multimodal characterization of pancreatic islets," *Lab on a Chip*, vol. 9, no. 1, pp. 97–106, 2009.

[135] A. F. Adewola, D. Lee, T. Harvat et al., "Microfluidic perifusion and imaging device for multi-parametric islet function assessment," *Biomedical Microdevices*, vol. 12, no. 3, pp. 409–417, 2010.

[136] R. P. Robertson, C. Davis, J. Larsen, R. Stratta, and D. E. R. Sutherland, "Pancreas and islet transplantation for patients with diabetes," *Diabetes Care*, vol. 23, no. 1, pp. 112–116, 2000.

[137] S. M. Schieke, D. Phillips, J. P. McCoy et al., "The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity," *Journal of Biological Chemistry*, vol. 281, no. 37, pp. 27643–27652, 2006.

[138] C. Chen, Y. Liu, R. Liu et al., "TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species," *Journal of Experimental Medicine*, vol. 205, no. 10, pp. 2397–2408, 2008.

[139] G. S. Hotamisligil, "Endoplasmic reticulum stress and the inflammatory basis of metabolic disease," *Cell*, vol. 140, no. 6, pp. 900–917, 2010.

[140] M. Tanemura, A. Saga, K. Kawamoto et al., "Rapamycin induces autophagy in islets: relevance in islet transplantation," *Transplantation Proceedings*, vol. 41, no. 1, pp. 334–338, 2009.

[141] J. Y. Li, Y. Furuichi, N. Matsuoka, S. Mutoh, and T. Yanagihara, "Tacrolimus (FK506) attenuates biphasic cytochrome c release and Bad phosphorylation following transient cerebral ischemia in mice," *Neuroscience*, vol. 142, no. 3, pp. 789–797, 2006.

[142] N. Erin, R. A. W. Lehman, P. J. Boyer, and M. L. Billingsley, "In vitro hypoxia and excitotoxicity in human brain induce calcineurin-Bcl-2 interactions," *Neuroscience*, vol. 117, no. 3, pp. 557–565, 2003.

[143] S. Marcelli-Tourvieille, T. Hubert, E. Moerman et al., "In vivo and in vitro effect of sirolimus on insulin secretion," *Transplantation*, vol. 83, no. 5, pp. 532–538, 2007.

[144] A. Mita, C. Ricordi, A. Miki et al., "Anti-proinflammatory effects of sirolimus on human islet preparations," *Transplantation*, vol. 86, no. 1, pp. 46–53, 2008.

[145] L. Piemonti, P. Maffi, L. Monti et al., "Beta cell function during rapamycin monotherapy in long-term type 1 diabetes," *Diabetologia*, vol. 54, no. 2, pp. 433–439, 2011.

[146] R. Melzi, P. Maffi, R. Nano et al., "Rapamycin does not adversely affect intrahepatic islet engraftment in mice and improves early islet engraftment in humans," *Islets*, vol. 1, no. 1, pp. 42–49, 2009.

[147] M. Shimodahira, S. Fujimoto, E. Mukai et al., "Rapamycin impairs metabolism-secretion coupling in rat pancreatic islets by suppressing carbohydrate metabolism," *Journal of Endocrinology*, vol. 204, no. 1, pp. 37–46, 2010.

[148] M. Laugharne, S. Cross, S. Richards et al., "Sirolimus toxicity and vascular endothelial growth factor release from islet and renal cell lines," *Transplantation*, vol. 83, no. 12, pp. 1635–1638, 2007.

[149] C. T. Bussiere, J. R. T. Lakey, A. M. J. Shapiro, and G. S. Korbutt, "The impact of the mTOR inhibitor sirolimus on

the proliferation and function of pancreatic islets and ductal cells," *Diabetologia*, vol. 49, no. 10, pp. 2341–2349, 2006.

[150] B. W. Paty, J. S. Harmon, C. L. Marsh, and R. P. Robertson, "Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wistar rat islets," *Transplantation*, vol. 73, no. 3, pp. 353–357, 2002.

[151] L. Polastri, F. Galbiati, F. Bertuzzi et al., "Secretory defects induced by immunosuppressive agents on human pancreatic β-cells," *Acta Diabetologica*, vol. 39, no. 4, pp. 229–233, 2002.

[152] J. B. Redmon, L. K. Olson, M. B. Armstrong, M. J. Greene, and R. P. Robertson, "Effects of tacrolimus (FK506) on human insulin gene expression, insulin mRNA levels, and insulin secretion in HIT-T15 cells," *Journal of Clinical Investigation*, vol. 98, no. 12, pp. 2786–2793, 1996.

[153] V. M. Montori, A. Basu, P. J. Erwin, J. A. Velosa, S. E. Gabriel, and Y. C. Kudva, "Posttransplantation diabetes: a systematic review of the literature," *Diabetes Care*, vol. 25, no. 3, pp. 583–592, 2002.

[154] R. G. Radu, S. Fujimoto, E. Mukai et al., "Tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity," *American Journal of Physiology*, vol. 288, no. 2, pp. E365–E371, 2005.

[155] N. Rostambeigi, I. R. Lanza, P. P. Dzeja et al., "Unique cellular and mitochondrial defects mediate FK506-induced islet β-cell dysfunction," *Transplantation*, vol. 91, no. 6, pp. 615–623, 2011.

[156] J. D. Johnson, Z. Ao, P. Ao et al., "Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets," *Cell Transplantation*, vol. 18, no. 8, pp. 833–845, 2009.

[157] H. K. Song, D. H. Han, J. H. Song et al., "Influence of sirolimus on cyclosporine-induced pancreas islet dysfunction in rats," *American Journal of Transplantation*, vol. 9, no. 9, pp. 2024–2033, 2009.

[158] B. Kaplan, P. West, H. Neeley et al., "Use of low dose tacrolimus, mycophenolate mofetil and maintenance IL-2 receptor blockade in an islet transplant recipient," *Clinical Transplantation*, vol. 22, no. 2, pp. 250–253, 2008.

[159] N. R. Barshes, S. Wyllie, and J. A. Goss, "Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts," *Journal of Leukocyte Biology*, vol. 77, no. 5, pp. 587–597, 2005.

[160] C. E. Gleason, M. Gonzalez, J. S. Harmon, and R. Paul Robertson, "Determinants of glucose toxicity and its reversibility in the pancreatic islet β-cell line, HIT-T15," *American Journal of Physiology*, vol. 279, no. 5, pp. E997–E1002, 2000.

[161] V. Poitout and R. P. Robertson, "Minireview: decondary β-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity," *Endocrinology*, vol. 143, no. 2, pp. 339–342, 2002.

[162] A. Rajab, "Islet transplantation: alternative sites," *Current Diabetes Reports*, vol. 10, no. 5, pp. 332–337, 2010.

[163] S. Merani, C. Toso, J. Emamaullee, and A. M. J. Shapiro, "Optimal implantation site for pancreatic islet transplantation," *British Journal of Surgery*, vol. 95, no. 12, pp. 1449–1461, 2008.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 896751, 14 pages
doi:10.1155/2012/896751

Review Article

Mitochondria: Redox Metabolism and Dysfunction

Jia Kang[1,2] and Shazib Pervaiz[1,2,3,4]

[1] *ROS, Apoptosis and Cancer Biology Laboratory, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597*
[2] *NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117597*
[3] *Cancer and Stem Cell Biology Program, Duke-NUS Graduate Medical School, Singapore 169857*
[4] *Cell and Systems Biology (CSB), Singapore-MIT Alliance, Singapore 637460*

Correspondence should be addressed to Shazib Pervaiz, phssp@nus.edu.sg

Received 23 December 2011; Accepted 5 February 2012

Academic Editor: Renée Ventura-Clapier

Mitochondria are the main intracellular location for fuel generation; however, they are not just power plants but involved in a range of other intracellular functions including regulation of redox homeostasis and cell fate. Dysfunction of mitochondria will result in oxidative stress which is one of the underlying causal factors for a variety of diseases including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancer. In this paper, generation of reactive oxygen/nitrogen species (ROS/RNS) in the mitochondria, redox regulatory roles of certain mitochondrial proteins, and the impact on cell fate will be discussed. The current state of our understanding in mitochondrial dysfunction in pathological states and how we could target them for therapeutic purpose will also be briefly reviewed.

1. Introduction

Mitochondria are the main intracellular location for generating adenosine triphosphate (ATP), the fuel for cell's metabolic needs, and therefore are referred to as the power plants of the cell. Energy is stored in the form of phosphate bond and is released when ATP is hydrolyzed to adenosine diphosphate (ADP) to meet the requirement of a number of energy demanding cellular processes. ATP is generated through cellular respiration, including a set of chemical reactions named as the tricarboxylic acid (TCA) cycle and oxidative phosphorylation that take place in mitochondria; therefore, mitochondria play a crucial regulatory role in cellular metabolism [1]. However, mitochondria are far more than just power suppliers. They are also involved in many other cellular functions, including calcium signaling [2], heme [3] and steroid synthesis [4], regulation of membrane potential [1], proliferation [5] or apoptosis [6], and redox homeostasis maintenance [7], to name just a few. Mitochondria are the major sites for free radical species production, including both reactive oxygen species (ROS) and reactive nitrogen species (RNS). On one hand, free radical species are indispensable for proper cell signaling; on the other hand, excessive generation of ROS results in cell/tissue injury and death. In this paper, the underlying mechanisms for generation of free radical species in the mitochondria and how some mitochondrial proteins act as redox regulators will be of prime focus. In the past decade, more and more pieces of evidence are surfacing to point the role of ROS as critical mediators of the balance between cell proliferation and cell death [8–10]. Therefore, the involvement of mitochondria in cell death, especially from the noncanonical view where mitochondria regulate cell death through manipulating the redox millieu will also be reviewed. Due to the fundamental regulatory role of mitochondria, disturbances of the integrity of their functions will result in cellular dysfunction leading to various pathological states or even death. Therefore, a good understanding of the basic mitochondrial biology will help in therapeutic design for better disease management.

2. Mitochondrial Functions

2.1. Mitochondria Structure and Metabolism

2.1.1. Mitochondria Structure. Mitochondria are essential to sustain life as around 98% of the oxygen that we breathe

in is consumed by mitochondria for energy production. In order to understand the basic principles of mitochondrial bioenergetics, it is necessary to have a brief overview of its structure first. Mitochondria, as thought to have evolved from a bacterial progenitor and having their own mitochondrial DNA pool [11], are bounded by two membrane systems, including an outer and an inner membrane, and the space in between is referred to as the intermembrane space; however, the two layers of membranes occasionally come into contact with each other to form junctional complexes. The inner mitochondrial membrane, with multiple inward folding known as cristae which house membrane-bound mitochondrial enzymes, serves as the major barrier between mitochondria and cytoplasm since it is largely impermeable, thereby preventing small molecules and ionic species from entering the mitochondrial matrix [1].

2.1.2. TCA Cycle and Electron Transport Chain. The tricarboxylic acid (TCA) cycle and the electron transport chain contribute to the key enzymatic components of the mitochondria. During the process of breaking down carbon substrates into acetyl CoA, reducing equivalents (NADH and FADH) are produced, which are then fed to the electron transport chain consisting of Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (ubiquinol cytochrome c reductase), and Complex IV (cytochrome c oxidase). Electrons move from the reducing equivalents to Complexes I and II, respectively, which are then passed onto ubisemiquinone for shuttling to Complex III followed by Complex IV through cytochrome c. In the meanwhile, an electrochemical proton gradient is generated when protons are transferred across the inner mitochondrial membrane into the intermembrane space coupled to the electron transfer at Complexes I, III, and IV. This is known as the proton-motive force, generating the mitochondrial transmembrane potential, which is usually about negative 150–180 mV as compared to the cytoplasm. The influx of the protons through the proton translocating F_1F_0-ATP synthase, driven by this force, is coupled to a chemical reaction that phosphorylates ADP to ATP [12]. An electrogenic transporter, adenine nucleotide translocase (ANT), then transports ATP out of the mitochondria to places where energy demanding cellular processes occur [1].

2.2. Mitochondria and Redox Homeostasis Maintenance

2.2.1. Sources of Mitochondrial Reactive Radical Species

Reactive Oxygen Species (ROS). Mitochondria are the major sources of free radical species production since unpaired electrons are generated in the process of oxidative phosphorylation. Partial reduction of molecular oxygen by the unpaired electrons leads to the production of superoxide anions ($O_2^{\bullet -}$) which are one of the reactive oxygen species (ROS) and readily converted to hydrogen peroxide (H_2O_2) by magnesium superoxide dismutase (MnSOD) residing in mitochondria matrix. H_2O_2 can be converted subsequently to the highly reactive oxygen species, hydroxyl radical ($^{\bullet}OH$), through the Fenton reaction [7]. Unlike H_2O_2, $O_2^{\bullet -}$ does not diffuse that readily across the membrane and thus for $O_2^{\bullet -}$ produced in the matrix, the activity of MnSOD is critical to prevent the mitochondrial matrix components from oxidative damage.

Although, it is well known that ROS can be generated as byproducts of oxidative phosphorylation, the question as to the specific site(s) along the electron transport chain responsible for ROS generation has always been a hotly debated topic. It is traditionally believed that under physiological conditions, Complex I is the main site for mitochondrial ROS production, where $O_2^{\bullet -}$ is produced on the matrix side and rapidly dismutated to H_2O_2 [13, 14]. In addition, Complex III has also been reported as a site for $O_2^{\bullet -}$ production [15, 16]. It is demonstrated that under ischemic and apoptotic conditions, $O_2^{\bullet -}$ production is triggered at Complex III. This may happen through inhibition of Complex IV as well as over reduction of the electron transport chain in the event of mounting hypoxic stress [17]. In a more recent review [18], the relative contribution of each complex towards $O_2^{\bullet -}$ production has been clearly quantitated with Complexes I (producing $O_2^{\bullet -}$ to the matrix) and III (producing $O_2^{\bullet -}$ to both the matrix and intermembrane space) having the greatest maximum capacities while Complex II has normally negligible rates.

Another major source of ROS production in the cell is the NADPH oxidase (Nox) family proteins, which are enzyme complexes catalyzing the electron transfer from NADPH to molecular oxygen, generating $O_2^{\bullet -}$ and H_2O_2. The relative quantitative contribution of mitochondria and NADPH oxidase in cellular ROS production is expected to vary greatly from one cell type to another. In certain cells including the phagocytic neutrophils as well as nonphagocytic fibroblasts, vascular smooth muscle cells and endothelial cells, cellular ROS production is largely contributed by NADPH oxidase [18–20]. It has been recently reported that one of the Nox isoforms, Nox4, is expressed in the mitochondria in the rat renal cortex [21], cardiac myocytes [22, 23], and in the mitochondria-enriched heavy membrane fractions of the chronic myeloid leukemia cells that overexpress the antiapoptotic protein Bcl-2 (CEM/Bcl-2) [24]. Nox4 expression was shown to correlate with dihydroethidium staining for $O_2^{\bullet -}$. In Nox4-transgenic mice cysteine residues of mitochondrial proteins were also more oxidized [23]. However, there are no direct and specific measurements of Nox4 activity in the mitochondria up to date. Meanwhile, the cytoplasmic Nox4 may be involved in the activation of PKCε, mitoK_{ATP} and modulation of thioredoxin 2 activity resulting in redox-sensitive upregulation of mitochondrial ROS production through the electron transport chain [25]. Apart from the electron transport chain and the NOX family, several other sites in the mitochondria have also been reported to generate $O_2^{\bullet -}$, including pyruvate dehydrogenase, α-ketaglutarate dehydrogenase [26], glycerol-3-phosphate dehydrogenase, and fatty acid β-oxidation [18].

Recently, new findings that supported the concept of mitochondrial $O_2^{\bullet -}$ flashes have gained much interest and revealed several previously unknown aspects of mitochondrial dynamics. Transient quantal $O_2^{\bullet -}$ flashes were observed in excitable cells such as muscle cells and neurons *in vivo*

and they are associated with mitochondrial permeability transition pore (mPTP) opening, which presents a new facet in physiological ROS production [27–29].

Reactive Nitrogen Species (RNS). Apart from ROS generation, there is evidence to suggest the expression of a mitochondrial specific nitric oxide synthase (NOS) leading to $^{\bullet}NO$ production. It was first described in 1997 by Ghafourifar and Richter that $^{\bullet}NO$ was generated from isolated mitochondria when they were loaded with calcium and mitochondrial potential dropped in some of the treated mitochondria [30]. Another group provided more direct evidence by showing images of calcium regulated $^{\bullet}NO$ production within the mitochondria of permeabilized cells, through the use of the $^{\bullet}NO$-sensitive chromophore, DAF-2 [31].

The impact of $^{\bullet}NO$ generation on mitochondrial functions remains controversial, largely depending on the amount of $^{\bullet}NO$ that is produced as well as the conditions under which it is produced. It has been shown that mitochondrial respiration can be partially inhibited with even the modest levels of $^{\bullet}NO$ causing an increase in mitochondrial ROS production. This also results in mitochondrial depolarization resulting in a decrease in mitochondrial calcium uptake [32]. The inhibitory effect of $^{\bullet}NO$ on mitochondrial respiration has been shown to mainly result from inactivation of cytochrome c oxidase (Complex IV) [33–40], which is the rate-limiting step of the electron transport chain. Being a double-edged sword, $^{\bullet}NO$ was demonstrated to play a role in mitochondrial biogenesis as well, which gets stimulated by $^{\bullet}NO$ generation through a cGMP-dependent upregulation of PGC1α expression, which in turn increases the expression of mtTFA and NRF-1 resulting in an increased mitochondria biosynthesis observed in adipocytes and hepatocytes [41]. Another group also showed a physiological role for $^{\bullet}NO$ generation in mitochondrial mass regulation where endothelial-$^{\bullet}NO$-synthase- (eNOS-) deficient mice showed deficiencies in mitochondrial enzymes [42]. In addition, protective effect of $^{\bullet}NO$ on mitochondria and cells is also observed against ischemia/reperfusion (I/R) injury (late preconditioning). It has been suggested that partial temporary inhibition of Complex I might be involved in this effect via inhibition of ROS burst in reperfusion. Furthermore, treatment with diet inorganic nitrates or administration of nitrites or S-nitroso-2-mercaptopropionyl glycine (SNO-MPG) confers cardioprotection against the injury via temporary S-nitrosylation of various cellular protein targets [43].

On the contrary, under some pathological stimuli, excessive production of $^{\bullet}NO$ could result in severe tissue damage. Several groups have suggested that in the face of a high concentration of $^{\bullet}NO$ with a relatively low oxygen concentration, respiration can be inhibited resulting in an increased $O_2^{\bullet-}$ production, which will react with the freely membrane diffusible $^{\bullet}NO$ to form a much more reactive radical species, peroxynitrite ($ONOO^-$). Peroxynitrite can also be formed through an alternative route via the reaction of nitroxyl anion (NO^-) and molecular oxygen [44]. Nitroxyl anion can be derived from $^{\bullet}NO$ through one electron reduction by the electron donors including cytochrome c [45] or ubiquinol [46]. Due to its ability to diffuse across the mitochondrial membranes, $ONOO^-$ can result in oxidative damage of critical components throughout the mitochondria via oxidation, nitration, and/or nitrosation. For example, thiols present in Complex I can be oxidized leading to the formation of an S-nitrosothiol derivative and inactivation of the complex [47–49]. Complexes II and V were also shown to be inactivated by $ONOO^-$ [34, 44, 48–53]. MnSOD, the matrix antioxidant enzyme, is also a target for nitration leading to a decrease in its enzymatic activity [54]. Adenine nucleotide translocase (ANT) [55], creatine kinase [56], nicotinamide nucleotide transhydrogenase [57], aconitase [58], and components of the pyridine nucleotide-dependent calcium release pathway [59] are all targets of $ONOO^-$. Therefore, $ONOO^-$ has profound effects on mitochondrial metabolism, calcium homeostasis, and the mitochondrial permeability transition pore [42]. In addition, $ONOO^-$ has been shown to uncouple eNOS, leading to a switch from $^{\bullet}NO$ to $O_2^{\bullet-}$ production and an increase in mitochondrial ROS levels as well [42, 60].

Of particular note, carbon monoxide (CO), an endogeneous gas produced by heme oxygenase (HO) that catalyzes heme degradation, is able to induce the production of ROS and RNS as well due to its high affinity for reduced transition metals such as Fe^{2+}. For example, it binds to Complex IV and slows the terminal transfer rate of electrons to molecular O_2 leading to enhanced $O_2^{\bullet-}$ production [61].

2.2.2. Mitochondria in Redox Regulation. Since mitochondria are major sources for ROS production, it is not surprising that they are well equipped with antioxidant defenses, including a large pool of glutathione, glutathione peroxidase, glutathione reductase, MnSOD, catalase, and the thioredoxin system [1, 62].

Although excessive levels of ROS will lead to protein oxidation and lipid peroxidation causing damage to mitochondrial membrane, proteins, and DNA, especially when the mitochondrial DNA is not protected with associated histones, lower levels of ROS have been demonstrated to be essential signaling molecules [7, 63, 64]. A new concept is now emerging that mitochondrial ROS production is likely to be highly regulated as a part of physiological mitochondrial functions and the underlying molecular mechanisms are being gradually uncovered [7]. In this paper, a few mitochondrial proteins that act as redox regulators will be discussed as examples, including the antiapoptotic protein Bcl-2, cytochrome c oxidase (COX), and the small GTPase Rac1.

Bcl-2 and Its Effect on Mitochondrial ROS Generation. Bcl-2, one of the antiapoptotic members of the Bcl-2 family proteins residing on the outer mitochondrial membrane [65], is best known for its ability to inhibit apoptotic execution, that is, to form homo- and heterodimers to prevent the oligomerization of proapoptotic Bcl-2 family members, thus antagonizing the induction of mitochondrial outer membrane permeabilization (MOMP) [66]. However, recent evidence points to a new facet of Bcl-2 biology in redox

regulation. The involvement of Bcl-2 in redox regulation was first demonstrated by Hockenbery et al. that Bcl-2 overexpression protected against ROS-induced apoptosis [67]. Soon after other studies also revealed the protective capacity of Bcl-2 against various ROS triggers [68–73]. However, Bcl-2 itself was later found out to possess no intrinsic antioxidant ability [74] implying that rather its overexpression indirectly induces an enhancement in antioxidant capacity of the cells when they undergo overt oxidative stress [66, 75] and this is supported by the findings that Bcl-2-mediated protection is associated with upregulation of the cellular enzymatic and nonenzymatic antioxidant defense machineries including the glutathione system, catalase, and NAD(P)H [74, 76–79]. More recently, a clearer picture of Bcl-2 with respect to its ability to regulate redox status is emerging, and a prooxidant role of Bcl-2 is established under normal physiological states [77, 80–84]. Based on this prooxidant property of Bcl-2, it implies that the enhanced antioxidant capacity that was observed with Bcl-2 overexpression could be an adaptive response to the chronic but mild oxidative intracellular milieu [70, 74, 76, 79, 83] and this serves as a first-line defense in the event of acute oxidative insults maintaining the ROS levels within a threshold optimal for cell survival [66, 75, 81, 85].

The underlying mechanisms on how Bcl-2 exerts its prooxidant activity, however, have not been fully elucidated. It was first hypothesized that the prooxidant milieu in Bcl-2 overexpressing mitochondria resulted from an altered dynamics of the oxidative phosphorylation. An increase in mitochondrial size and associated matrix content was observed with Bcl-2 overexpression and this indicated an increase in the number of electron donors and a subsequent increase in the chance of electrons leaking out of the electron transport chain to form $O_2^{\bullet -}$ [77, 86]. However, the exact mechanism linking Bcl-2 expression levels to mitochondrial size and matrix content was not addressed in the above-mentioned studies. More recently, our group has established the inherent ability of Bcl-2 to generate intramitochondrial $O_2^{\bullet -}$ by engaging mitochondrial respiration in tumor cells. An increased mitochondrial oxygen consumption rate and cytochrome c oxidase (COX or Complex IV) activity was observed in Bcl-2 overexpressing cells [81, 85, 87]. It is plausible that the increased mitochondrial respiration rate results in an increased electron flux across the electron transport chain and an increased probability of leakage of electrons onto molecular oxygen thus leading to an increase in the by-production of $O_2^{\bullet -}$. Indeed, either silencing of Bcl-2 with siRNA or functional inhibition of Bcl-2 with the BH3 mimetic, HA14-1, in those cells, reversed both the oxygen consumption rate as well as the $O_2^{\bullet -}$ levels [81]. This is further supported by the observation that mitochondrial respiratory rate and $O_2^{\bullet -}$ levels correlated with Bcl-2 expression levels across different tumor cell lines with various endogeneous Bcl-2 levels [85]. Of note, Bcl-2 overexpression promoted the mitochondrial localization of COX Va and Vb, which are nuclear encoded subunits of Complex IV, which could explain for the significantly increased Complex IV activity in these cells [85]; it has been previously shown that mitochondrial level of COX Vb correlated with the COX holoenzyme activity [88]. Of particular note, the increased $O_2^{\bullet -}$ release as induced by Bcl-2 overexpression might seem contradictory to what we mentioned in the earlier section that inhibition of ETC by $^{\bullet}NO$ also results in $O_2^{\bullet -}$ release. However, the former occurs as a result of increased electron flux across the electron transport chain and an increased probability of leakage of electrons onto molecular oxygen while the latter is the result of inhibition of reduction step along the ETC leading to promotion of the reaction of oxygen with accumulated reductants [89].

More recently, our group has identified another functional player in Bcl-2-mediated prooxidant state, the small GTPase Rac1 [24]. Rac1 is known to be involved in the assembly and activation of NADPH oxidase complex leading to $O_2^{\bullet -}$ production. It was first discovered that introduction of the dominant negative mutant Rac1N17 neutralized the prooxidant activity of Bcl-2 [82]. Later on, a physical interaction was observed between these two proteins in the outer mitochondrial membrane of tumor cells which could be blocked with the BH3 mimetics and Bcl-2 BH3 domain peptides. The intramitochondrial $O_2^{\bullet -}$ production in Bcl-2 overexpressing cells was also reversed by BH3 peptides, which can also be achieved with silencing or functional inhibition of Rac1 [24].

These data provide evidence for the existence of functional complexes within the mitochondria involving Bcl-2, but the precise mechanism of interaction and how disruption of these interaction(s) could impact cell fate remains to be elucidated. Of course, apart from the above-mentioned proteins, there are many others that act as mitochondrial redox regulators including the master transcription factor for cellular antioxidant defense machinery, the transcription factor NF-E2-related factor 2 (Nrf2), which gets activated under cellular oxidative stress conditions such as GSH depletion, $^{\bullet}NO$, and nitrosative stress [90–92]. Interestingly, a recent paper provided the first evidence that activation of Nrf2 can upregulate Bcl-2 as well [93].

2.3. Mitochondria and Cell Fate Regulation. Since mitochondria are fundamental energy generators, severe damage to mitochondria will inevitably cause disorders in cellular functions. Once ionic gradients and intracellular osmolarity cannot be maintained, cells will swell and die through a death process known as necrosis [94]. However, for some immortalized cells, they survive reasonably well on ATP generated from glycolysis even when mitochondrial respiration is completely inhibited [1, 95, 96]. Apart from dying passively when the ATP supply fails, cells can also actively undergo a "suicide" program through the mitochondria-mediated apoptotic pathway upon compromise in the mitochondrial outer and/or inner membrane permeability [97]. Furthermore, there is another form of cell death named autophagy, which degrades cellular organelles and proteins promoting either survival or death depending on the stress conditions. Various studies have indicated the involvement of ROS and mitochondria in autophagic regulation [98, 99]; however, due to space constraints, it is not covered in this review.

2.3.1. Apoptosis. For the past two decade or so, mitochondria have been extensively studied for its essential role in defining the balance between cell life and death. It was first demonstrated in 1994, that cytochrome c, once it is released from the intermembrane space of the mitochondria, can initiate an enzyme cascade of cellular self-destruction. Other death amplification factors, such as the inhibitors of apoptosis (the IAP family which prevent accidental caspase activation presumably), smac or *diablo* (which inhibits IAPs to permit the apoptotic cascade to proceed), procaspase-9, and AIF (the flavoprotein apoptosis inducing factor) are also released to participate in the death execution pathway [97].

2.3.2. Redox Status in Cell Fate Decision. Although overwhelming amount of ROS are definitely detrimental to the cells, emerging evidence has demonstrated that when they are present in nonlethal concentrations, they can function as proliferative and/or survival signals [8]. A mild increase in the $O_2^{\bullet -}$ has been shown to confer survival advantage to the tumor cells under apoptotic triggers [8, 10, 100–103]. Furthermore, it has been demonstrated by our group that cell fate is tightly regulated as a function of the ratio of $O_2^{\bullet -}$ and H_2O_2. A tilt in the balance of the two reactive oxygen species towards $O_2^{\bullet -}$ leads to survival signaling, while the reverse sensitizes cells to apoptotic triggers [8–10]. Corroborating the survival advantage of a mild prooxidant status, the antiapoptotic activity of Bcl-2 can be contributed to its noncanonical ability to induce intramitochondrial $O_2^{\bullet -}$ production. Indeed, Bcl-2 BH3 peptides, which reversed the prooxidant state of Bcl-2-overexpressing tumor cells, sensitized those cells to drug-induced apoptosis. Similarly, silencing or pharmacological inhibition of Rac1 also compromised Bcl-2-induced increase in intramitochondrial $O_2^{\bullet -}$ levels leading to sensitization of those tumor cells to apoptotic triggers [24].

3. Mitochondrial Dysfunction in Pathology and Therapeutic Targeting

Mitochondria, as one of the major ROS producers within the cell, have been rendered susceptible to oxidative damage when the antioxidant defense machinery fails to meet their ROS scavenging tasks; therefore, they are implicated in the pathology of various diseases including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancer [104–114].

3.1. Neurodegenerative Diseases. ROS-mediated mitochondrial dysfunctions and apoptosis have been demonstrated as causal factors in the pathology of several neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD), and Amyotrophic lateral sclerosis (ALS). Oxidative damage, as indicated by malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), have been identified in patients diagnosed with PD (substantia nigra), AD (hippocampus and cortex) as well as ALS (spinal fluid) [115–118]. The levels of Iron are also found to be elevated in the substantia nigra of patients with PD, which could serve as a catalyst for the Fenton's reaction in producing hydroxyl radicals [119–121], whereas the activities of antioxidant defense enzymes such as glutathione peroxidase (GPx) and reductase (GR), superoxide dismutase (SOD) and catalase (CAT) are reduced in the affected brain regions of patients with AD [117, 122, 123]. Similarly, patients with PD demonstrate diminished levels of GSH in the dopaminergic neurons of substantia nigra [118, 119, 124].

Transgenic animals that develop neurodegenerative diseases have been utilized as models to clarify the roles of oxidative stress in the pathogenesis of these diseases. Transgenic mice that harbor an ALS-linked mutant CuZnSOD gene show progressive accumulation of 8-OHdG, one of the best markers for oxidative DNA damage, in ventral horn neurons. The immunoreactivity for this marker indicates the existence of oxidative damage to mitochondrial DNA in spinal motoneurons starting from very early stage of the disease, and probably contributing to the subsequent motoneuron death [125]. In another model of GPx deficient mice, administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) led to enhanced toxicity to dopaminergic neurons indicating the involvement of ROS in the early pathogenesis of PD [126]. Triple-transgenic mice that mimic AD progression in humans also demonstrate reduced levels of GSH and vitamin E as well as increased extent of lipid peroxidation during the stage of Amyloid β (Aβ) oligomerization before the onset of Aβ plaques and neurofibrillary tangles [127].

Since oxidative stress resulting from depletion of the cellular antioxidant glutathione occured during the early stages of neurodegeneration, a lot of effort has been dedicated to study the effects of antioxidants in combating oxidative stress and preserving the integrity of mitochondria for the treatment of neurodegenerative diseases. Several examples of antioxidants that have been demonstrated to attenuate disease progression will be discussed below.

Curcumin. Curcumin is a polyphenol that belongs to the ginger family (Zingiberaceae). Curcumin treatment of dopaminergic neuronal cells and mice restores GSH pools, which protects against oxidative stress and preserves mitochondrial complex I activity, thereby suggesting its therapeutic benefits in the treatment of PD [128]. Protective effects of curcumin have also been shown against MPP (+)-induced cytotoxicity and apoptosis by upregulation of Bcl-2 expression and restoring mitochondrial membrane potential [129]. In addition, tetrahydrocurcumin (THC), a metabolite of curcumin, has been shown to protect against Aβ-induced ROS burst, preserve mitochondrial membrane potential, and prevent caspase activation in rat primary hippocampal cultures [130].

Apart from oxidative stress, nitrosative stress, largely mediated by reactive nitrogen species (RNS) such as $OONO^-$, is also crucial in PD development by inducing mitochondrial dysfunction through inhibition of brain mitochondrial complex I activity, decrease of mitochondrial membrane potential, and compromise of mitochondrial integrity. The glutamoyl diester bioconjugate of curcumin has been shown

to restore Complex I activity and protect against protein nitration [131].

Epigallocatechin-3-Gallate (EGCG). EGCG is the most abundant polyphenol found in green tea. Apart from its iron-chelating property, the antioxidant capacity of EGCG has been demonstrated at the level of mitochondria where it not only enhances the activities of both TCA cycle enzymes and ETC complexes but also upregulates the antioxidant system in aged brain [132]. EGCG has also been shown to increase the activity of SOD and catalase in mice striatum [133]. The molecular mechanism underlying the upregulation of the antioxidant defense machinery is due to the ability of EGCG to induce the activation of Nrf2, a master transcription factor for antioxidant and phase II detoxifying enzymes [134].

Although these molecules demonstrate protective effects in animal models of the neurodegenerative diseases, their beneficial effects in humans have not been clearly demonstrated in clinical trials, conceivably due to the difficulties in penetrating the blood-brain barrier; therefore a lot of effort is currently being spent in developing better delivery systems for specific targeting, especially to mitochondria, where their pharmacological activity is mostly required to increase the therapeutic efficacy [135–138].

3.2. Diabetes, Diabetic Complications, and Cardiovascular Diseases. Despite the fact that diabetes mellitus (DM) is a heterogeneous, multifactorial and chronic disease, it can be stated without doubt that DM is linked to acute and continuous overproduction of ROS and characterized by mitochondrial impairment. DM is also marked with chronic inflammation that further weakens intracellular antioxidant defense. Reduced levels of antioxidants such as GSH, vitamins C and E are observed in diabetic patients [139, 140]. Dysfunction of mitochondrial complex I and subsequent increase in ROS production together with decreases in antioxidant levels and membrane potential have been reported in diabetic patients [141]. Risk factors such as aging, obesity, and unhealthy diet contribute to an oxidative environment which impairs insulin signaling and mitochondrial function leading to diabetes development, and the resulting hyperglycemia in turn contributes to the maintenance and progression of the overall oxidative stress through mechanisms such as glycation of antioxidant enzymes [111] and overproduction of O_2^- by the mitochondrial ETC, which in turn activate a variety of proinflammatory signals [111, 142–144].

Malfunctioning of mitochondrial oxidative phosphorylation has been considered as one of the main culprits in the development of diabetic complications as well, such as renal dysfunction [145]. In addition, it has been reported that the activities and expression of antioxidant enzymes are decreased in diabetic microvascular disease [146, 147] and a specific polymorphic MnSOD gene is correlated with diabetic nephropathy development [148]. Protective effects of catalase overexpression have also been demonstrated in the experimental models of type 2 diabetic nephropathy, thereby implicating H_2O_2 [149]. Majority of the type 2 diabetic patients with insulin resistance also exhibit a significantly higher risk of developing cardiovascular disease (CVD) [150]. Human atherosclerotic samples show higher extent of mitochondrial DNA damage, which correlates with greater ROS production. In apoE-null mice, mitochondrial damage has been shown to precede the development of atherosclerosis. In the same model, heterozygous deficiency of MnSOD also increases vascular mitochondrial dysfunction [151].

Since oxidative stress has been considered as one of the main contributing factors for the onset and progression of diabetes, diabetic complications, and CVD, the need to eradicate ROS especially from mitochondria is of great therapeutic importance [152]. Although classical antioxidants such as vitamins C and E do not show significant improvement in disease conditions [153], recent reports show that a subgroup of type 2 diabetic patients with haptoglobin (Hp) 2-2 genotype can benefit from *vitamin E* supplementation suggesting that tailored treatment regimens for different subgroups of patients could be more favorable [154, 155]. *MitoQ* is an antioxidant that is selectively targeted to and accumulates in mitochondria due to its covalent attachment to the lipophilic triphenylphosphonium cation [156]. MitoQ administration to Ins2(+/) (AkitaJ) mice improves the tubular and glomerular function and reduces urinary albumin levels and interstitial fibrosis implicating their therapeutic benefits in treating diabetic nephropathy [157].

3.3. Cancer

3.3.1. Prooxidant Theory of Carcinogenesis. We have discussed in the previous Section 2.3.2 how redox status can affect cell fate decision and how some mitochondrial proteins can act as redox mediators. Over the past decade, our group has been working on the underlying mechanisms and translational relevance of redox signaling in the context of carcinogenesis. We have established that cell fate is tightly regulated as a function of the ratio of $O_2^{\bullet -}$ and H_2O_2. A tilt in the balance of the two reactive oxygen species towards $O_2^{\bullet -}$ leads to survival signaling while the reverse sensitizes cells to apoptotic triggers [8–10].

3.3.2. Mitochondria as Therapeutic Targets in Cancer. Since mitochondria are key regulators for energy metabolism, ROS production and cell fate [158], targeting mitochondria to elicit cell death would therefore be a good strategy in cancer therapeutics especially in those cancer cells where upstream apoptotic signaling is malfunctional [159]. In addition, the bioenergetic differences between nontransformed and cancer cells confer the selectivity and specificity in targeting small molecule agents to the mitochondria of desired cancer cells. For example, lipophilic cations preferentially accumulate in the mitochondrial matrix of cancer cells due to their increased mitochondrial membrane potential resulting from increased glycolytic rates as compared to their normal counterparts [160, 161]. In addition, the addiction of cancer cells to glycolysis for ATP supply, described as the Warburg effect, also renders them more susceptible to apoptotic induction when their cellular bioenergetic pathways are intervened [161].

Direct Targeting of Mitochondrial ETC. Since mitochondrial ETC is essential in energy production and ROS generation, there are a plethora of agents that target ETC directly for cancer therapeutics [106]. Rotenone is a naturally derived hydrophobic pesticide, which binds and inactivates mitochondrial Complex I irreversibly leading to a blockage of oxidative phosphorylation and an increase in ROS generation [162, 163] and finally apoptotic induction [164–167]. Therapeutic benefits of Rotenone have been demonstrated in human breast cancer cells [168], neuroblastomas [169], promyelocytic leukemias [170], and human B-cell lymphomas [167]. Tamoxifen and estradiol have also been reported to act on the flavin mononucleotide site of complex I leading to mitochondrial failure independent of estrogen receptors [171], and particularly in MCF-7 breast cancer cells tamoxifen is able to induce an increase in ROS levels, a decrease in mitochondrial membrane potential and release of cytochrome c [172]. 3-nitropropionic acid is a toxin found in fungi and plants that can bind covalently to complex II [173–175] and its toxicity to tumor cells is linked to cellular energy depletion and oxidative stress due to the generation of O_2^-, H_2O_2, and $OONO^-$ [176, 177]. In addition, an analog of vitamin E, α-tocopheryl succinate (α-TOS) is able to interfere with the ubiquinone binding site on Complex II [178] leading to cell cycle arrest and apoptosis in a host of established cancer cell lines of different origin as well as in *in vivo* experimental animal models [179–185]. Although α-TOS has been shown to be largely nontoxic to normal tissues [183], its efficiency has not yet been tested in human cancer patients due to the difficulties in administration [186]. Antimycin A is a secondary metabolite produced by *Streptomyces kitazawensis* [187] which binds to the Qi site of Complex III [188, 189] leading to collapse of the proton gradient [188], ROS production, and apoptosis [190]. Fenretinide is a synthetic analog of retinoic acid which is able to downregulate Complex IV subunit III mRNA levels leading to a decrease in Complex IV activity [191]. Fenretinide induces apoptosis through elevated ROS production, cytochrome c release, and induction of mitochondrial permeability transition [192, 193], which could be prevented by the addition of antioxidants [194, 195]. It is likely that fenretinide inhibits at least one of the complexes along the ETC, although the exact prooxidant mechanisms are yet to be elucidated [194, 196]. *In vivo* therapeutic benefits of fenretinide have been demonstrated in both carcinogen-induced or xenograft animal models [197].

Direct targeting of mitochondrial ETC increases ROS production from the mitochondria of cancer cells which results in increased susceptibility of those glycolytic addicted cells to apoptotic induction. However, the critical points to be taken into consideration when using mitochondrial respiration "poisons" are their *in vivo* toxicity and therapeutic indexes. Almost half of the studies as discussed above failed to actually demonstrate nontoxicity of the agents to nontransformed cells *in vivo* except for α-TOS and fenretinide.

Direct Targeting of Bcl-2 Family Proteins. Apart from targeting the mitochondrial ETC, there is another group of proteins that are of particular interest due to their regulatory roles in apoptosis, which is the Bcl-2 family. We have discussed in Sections 2.2.2 and 2.3.2 that the ratio between the pro- and antiapoptotic members of Bcl-2 family is critical in cell fate decision. In addition, Bcl-2, an antiapoptotic member of the family and a resident protein of mitochondria, is able to modulate redox status which could be utilized in cancer therapeutics as well. Promising therapeutic strategies that aim at overcoming the problem of Bcl-2 overexpression (which happens in a number of cancers) including Bcl-2 antisense and BH3 mimetics have been recently reviewed by our group [66]. Furthermore, treatment of membrane active segments of the proapoptotic member Bax can also induce apoptosis in tumor cells [198].

Indirect Targeting of Mitochondrial Apoptotic Pathway. There are another group of drugs that do not target mitochondria directly but rather modulate mitochondrial proteins and/or induce ROS production leading to induction of intrinsic mitochondrial apoptotic pathway in cancer cells. These include clinically used chemotherapies such as irinotecan, topotecan [199], etoposide [200], vinblastine [201], and arsenic trioxide [202], which induce mitochondrial apoptotic pathway as well as those currently undergoing clinical evaluation such as betulinic acid [203], curcumin [204], camptothecin derivatives [199], and triapine [205], where betulinic acid and triapine-induced apoptosis has been attributed to ROS production.

4. Concluding Remarks

Mitochondria are essential regulators of cellular energy metabolism, redox homeostasis, and cell fate decision, and their dysfunction inevitably leads to various pathological states including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancer as briefly discussed in this review. Oxidative stress is the underlying causal factor in majority if not all of the diseases listed above; therefore, therapeutic strategies that aim at manipulating the redox metabolism represent promising options which have been and will still be at the center stage of targeted drug development.

References

[1] M. R. Duchen, "Mitochondria in health and disease: perspectives on a new mitochondrial biology," *Molecular Aspects of Medicine*, vol. 25, no. 4, pp. 365–451, 2004.

[2] G. Hajnóczky, G. Csordás, S. Das et al., "Mitochondrial calcium signalling and cell death: approaches for assessing the role of mitochondrial Ca2+ uptake in apoptosis," *Cell Calcium*, vol. 40, no. 5-6, pp. 553–560, 2006.

[3] T. Oh-Hama, "Evolutionary consideration on 5-aminolevulinate synthase in nature," *Origins of Life and Evolution of the Biosphere*, vol. 27, no. 4, pp. 405–412, 1997.

[4] M. F. Rossier, "T channels and steroid biosynthesis: in search of a link with mitochondria," *Cell Calcium*, vol. 40, no. 2, pp. 155–164, 2006.

[5] H. M. McBride, M. Neuspiel, and S. Wasiak, "Mitochondria: more than just a powerhouse," *Current Biology*, vol. 16, no. 14, pp. R551–R560, 2006.

[6] D. R. Green, "Apoptotic pathways: the roads to ruin," *Cell*, vol. 94, no. 6, pp. 695–698, 1998.

[7] R. B. Hamanaka and N. S. Chandel, "Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes," *Trends in Biochemical Sciences*, vol. 35, no. 9, pp. 505–513, 2010.

[8] S. Pervaiz and M. V. Clement, "Superoxide anion: oncogenic reactive oxygen species?" *International Journal of Biochemistry and Cell Biology*, vol. 39, no. 7-8, pp. 1297–1304, 2007.

[9] M. V. Clément and S. Pervaiz, "Intracellular superoxide and hydrogen peroxide concentrations: a critical balance that determines survival or death," *Redox Report*, vol. 6, no. 4, pp. 211–214, 2001.

[10] M. V. Clément, A. Ponton, and S. Pervaiz, "Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu," *FEBS Letters*, vol. 440, no. 1-2, pp. 13–18, 1998.

[11] C. Leblanc, O. Richard, B. Kloareg, S. Viehmann, K. Zetsche, and C. Boyen, "Origin and evolution of mitochondria: what have we learnt from red algae?" *Current Genetics*, vol. 31, no. 3, pp. 193–207, 1997.

[12] D. Stock, A. G. W. Leslie, and J. E. Walker, "Molecular architecture of the rotary motor in ATP synthase," *Science*, vol. 286, no. 5445, pp. 1700–1705, 1999.

[13] E. Cadenas, A. Boveris, C. I. Ragan, and A. O. M. Stoppani, "Production of superoxide radicals and hydrogen peroxide by NADH ubiquinone reductase and ubiquinol cytochrome c reductase from beef heart mitochondria," *Archives of Biochemistry and Biophysics*, vol. 180, no. 2, pp. 248–257, 1977.

[14] D. Han, E. Williams, and E. Cadenas, "Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space," *Biochemical Journal*, vol. 353, part 2, pp. 411–416, 2001.

[15] J. St-Pierre, J. A. Buckingham, S. J. Roebuck, and M. D. Brand, "Topology of superoxide production from different sites in the mitochondrial electron transport chain," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 44784–44790, 2002.

[16] E. B. Tahara, F. D. T. Navarete, and A. J. Kowaltowski, "Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation," *Free Radical Biology and Medicine*, vol. 46, no. 9, pp. 1283–1297, 2009.

[17] C. Piskernik, S. Haindl, T. Behling et al., "Antimycin A and lipopolysaccharide cause the leakage of superoxide radicals from rat liver mitochondria," *Biochimica et Biophysica Acta*, vol. 1782, no. 4, pp. 280–285, 2008.

[18] M. D. Brand, "The sites and topology of mitochondrial superoxide production," *Experimental Gerontology*, vol. 45, no. 7-8, pp. 466–472, 2010.

[19] P. Ježek and L. Hlavatá, "Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism," *International Journal of Biochemistry and Cell Biology*, vol. 37, no. 12, pp. 2478–2503, 2005.

[20] J. M. Li and A. M. Shah, "ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 14, no. 8, supplement 3, pp. S221–S226, 2003.

[21] K. Block, Y. Gorin, and H. E. Abboud, "Subcellular localization of Nox4 and regulation in diabetes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 34, pp. 14385–14390, 2009.

[22] J. Kuroda, T. Ago, S. Matsushima, P. Zhai, M. D. Schneider, and J. Sadoshima, "NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 35, pp. 15565–15570, 2010.

[23] T. Ago, J. Kuroda, J. Pain, C. Fu, H. Li, and J. Sadoshima, "Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes," *Circulation Research*, vol. 106, no. 7, pp. 1253–1264, 2010.

[24] R. Velaithan, J. Kang, J. L. Hirpara et al., "The small GTPase Rac1 is a novel binding partner of Bcl-2 and stabilizes its antiapoptotic activity," *Blood*, vol. 117, no. 23, pp. 6214–6226, 2011.

[25] A. K. Doughan, D. G. Harrison, and S. I. Dikalov, "Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction," *Circulation Research*, vol. 102, no. 4, pp. 488–496, 2008.

[26] A. A. Starkov, G. Fiskum, C. Chinopoulos et al., "Mitochondrial α-ketoglutarate dehydrogenase complex generates reactive oxygen species," *Journal of Neuroscience*, vol. 24, no. 36, pp. 7779–7788, 2004.

[27] H. Fang, M. Chen, Y. Ding et al., "Imaging superoxide flash and metabolism-coupled mitochondrial permeability transition in living animals," *Cell Research*, vol. 21, no. 9, pp. 1295–1304, 2011.

[28] W. Wang, H. Fang, L. Groom et al., "Superoxide Flashes in Single Mitochondria," *Cell*, vol. 134, no. 2, pp. 279–290, 2008.

[29] M. P. Mattson, "A reaction to mitochondria in action," *Cell Research*, vol. 21, no. 9, pp. 1279–1282, 2011.

[30] P. Ghafourifar and C. Richter, "Nitric oxide synthase activity in mitochondria," *FEBS Letters*, vol. 418, no. 3, pp. 291–296, 1997.

[31] E. N. Dedkova, X. Ji, S. L. Lipsius, and L. A. Blatter, "Mitochondrial calcium uptake stimulates nitric oxide production in mitochondria of bovine vascular endothelial cells," *American Journal of Physiology*, vol. 286, no. 2, pp. C406–C415, 2004.

[32] R. D. Rakhit, M. H. Mojet, M. S. Marber, and M. R. Duchen, "Mitochondria as targets for nitric oxide-induced protection during simulated ischemia and reoxygenation in isolated neonatal cardiomyocytes," *Circulation*, vol. 103, no. 21, pp. 2617–2623, 2001.

[33] J. J. Poderoso, M. C. Carreras, C. Lisdero, N. Riobó, F. Schöpfer, and A. Boveris, "Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles," *Archives of Biochemistry and Biophysics*, vol. 328, no. 1, pp. 85–92, 1996.

[34] A. Cassina and R. Radi, "Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport," *Archives of Biochemistry and Biophysics*, vol. 328, no. 2, pp. 309–316, 1996.

[35] M. W. Cleeter, J. M. Cooper, V. M. Darley-Usmar, S. Moncada, and A. H. Schapira, "Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide Implications for neurodegenerative diseases," *FEBS Letters*, vol. 345, no. 1, pp. 50–54, 1994.

[36] G. C. Brown, "Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase," *Biochimica et Biophysica Acta*, vol. 1504, no. 1, pp. 46–57, 2001.

[37] V. Borutaité and G. C. Brown, "Rapid reduction of nitric oxide by mitochondria, and reversible inhibition of

mitochondrial respiration by nitric oxide," *Biochemical Journal*, vol. 315, no. 1, pp. 295–299, 1996.

[38] Y. Takehara, T. Kanno, T. Yoshioka, M. Inoue, and K. Utsumi, "Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide," *Archives of Biochemistry and Biophysics*, vol. 323, no. 1, pp. 27–32, 1995.

[39] J. Torres, V. Darley-Usmar, and M. T. Wilson, "Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration," *Biochemical Journal*, vol. 312, no. 1, pp. 169–173, 1995.

[40] G. C. Brown, "Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase," *FEBS Letters*, vol. 356, no. 2-3, pp. 295–298, 1994.

[41] E. Nisoli, E. Clementi, C. Paolucci et al., "Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide," *Science*, vol. 299, no. 5608, pp. 896–899, 2003.

[42] R. Radi, A. Cassina, R. Hodara, C. Quijano, and L. Castro, "Peroxynitrite reactions and formation in mitochondria," *Free Radical Biology and Medicine*, vol. 33, no. 11, pp. 1451–1464, 2002.

[43] L. S. Burwell, S. M. Nadtochiy, and P. S. Brookes, "Cardioprotection by metabolic shut-down and gradual wake-up," *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 6, pp. 804–810, 2009.

[44] R. Radi, M. Rodriguez, L. Castro, and R. Telleri, "Inhibition of mitochondrial electron transport by peroxynitrite," *Archives of Biochemistry and Biophysics*, vol. 308, no. 1, pp. 89–95, 1994.

[45] M. A. Sharpe and C. E. Cooper, "Reactions of nitric oxide with mitochondrial cytochrome c: a novel mechanism for the formation of nitroxyl anion and peroxynitrite," *Biochemical Journal*, vol. 332, no. 1, pp. 9–19, 1998.

[46] J. J. Poderoso, M. C. Carreras, F. Schöpfer et al., "The reaction of nitric oxide with ubiquinol: kinetic properties and biological significance," *Free Radical Biology and Medicine*, vol. 26, no. 7-8, pp. 925–935, 1999.

[47] E. Clementi, G. C. Brown, M. Feelisch, and S. Moncada, "Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7631–7636, 1998.

[48] N. A. Riobó, E. Clementi, M. Melani et al., "Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation," *Biochemical Journal*, vol. 359, no. 1, pp. 139–145, 2001.

[49] V. Borutaite, A. Budriunaite, and G. C. Brown, "Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols," *Biochimica et Biophysica Acta*, vol. 1459, no. 2-3, pp. 405–412, 2000.

[50] C. Szabó and A. L. Salzman, "Endogenous peroxynitrite is involved in the inhibition of mitochondrial respiration in immune-stimulated J774.2 macrophages," *Biochemical and Biophysical Research Communications*, vol. 209, no. 2, pp. 739–743, 1995.

[51] J. P. Bolanos, S. J. R. Heales, J. M. Land, and J. B. Clark, "Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture," *Journal of Neurochemistry*, vol. 64, no. 5, pp. 1965–1972, 1995.

[52] I. Lizasoain, M. A. Moro, R. G. Knowles, V. Darley-Usmar, and S. Moncada, "Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose," *Biochemical Journal*, vol. 314, no. 3, pp. 877–880, 1996.

[53] Y. W. Xie and M. S. Wolin, "Role of nitric oxide and its interaction with superoxide in the suppression of cardiac muscle mitochondrial respiration: involvement in response to hypoxia/reoxygenation," *Circulation*, vol. 94, no. 10, pp. 2580–2586, 1996.

[54] C. Quijano, D. Hernandez-Saavedra, L. Castro, J. M. McCord, B. A. Freeman, and R. Radi, "Reaction of peroxynitrite with Mn-Superoxide dismutase. Role of the metal center in decomposition kinetics and nitration," *Journal of Biological Chemistry*, vol. 276, no. 15, pp. 11631–11638, 2001.

[55] H. L. A. Vieira, A. S. Belzacq, D. Haouzi et al., "The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal," *Oncogene*, vol. 20, no. 32, pp. 4305–4316, 2001.

[56] E. A. Konorev, N. Hogg, and B. Kalyanaraman, "Rapid and irreversible inhibition of creatine kinase by peroxynitrite," *FEBS Letters*, vol. 427, no. 2, pp. 171–174, 1998.

[57] P. Forsmark-Andrée, B. Persson, R. Radi, G. Dallner, and L. Ernster, "Oxidative modification of nicotinamide nucleotide transhydrogenase in submitochondrial particles: effect of endogenous ubiquinol," *Archives of Biochemistry and Biophysics*, vol. 336, no. 1, pp. 113–120, 1996.

[58] L. Castro, M. Rodriguez, and R. Radi, "Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide," *Journal of Biological Chemistry*, vol. 269, no. 47, pp. 29409–29415, 1994.

[59] M. Schweizer and C. Richter, "Peroxynitrite stimulates the pyridine nucleotide-linked Ca2+ release from intact rat liver mitochondria," *Biochemistry*, vol. 35, no. 14, pp. 4524–4528, 1996.

[60] N. Kuzkaya, N. Weissmann, D. G. Harrison, and S. Dikalov, "Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase," *Journal of Biological Chemistry*, vol. 278, no. 25, pp. 22546–22554, 2003.

[61] C. A. Piantadosi, "Carbon monoxide, reactive oxygen signaling, and oxidative stress," *Free Radical Biology and Medicine*, vol. 45, no. 5, pp. 562–569, 2008.

[62] A. Patenaude, M. R. Ven Murthy, and M. E. Mirault, "Mitochondrial thioredoxin system: effects of TrxR2 overexpression on redox balance, cell growth, and apoptosis," *Journal of Biological Chemistry*, vol. 279, no. 26, pp. 27302–27314, 2004.

[63] E. Naik and V. M. Dixit, "Mitochondrial reactive oxygen species drive proinflammatory cytokine production," *Journal of Experimental Medicine*, vol. 208, no. 3, pp. 417–420, 2011.

[64] A. B. Hwang and S. J. Lee, "Regulation of life span by mitochondrial respiration: the HIF-1 and ROS connection," *Aging*, vol. 3, no. 3, pp. 304–310, 2011.

[65] S. Krajewski, S. Tanaka, S. Takayama, M. J. Schibler, W. Fenton, and J. C. Reed, "Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes," *Cancer Research*, vol. 53, no. 18, pp. 4701–4714, 1993.

[66] I. C.C. Low, J. Kang, and S. Pervaiz, "Bcl-2: a prime regulator of mitochondrial redox metabolism in cancer cells," *Antioxidants and Redox Signaling*, vol. 15, no. 12, pp. 2975–2987, 2011.

[67] D. M. Hockenbery, Z. N. Oltvai, X. M. Yin, C. L. Milliman, and S. J. Korsmeyer, "Bcl-2 functions in an antioxidant

pathway to prevent apoptosis," *Cell*, vol. 75, no. 2, pp. 241–251, 1993.

[68] S. R. Chen, D. D. Dunigan, and M. B. Dickman, "Bcl-2 family members inhibit oxidative stress-induced programmed cell death in Saccharomyces cerevisiae," *Free Radical Biology and Medicine*, vol. 34, no. 10, pp. 1315–1325, 2003.

[69] D. J. Kane, T. A. Sarafian, R. Anton et al., "Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species," *Science*, vol. 262, no. 5137, pp. 1274–1277, 1993.

[70] N. Mirkovic, D. W. Voehringer, M. D. Story, D. J. McConkey, T. J. McDonnell, and R. E. Meyn, "Resistance to radiation-induced apoptosis in Bcl-2-expressing cells is reversed by depleting cellular thiols," *Oncogene*, vol. 15, no. 12, pp. 1461–1470, 1997.

[71] K. M. Myers, G. Fiskum, Y. Liu, S. J. Simmens, D. E. Bredesen, and A. N. Murphy, "Bcl-2 protects neural cells from cyanide/aglycemia-induced lipid oxidation, mitochondrial injury, and loss of viability," *Journal of Neurochemistry*, vol. 65, no. 6, pp. 2432–2440, 1995.

[72] N. Zamzami, I. Marzo, S. A. Susin et al., "The thiol crosslinking agent diamide overcomes the apoptosis-inhibitory effect of Bcl-2 by enforcing mitochondrial permeability transition," *Oncogene*, vol. 16, no. 8, pp. 1055–1063, 1998.

[73] L. T. Zhong, T. Sarafian, D. J. Kane et al., "bcl-2 inhibits death of central neural cells induced by multiple agents," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 10, pp. 4533–4537, 1993.

[74] M. Lee, D. H. Hyun, K. A. Marshall et al., "Effect of overexpression of Bcl-2 on cellular oxidative damage, nitric oxide production, antioxidant defenses, and the proteasome," *Free Radical Biology and Medicine*, vol. 31, no. 12, pp. 1550–1559, 2001.

[75] Z. X. Chen and S. Pervaiz, "BCL-2: pro-or anti-oxidant?" *Frontiers in Bioscience*, vol. 1, pp. 263–268, 2009.

[76] L. M. Ellerby, H. M. Ellerby, S. M. Park et al., "Shift of the cellular oxidation-reduction potential in neural cells expressing Bcl-2," *Journal of Neurochemistry*, vol. 67, no. 3, pp. 1259–1267, 1996.

[77] A. J. Kowaltowski and G. Fiskum, "Redox mechanisms of cytoprotection by Bcl-2," *Antioxidants and Redox Signaling*, vol. 7, no. 3-4, pp. 508–514, 2005.

[78] A. J. Kowaltowski, A. E. Vercesi, and G. Fiskum, "Bcl-2 prevents mitochondrial permeability transition and cytochrome c release via maintenance of reduced pyridine nucleotides," *Cell Death and Differentiation*, vol. 7, no. 10, pp. 903–910, 2000.

[79] M. C. Papadopoulos, I. L. Koumenis, L. Xu, and R. G. Giffard, "Potentiation of murine astrocyte antioxidant defence by bcl-2: protection in part reflects elevated glutathione levels," *European Journal of Neuroscience*, vol. 10, no. 4, pp. 1252–1260, 1998.

[80] J. S. Armstrong and D. P. Jones, "Glutathione depletion enforces the mitochondrial permeability transition and causes cell death in Bcl-2 overexpressing HL60 cells," *The FASEB Journal*, vol. 16, no. 10, pp. 1263–1265, 2002.

[81] Z. X. Chen and S. Pervaiz, "Bcl-2 induces pro-oxidant state by engaging mitochondrial respiration in tumor cells," *Cell Death and Differentiation*, vol. 14, no. 9, pp. 1617–1627, 2007.

[82] M. V. Clément, J. L. Hirpara, and S. Pervaiz, "Decrease in intracellular superoxide sensitizes Bcl-2-overexpressing tumor cells to receptor and drug-induced apoptosis independent of the mitochondria," *Cell Death and Differentiation*, vol. 10, no. 11, pp. 1273–1285, 2003.

[83] M. D. Esposti, I. Hatzinisiriou, H. McLennan, and S. Ralph, "Bcl-2 and mitochondrial oxygen radicals. New approaches with reactive oxygen species-sensitive probes," *Journal of Biological Chemistry*, vol. 274, no. 42, pp. 29831–29837, 1999.

[84] H. M. Steinman, "The Bcl-2 oncoprotein functions as a pro-oxidant," *Journal of Biological Chemistry*, vol. 270, no. 8, pp. 3487–3490, 1995.

[85] Z. X. Chen and S. Pervaiz, "Involvement of cytochrome c oxidase subunits Va and Vb in the regulation of cancer cell metabolism by Bcl-2," *Cell Death and Differentiation*, vol. 17, no. 3, pp. 408–420, 2010.

[86] A. J. Kowaltowski, R. G. Cosso, C. B. Campos, and G. Fiskum, "Effect of Bcl-2 overexpression on mitochondrial structure and function," *Journal of Biological Chemistry*, vol. 277, no. 45, pp. 42802–42807, 2002.

[87] I. C. C. Low, Z. X. Chen, and S. Pervaiz, "Bcl-2 modulates resveratrol-induced ROS production by regulating mitochondrial respiration in tumor cells," *Antioxidants and Redox Signaling*, vol. 13, no. 6, pp. 807–819, 2010.

[88] J. Li Campian, X. Gao, M. Qian, and J. W. Eaton, "Cytochrome c oxidase activity and oxygen tolerance," *Journal of Biological Chemistry*, vol. 282, no. 17, pp. 12430–12438, 2007.

[89] A. J. Lambert and M. D. Brand, "Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I)," *Journal of Biological Chemistry*, vol. 279, no. 38, pp. 39414–39420, 2004.

[90] G. E. Mann, B. Bonacasa, T. Ishii, and R. C. Siow, "Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones," *Current Opinion in Pharmacology*, vol. 9, no. 2, pp. 139–145, 2009.

[91] G. E. Mann, D. J. Rowlands, F. Y. L. Li, P. de Winter, and R. C. M. Siow, "Activation of endothelial nitric oxide synthase by dietary isoflavones: role of NO in Nrf2-mediated antioxidant gene expression," *Cardiovascular Research*, vol. 75, no. 2, pp. 261–274, 2007.

[92] X. Cheng, R. C. M. Siow, and G. E. Mann, "Impaired redox signaling and antioxidant gene expression in endothelial cells in diabetes: a role for mitochondria and the nuclear factor-E2-related factor 2-Kelch-like ECH-associated protein 1 defense pathway," *Antioxidants and Redox Signaling*, vol. 14, no. 3, pp. 469–487, 2011.

[93] S. K. Niture and A. K. Jaiswal, "Nrf2 up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis," *The Journal of Biological Chemistry*, vol. 287, no. 13, pp. 9873–9886, 2012.

[94] G. Kroemer, B. Dallaporta, and M. Resche-Rigon, "The mitochondrial death/life regulator in apoptosis and necrosis," *Annual Review of Physiology*, vol. 60, pp. 619–642, 1998.

[95] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[96] J. W. Kim and C. V. Dang, "Cancer's molecular sweet tooth and the warburg effect," *Cancer Research*, vol. 66, no. 18, pp. 8927–8930, 2006.

[97] K. C. Zimmermann, C. Bonzon, and D. R. Green, "The machinery of programmed cell death," *Pharmacology and Therapeutics*, vol. 92, no. 1, pp. 57–70, 2001.

[98] S. Marchi, C. Giorgi, J. M. Suski et al., "Mitochondria-ros crosstalk in the control of cell death and aging," *Journal of Signal Transduction*, vol. 2012, Article ID 329635, 17 pages, 2012.

[99] Z.-Y. Li, Y. Yang, M. Ming, and B. Liu, "Mitochondrial ROS generation for regulation of autophagic pathways in cancer,"

Biochemical and Biophysical Research Communications, vol. 414, no. 1, pp. 5–8, 2011.

[100] K. A. Ahmad, M. V. Clement, I. M. Hanif, and S. Pervaiz, "Resveratrol Inhibits Drug-Induced Apoptosis in Human Leukemia Cells by Creating an Intracellular Milieu Nonpermissive for Death Execution," *Cancer Research*, vol. 64, no. 4, pp. 1452–1459, 2004.

[101] K. A. Ahmad, M. V. Clement, and S. Pervaiz, "Pro-oxidant activity of low doses of resveratrol inhibits hydrogen peroxide—induced apoptosis," *Annals of the New York Academy of Sciences*, vol. 1010, pp. 365–373, 2003.

[102] M. V. Clément, S. Sivarajah, and S. Pervaiz, "Production of intracellular superoxide mediates dithiothreitol-dependent inhibition of apoptotic cell death," *Antioxidants and Redox Signaling*, vol. 7, no. 3-4, pp. 456–464, 2005.

[103] S. Pervaiz, J. K. Ramalingam, J. L. Hirpara, and M. V. Clément, "Superoxide anion inhibits drug-induced tumor cell death," *FEBS Letters*, vol. 459, no. 3, pp. 343–348, 1999.

[104] N. Ghosh, R. Ghosh, and S. C. Mandal, "Antioxidant protection: a promising therapeutic intervention in neurodegenerative disease," *Free Radical Research*, vol. 45, no. 8, pp. 888–905, 2011.

[105] M. G. Perrelli, P. Pagliaro, and C. Penna, "Ischemia/reperfusion injury and cardioprotective mechanisms: role of mitochondria and reactive oxygen species," *World Journal of Cardiology*, vol. 3, no. 6, pp. 186–200, 2011.

[106] G. Ferrin, C. I. Linares, and J. Muntane, "Mitochondrial drug targets in cell death and cancer," *Current Pharmaceutical Design*, vol. 17, no. 20, pp. 2002–2016, 2011.

[107] V. M. Victor, M. Rocha, C. Bañuls, L. Bellod, and A. Hernandez-Mijares, "Mitochondrial dysfunction and targeted drugs: a focus on diabetes," *Current Pharmaceutical Design*, vol. 17, no. 20, pp. 1986–2001, 2011.

[108] A. Chatterjee, S. Dasgupta, and D. Sidransky, "Mitochondrial subversion in cancer," *Cancer Prevention Research*, vol. 4, no. 5, pp. 638–654, 2011.

[109] T. Ishii, M. Miyazawa, P. S. Hartman, and N. Ishii, "Mitochondrial superoxide anion ($O_2^{\bullet -}$) inducible "mev-1" animal models for aging research," *BMB Reports*, vol. 44, no. 5, pp. 298–305, 2011.

[110] I. Al Ghouleh, N. K.H. Khoo, U. G. Knaus et al., "Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling," *Free Radical Biology and Medicine*, vol. 51, no. 7, pp. 1271–1288, 2011.

[111] J. L. Rains and S. K. Jain, "Oxidative stress, insulin signaling, and diabetes," *Free Radical Biology and Medicine*, vol. 50, no. 5, pp. 567–575, 2011.

[112] G. Serviddio, A. D. Romano, T. Cassano, F. Bellanti, E. Altomare, and G. Vendemiale, "Principles and therapeutic relevance for targeting mitochondria in aging and neurodegenerative diseases," *Current Pharmaceutical Design*, vol. 17, no. 20, pp. 2036–2055, 2011.

[113] M. E. Widlansky and D. D. Gutterman, "Regulation of endothelial function by mitochondrial reactive oxygen species," *Antioxidants and Redox Signaling*, vol. 15, no. 6, pp. 1517–1530, 2011.

[114] S.-D. Chen, D.-I. Yang, T.-K. Lin, F.-Z. Shaw, C.-W. Liou, and Y.-C. Chuang, "Roles of oxidative stress, apoptosis, PGC-1 and mitochondrial biogenesis in cerebral ischemia," *International Journal of Molecular Sciences*, vol. 12, no. 10, pp. 7199–7215, 2011.

[115] K. Hensley, M. L. Maidt, Z. Yu, H. Sang, W. R. Markesbery, and R. A. Floyd, "Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation," *Journal of Neuroscience*, vol. 18, no. 20, pp. 8126–8132, 1998.

[116] D. Allan Butterfield, A. Castegna, C. M. Lauderback, and J. Drake, "Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death," *Neurobiology of Aging*, vol. 23, no. 5, pp. 655–664, 2002.

[117] D. T. Dexter, C. J. Carter, F. R. Wells et al., "Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease," *Journal of Neurochemistry*, vol. 52, no. 2, pp. 381–389, 1989.

[118] W. A. Pedersen, W. Fu, J. N. Keller et al., "Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients," *Annals of Neurology*, vol. 44, no. 5, pp. 819–824, 1998.

[119] R. K. B. Pearce, A. Owen, S. Daniel, P. Jenner, and C. D. Marsden, "Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease," *Journal of Neural Transmission*, vol. 104, no. 6-7, pp. 661–677, 1997.

[120] E. Sofic, W. Paulus, K. Jellinger, P. Riederer, and M. B. H. Youdim, "Selective increase of iron in substantia nigra zona compacta of Parkinsonian brains," *Journal of Neurochemistry*, vol. 56, no. 3, pp. 978–982, 1991.

[121] K. A. Jellinger, E. Kienzl, G. Rumpelmaier et al., "Iron and ferritin in substantia nigra in Parkinson's disease," *Advances in neurology*, vol. 60, pp. 267–272, 1993.

[122] F. P. Zemlan, O. J. Thienhaus, and H. B. Bosmann, "Superoxide dismutase activity in Alzheimer's disease: possible mechanism for paired helical filament formation," *Brain Research*, vol. 476, no. 1, pp. 160–162, 1989.

[123] M. A. Pappolla, R. A. Omar, K. S. Kim, and N. K. Robakis, "Immunohistochemical evidence of antioxidant stress in Alzheimer's disease," *American Journal of Pathology*, vol. 140, no. 3, pp. 621–628, 1992.

[124] T. L. Perry, D. V. Godin, and S. Hansen, "Parkinson's disease: a disorder due to nigral glutathione deficiency?" *Neuroscience Letters*, vol. 33, no. 3, pp. 305–310, 1982.

[125] H. Warita, T. Hayashi, T. Murakami, Y. Manabe, and K. Abe, "Oxidative damage to mitochondrial DNA in spinal motoneurons of transgenic ALS mice," *Molecular Brain Research*, vol. 89, no. 1-2, pp. 147–152, 2001.

[126] J. Zhang, D. G. Graham, T. J. Montine, and Y. S. Ho, "Enhanced N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice deficient in CuZn-superoxide dismutase or glutathione peroxidase," *Journal of Neuropathology and Experimental Neurology*, vol. 59, no. 1, pp. 53–61, 2000.

[127] R. Resende, P. I. Moreira, T. Proença et al., "Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease," *Free Radical Biology and Medicine*, vol. 44, no. 12, pp. 2051–2057, 2008.

[128] B. Jagatha, R. B. Mythri, S. Vali, and M. M. S. Bharath, "Curcumin treatment alleviates the effects of glutathione depletion in vitro and in vivo: therapeutic implications for Parkinson's disease explained via in silico studies," *Free Radical Biology and Medicine*, vol. 44, no. 5, pp. 907–917, 2008.

[129] J. Chen, X. Q. Tang, J. L. Zhi et al., "Curcumin protects PC12 cells against 1-methyl-4-phenylpyridinium ion-induced apoptosis by bcl-2-mitochondria-ROS-iNOS pathway," *Apoptosis*, vol. 11, no. 6, pp. 943–953, 2006.

[130] S. Mishra, M. Mishra, P. Seth, and S. Kumar Sharma, "Tetrahydrocurcumin confers protection against amyloid β-induced toxicity," *NeuroReport*, vol. 22, no. 1, pp. 23–27, 2011.

[131] R. B. Mythri, G. Harish, S. K. Dubey, K. Misra, and M. M. Srinivas Bharath, "Glutamoyl diester of the dietary polyphenol curcumin offers improved protection against peroxynitrite-mediated nitrosative stress and damage of brain mitochondria in vitro: implications for Parkinson's disease," *Molecular and Cellular Biochemistry*, vol. 347, no. 1-2, pp. 135–143, 2011.

[132] R. Srividhya, K. Zarkovic, M. Stroser, G. Waeg, N. Zarkovic, and P. Kalaiselvi, "Mitochondrial alterations in aging rat brain: effective role of (-)-epigallo catechin gallate," *International Journal of Developmental Neuroscience*, vol. 27, no. 3, pp. 223–231, 2009.

[133] Y. Levites, O. Weinreb, G. Maor, M. B. H. Youdim, and S. Mandel, "Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration," *Journal of Neurochemistry*, vol. 78, no. 5, pp. 1073–1082, 2001.

[134] H.-K. Na and Y.-J. Surh, "Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG," *Food and Chemical Toxicology*, vol. 46, no. 4, pp. 1271–1278, 2008.

[135] M. P. Murphy, "Selective targeting of bioactive compounds to mitochondria," *Trends in Biotechnology*, vol. 15, no. 8, pp. 326–330, 1997.

[136] Y. Yamada, H. Akita, K. Kogure, H. Kamiya, and H. Harashima, "Mitochondrial drug delivery and mitochondrial disease therapy—an approach to liposome-based delivery targeted to mitochondria," *Mitochondrion*, vol. 7, no. 1-2, pp. 63–71, 2007.

[137] L. F. Yousif, K. M. Stewart, and S. O. Kelley, "Targeting mitochondria with organelle-specific compounds: strategies and applications," *ChemBioChem*, vol. 10, no. 12, pp. 1939–1950, 2009.

[138] L. F. Yousif, K. M. Stewart, K. L. Horton, and S. O. Kelley, "Mitochondria-penetrating peptides: sequence effects and model cargo transport," *ChemBioChem*, vol. 10, no. 12, pp. 2081–2088, 2009.

[139] S. K. Jain, S. N. Levine, J. Duett, and B. Hollier, "Reduced vitamin E and increased lipofuscin products in erythrocytes of diabetic rats," *Diabetes*, vol. 40, no. 10, pp. 1241–1244, 1991.

[140] S. K. Jain and R. McVie, "Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients," *Metabolism: Clinical and Experimental*, vol. 43, no. 3, pp. 306–309, 1994.

[141] A. Hernandez-Mijares, M. Rocha, N. Apostolova et al., "Mitochondrial complex i impairment in leukocytes from type 2 diabetic patients," *Free Radical Biology and Medicine*, vol. 50, no. 10, pp. 1215–1221, 2011.

[142] M. Brownlee, "Biochemistry and molecular cell biology of diabetic complications," *Nature*, vol. 414, no. 6865, pp. 813–820, 2001.

[143] F. K. Ahmad, Z. He, and G. L. King, "Molecular targets of diabetic cardiovascular complications," *Current Drug Targets*, vol. 6, no. 4, pp. 487–494, 2005.

[144] X. Du, D. Edelstein, S. Obici, N. Higham, M. H. Zou, and M. Brownlee, "Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation," *Journal of Clinical Investigation*, vol. 116, no. 4, pp. 1071–1080, 2006.

[145] E. Martín-Hernández, M. T. García-Silva, J. Vara et al., "Renal pathology in children with mitochondrial diseases," *Pediatric Nephrology*, vol. 20, no. 9, pp. 1299–1305, 2005.

[146] S. Krauss, C. Y. Zhang, L. Scorrano et al., "Superoxide-mediated activation of uncoupling protein 2 causes pancreatic β cell dysfunction," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1831–1842, 2003.

[147] A. Ceriello, A. Morocutti, F. Mercuri et al., "Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy," *Diabetes*, vol. 49, no. 12, pp. 2170–2177, 2000.

[148] A. Möllsten, S. L. Marklund, M. Wessman et al., "A functional polymorphism in the manganese superoxide dismutase gene and diabetic nephropathy," *Diabetes*, vol. 56, no. 1, pp. 265–269, 2007.

[149] M. L. Brezniceanu, F. Liu, C. C. Wei et al., "Catalase overexpression attenuates angiotensinogen expression and apoptosis in diabetic mice," *Kidney International*, vol. 71, no. 9, pp. 912–923, 2007.

[150] S. M. Haffner, S. Lehto, T. Rönnemaa, K. Pyörälä, and M. Laakso, "Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction," *The New England Journal of Medicine*, vol. 339, no. 4, pp. 229–234, 1998.

[151] C. F. Semenkovich, "Insulin resistance and atherosclerosis," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 1813–1822, 2006.

[152] T. Nishikawa, D. Edelstein, X. L. Du et al., "Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage," *Nature*, vol. 404, no. 6779, pp. 787–790, 2000.

[153] A. Ceriello and R. Testa, "Antioxidant anti-inflammatory treatment in type 2 diabetes," *Diabetes Care*, vol. 32, pp. S232–236, 2009.

[154] S. Blum, M. Vardi, J. B. Brown et al., "Vitamin e reduces cardiovascular disease in individuals with diabetes mellitus and the haptoglobin 2-2 genotype," *Pharmacogenomics*, vol. 11, no. 5, pp. 675–684, 2010.

[155] U. Milman, S. Blum, C. Shapira et al., "Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype: a prospective double-blinded clinical trial," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 2, pp. 341–347, 2008.

[156] K. Green, M. D. Brand, and M. P. Murphy, "Prevention of Mitochondrial Oxidative Damage As A Therapeutic Strategy in Diabetes," *Diabetes*, vol. 53, no. 1, pp. S110–S118, 2004.

[157] B. K. Chacko, C. Reily, A. Srivastava et al., "Prevention of diabetic nephropathy in $Ins2^{+/-AkitaJ}$ mice by the mitochondria-targeted therapy MitoQ," *Biochemical Journal*, vol. 432, no. 1, pp. 9–19, 2010.

[158] D. R. Green and J. C. Reed, "Mitochondria and apoptosis," *Science*, vol. 281, no. 5381, pp. 1309–1312, 1998.

[159] K. M. Debatin, D. Poncet, and G. Kroemer, "Chemotherapy: targeting the mitochondrial cell death pathway," *Oncogene*, vol. 21, no. 57, pp. 8786–8803, 2002.

[160] J. S. Armstrong, "Mitochondria: a target for cancer therapy," *British Journal of Pharmacology*, vol. 147, no. 3, pp. 239–248, 2006.

[161] V. Gogvadze, S. Orrenius, and B. Zhivotovsky, "Mitochondria in cancer cells: what is so special about them?" *Trends in Cell Biology*, vol. 18, no. 4, pp. 165–173, 2008.

[162] K. Radad, W. D. Rausch, and G. Gille, "Rotenone induces cell death in primary dopaminergic culture by increasing ROS production and inhibiting mitochondrial respiration," *Neurochemistry International*, vol. 49, no. 4, pp. 379–386, 2006.

[163] P. E. Lindahl and K. E. Öberg, "The effect of rotenone on respiration and its point of attack," *Experimental Cell Research*, vol. 23, no. 2, pp. 228–237, 1961.

[164] E. J. Wolvetang, K. L. Johnson, K. Krauer, S. J. Ralph, and A. W. Linnane, "Mitochondrial respiratory chain inhibitors induce apoptosis," *FEBS Letters*, vol. 339, no. 1-2, pp. 40–44, 1994.

[165] J. S. Isenberg and J. E. Klaunig, "Role of the mitochondrial membrane permeability transition (MPT) in rotenone-induced apoptosis in liver cells," *Toxicological Sciences*, vol. 53, no. 2, pp. 340–351, 2000.

[166] J. S. Isenberg, K. L. Kolaja, S. A. Ayoubi, J. B. Watkins, and J. E. Klaunig, "Inhibition of WY-14,643 induced hepatic lesion growth in mice by rotenone," *Carcinogenesis*, vol. 18, no. 8, pp. 1511–1519, 1997.

[167] J. S. Armstrong, B. Hornung, P. Lecane, D. P. Jones, and S. J. Knox, "Rotenone-induced G2/M cell cycle arrest and apoptosis in a human B lymphoma cell line PW," *Biochemical and Biophysical Research Communications*, vol. 289, no. 5, pp. 973–978, 2001.

[168] Y. T. Deng, H. C. Huang, and J. K. Lin, "Rotenone induces apoptosis in MCF-7 human breast cancer cell-mediated ROS through JNK and p38 signaling," *Molecular Carcinogenesis*, vol. 49, no. 2, pp. 141–151, 2010.

[169] W. G. Chung, C. L. Miranda, and C. S. Maier, "Epigallocatechin gallate (EGCG) potentiates the cytotoxicity of rotenone in neuroblastoma SH-SY5Y cells," *Brain Research*, vol. 1176, no. 1, pp. 133–142, 2007.

[170] S. Tada-Oikawa, Y. Hiraku, M. Kawanishi, and S. Kawanishi, "Mechanism for generation of hydrogen peroxide and change of mitochondrial membrane potential during rotenone-induced apoptosis," *Life Sciences*, vol. 73, no. 25, pp. 3277–3288, 2003.

[171] P. I. Moreira, J. Custódio, A. Moreno, C. R. Oliveira, and M. S. Santos, "Tamoxifen and estradiol interact with the flavin mononucleotide site of complex I leading to mitochondrial failure," *Journal of Biological Chemistry*, vol. 281, no. 15, pp. 10143–10152, 2006.

[172] A. Kallio, A. Zheng, J. Dahllund, K. M. Heiskanen, and P. Härkönen, "Role of mitochondria in tamoxifen-induced rapid death of MCF-7 breast cancer cells," *Apoptosis*, vol. 10, no. 6, pp. 1395–1410, 2005.

[173] T. A. Alston, L. Mela, and H. J. Bright, "3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 9, pp. 3767–3771, 1977.

[174] C. J. Coles, D. E. Edmondson, and T. P. Singer, "Inactivation of succinate dehydrogenase by 3-nitropropionate," *Journal of Biological Chemistry*, vol. 254, no. 12, pp. 5161–5167, 1979.

[175] L. S. Huang, G. Sun, D. Cobessi et al., "3-Nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme," *Journal of Biological Chemistry*, vol. 281, no. 9, pp. 5965–5972, 2006.

[176] A. Bacsi, M. Woodberry, W. Widger et al., "Localization of superoxide anion production to mitochondrial electron transport chain in 3-NPA-treated cells," *Mitochondrion*, vol. 6, no. 5, pp. 235–244, 2006.

[177] J. Wang, P. S. Green, and J. W. Simpkins, "Estradiol protects against ATP depletion, mitochondrial membrane potential decline and the generation of reactive oxygen species induced by 3-nitroproprionic acid in SK-N-SH human neuroblastoma cells," *Journal of Neurochemistry*, vol. 77, no. 3, pp. 804–811, 2001.

[178] L. F. Dong, P. Low, J. C. Dyason et al., "α-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II," *Oncogene*, vol. 27, no. 31, pp. 4324–4335, 2008.

[179] J. Neuzil, T. Weber, A. Schröder et al., "Induction of cancer cell apoptosis by α-tocopheryl succinate: molecular pathways and structural requirements," *The FASEB Journal*, vol. 15, no. 2, pp. 403–415, 2001.

[180] K. Wu, Y. Zhao, G. C. Li, and W. P. Yu, "c-Jun N-terminal kinase is required for vitamin E succinate-induced apoptosis in human gastric cancer cells," *World Journal of Gastroenterology*, vol. 10, no. 8, pp. 1110–1114, 2004.

[181] K. Wu, Y. Zhao, B. H. Liu et al., "RRR-α-tocopheryl succinate inhibits human gastric cancer SGC-7901 cell growth by inducing apoptosis and DNA synthesis arrest," *World Journal of Gastroenterology*, vol. 8, no. 1, pp. 26–30, 2002.

[182] W. Yu, Qiao Yin Liao, F. M. Hantash, B. G. Sanders, and K. Kline, "Activation of extracellular signal-regulated kinase and c-Jun-NH2-terminal kinase but not p38 mitogen-activated protein kinases is required for RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells," *Cancer Research*, vol. 61, no. 17, pp. 6569–6576, 2001.

[183] T. Weber, M. Lu, L. Andera et al., "Vitamin E succinate is a potent novel antineoplastic agent with high selectivity and cooperativity with tumor necrosis factorrelated apoptosis-inducing ligand (Apo2 ligand) in vivo," *Clinical Cancer Research*, vol. 8, no. 3, pp. 863–869, 2002.

[184] K. Kline, W. Yu, and B. G. Sanders, "Vitamin E and breast cancer," *Journal of Nutrition*, vol. 134, no. 12, supplement, pp. 3458S–3462S, 2004.

[185] W. Yu, B. G. Sanders, and K. Kline, "RRR-α-tocopheryl succinate-induced apoptosis of human breast cancer cells involves bax translocation to mitochondrial," *Cancer Research*, vol. 63, no. 10, pp. 2483–2491, 2003.

[186] X. F. Wang, L. Dong, Y. Zhao, M. Tomasetti, K. Wu, and J. Neuzil, "Vitamin E analogues as anticancer agents: lessons from studies with α-tocopheryl succinate," *Molecular Nutrition and Food Research*, vol. 50, no. 8, pp. 675–685, 2006.

[187] K. Nakayama, F. Okamoto, and Y. Harada, "Antimycin A: isolation from a new Streptomyces and activity against rice plant blast fungi," *The Journal of Antibiotics*, vol. 9, no. 2, pp. 63–66, 1956.

[188] M. L. Campo, K. W. Kinnally, and H. Tedeschi, "The effect of antimycin A on mouse liver inner mitochondrial membrane channel activity," *Journal of Biological Chemistry*, vol. 267, no. 12, pp. 8123–8127, 1992.

[189] A. Alexandre and A. L. Lehninger, "Bypasses of the antimycin A block of mitochondrial electron transport in relation to ubisemiquinone function," *Biochimica et Biophysica Acta*, vol. 767, no. 1, pp. 120–129, 1984.

[190] R. S. Balaban, S. Nemoto, and T. Finkel, "Mitochondria, oxidants, and aging," *Cell*, vol. 120, no. 4, pp. 483–495, 2005.

[191] K. R. You, J. Wen, S. T. Lee, and D. G. Kim, "Cytochrome c oxidase subunit III: a molecular marker for N-(4-hydroxyphenyl)retinamide-induced oxidative stress in hepatoma cells," *Journal of Biological Chemistry*, vol. 277, no. 6, pp. 3870–3877, 2002.

[192] J. M. Wu, A. M. DiPietrantonio, and T. C. Hsieh, "Mechanism of fenretinide (4-HPR)-induced cell death," *Apoptosis*, vol. 6, no. 5, pp. 377–388, 2001.

[193] X. Li, W. Ling, A. Pennisi, S. Khan, and S. Yaccoby, "Fenretinide inhibits myeloma cell growth, osteoclastogenesis and osteoclast viability," *Cancer Letters*, vol. 284, no. 2, pp. 175–181, 2009.

[194] S. Suzuki, M. Higuchi, R. J. Proske, N. Oridate, W. K. Hong, and R. Lotan, "Implication of mitochondria-derived reactive oxygen species, cytochrome C and caspase-3 in N-(4-Hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells," *Oncogene*, vol. 18, no. 46, pp. 6380–6387, 1999.

[195] H. J. Kim, N. Chakravarti, N. Oridate, C. Choe, F. X. Claret, and R. Lotan, "N-(4-hydroxyphenyl)retinamide-induced apoptosis triggered by reactive oxygen species is mediated by activation of MAPKs in head and neck squamous carcinoma cells," *Oncogene*, vol. 25, no. 19, pp. 2785–2794, 2006.

[196] N. Hail Jr., H. J. Kim, and R. Lotan, "Mechanisms of fenretinide-induced apoptosis," *Apoptosis*, vol. 11, no. 10, pp. 1677–1694, 2006.

[197] F. Formelli, A. B. Barua, and J. A. Olson, "Bioactivities of N-(4-hydroxyphenyl) retinamide and retinoyl β-glucuronide," *The FASEB Journal*, vol. 10, no. 9, pp. 1014–1024, 1996.

[198] J. G. Valero, L. Sancey, J. Kucharczak et al., "Bax-derived membrane-active peptides act as potent and direct inducers of apoptosis in cancer cells," *Journal of Cell Science*, vol. 124, no. 4, pp. 556–564, 2011.

[199] V. J. Venditto and E. E. Simanek, "Cancer therapies utilizing the camptothecins: a review of the in vivo literature," *Molecular Pharmaceutics*, vol. 7, no. 2, pp. 307–349, 2010.

[200] K. R. Hande, "Etoposide: four decades of development of a topoisomerase II inhibitor," *European Journal of Cancer*, vol. 34, no. 10, pp. 1514–1521, 1998.

[201] M. A. Jordan and L. Wilson, "Microtubules as a target for anticancer drugs," *Nature Reviews Cancer*, vol. 4, no. 4, pp. 253–265, 2004.

[202] D. Douer and M. S. Tollman, "Arsenic trioxide: new clinical experience with an old medication in hematologic malignancies," *Journal of Clinical Oncology*, vol. 23, no. 10, pp. 2396–2410, 2005.

[203] A. Petronellia, G. Pannitterib, and U. Testaa, "Triterpenoids as new promising anticancer drugs," *Anti-Cancer Drugs*, vol. 20, no. 10, pp. 880–892, 2009.

[204] A. Shehzad, F. Wahid, and Y. S. Lee, "Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials," *Archiv der Pharmazie*, vol. 343, no. 9, pp. 489–499, 2010.

[205] J. Kolesar, R. C. Brundage, M. Pomplun et al., "Population pharmacokinetics of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine) in cancer patients," *Cancer Chemotherapy and Pharmacology*, pp. 1–8, 2010.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 493894, 18 pages
doi:10.1155/2012/493894

Review Article

Health and Cellular Impacts of Air Pollutants: From Cytoprotection to Cytotoxicity

Karine Andreau, Melanie Leroux, and Aida Bouharrour

Laboratory of Molecular and Cellular Responses to Xenobiotics, Unit of Functional and Adaptive Biology (BFA), CNRS EAC 4413, University of Paris Diderot, Sorbonne Paris Cité, 75013 Paris, France

Correspondence should be addressed to Karine Andreau, karine.andreau@univ-paris-diderot.fr

Received 17 November 2011; Revised 17 January 2012; Accepted 18 January 2012

Academic Editor: Etienne Jacotot

Air pollution as one of the ravages of our modern societies is primarily linked to urban centers, industrial activities, or road traffic. These atmospheric pollutants have been incriminated in deleterious health effects by numerous epidemiological and *in vitro* studies. Environmental air pollutants are a heterogeneous mixture of particles suspended into a liquid and gaseous phase which trigger the disruption of redox homeostasis—known under the term of cellular oxidative stress—in relation with the establishment of inflammation and cell death *via* necrosis, apoptosis, or autophagy. Activation or repression of the apoptotic process as an adaptative response to xenobiotics might lead to either acute or chronic toxicity. The purpose of this paper is to highlight the central role of oxidative stress induced by air pollutants and to focus on the subsequent cellular impacts ranging from cytoprotection to cytotoxicity by decreasing or stimulating apoptosis, respectively.

1. Introduction

The air is fundamental and essential for living beings but epidemiological studies provide evidences of the harmful impacts of air pollution by increased cardiopulmonary morbidity and mortality as well as reproductive disorders and cancers [1, 2]. Some air toxics are released from natural sources but most are originated from anthropogenic sources, such as road traffic, construction, industrial, and agricultural activities [3]. Among almost two hundred hazardous air pollutants—mainly corresponding to suspended particulate matter and gases—only six are monitored by the Environmental Protection Agency (EPA) which sets the National Ambient Air Quality Standards (NAAQSs) for air particles, ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead (Table 1). In addition, some other air pollutants are subjected to a specific attention because of their deleterious health impacts, like asbestos, mercury, chlorofluorocarbons, and polycyclic aromatic hydrocarbons (PAHs) [4].

At the present time, air pollution is considered as a major inducer of harmful health effects, especially due to solids or liquid droplets suspended in air and termed particulate matter (PM). PMs are usually defined as PM_{10}, $PM_{2.5}$ and $PM_{0.1}$ that correspond to airborne particles with an aerodynamic diameter equal or less than 10, 2.5 and 0.1 microns, respectively. PM_{10} and $PM_{2.5}$ are often classified as the "coarse" fraction; $PM_{2.5}$–$PM_{0.1}$ as the "fine" fraction of particles (FP) and $PM_{0.1}$ correspond to the "ultrafine" fraction of particles (UFP). Although the transition from gasoline to diesel fuel has reduced emissions of carbon monoxide (CO), this has revealed new problems related to the emission of FP, UFP, and diesel exhaust particles (DEP). Engineered nanoparticles (NP), defined as particles having at least one dimension less than 100 nm, are in the same scale in size than atmospheric UFP, but NPs possess specific properties regarding their atomic scale capable of interacting directly with biological molecules. The surface parameter of PM is essential for understanding the biological effects of particulate pollution because size decrease is inversely correlated with the percentage of molecules on the surface and therefore with the surface reactivity. Thus, UFP and NP have a high surface reactivity that is responsible for the production of free radicals, for instance [5].

Table 1: Air pollutants. The National Ambient Air Quality Standards (NAAQSs) are set by the Environmental Protection Agency under authority of the Clean Air Act and define the maximum allowable concentrations of outdoor air pollutants in the USA. Units of NAAQS are parts per million (ppm) by volume, parts per billion (ppb-1 part in 1,000,000,000) by volume, milligrams per cubic meter of air (mg/m^3), and micrograms per cubic meter of air ($\mu g/m^3$). Average time refers to time for which the values of NAAQS should not be exceeded in the ambient air [14–16].

Pollutants	Main sources	NAAQS	
		Level	Average time
Asbestos	Electrical and building insulation (fiber cement)		
Carbon dioxide (CO_2)	Fossil fuel combustion		
Carbon monoxide (CO)	Incomplete combustion, exhaust from motor vehicles, emissions from certain industrial processes (agglomeration of ore, steel, waste incineration)	35 ppm (10 mg/m^3) 9 ppm (10 mg/m^3)	1 hour 8 hours
Chlorofluorocarbons (CFCs)	Use in consumer goods (aerosol propellants, foams, fire extinguishers, refrigerants)		
Dioxins and dioxin-like compounds	Byproducts of various industrial processes		
Polychlorinated dibenzo-p-dioxins (PCDDs)	Waste incineration, metal smelting and refining, chlorinated pesticides, and herbicides		
Polychlorinated dibenzofurans (PCDFs)	Environmental accidents contamination, waste incineration, chlorinated pesticides, and herbicides		
Polychlorinated biphenyls (PCBs)	Used as coolants and insulating fluids for transformers and capacitors, and as plasticizers in paints and cements, additives in flexible PVC coatings		
Hydrogen sulfide (H_2S)	Paper pulp production and oil refineries		
Methane (CH_4)	Coal mines exploitation, garbage landfills, livestock, gas distribution		
Nitrogen dioxide (NO_2)		100 ppb 53 ppb	1 hour Annual
Nitrogen monoxide (NO)			
Nitrogen oxides (NOx)	Fossil fuel combustion, industrial processes (nitric acid production, fertilizer manufacturing, surface treatment)		
Nitrous oxide (N_2O)	Fossil fuel combustion, some industrial processes, motor vehicles, soils, and oceans		
Ozone (O_3)	Tropospheric ozone formed from reaction between UV, NOx, and VOC	0.12 ppm (235 $\mu g/m^3$) 0.075 ppm (150 $\mu g/m^3$)	1 hour 8 hours
Particulate matter (PM)			
PM_{10}	Natural dust, sea salt, industrial, agriculture, and forestry activities	150 $\mu g/m^3$	24 hours
$PM_{2.5}$	Fossil fuel combustion, road traffic, and other transports, agriculture, and manufacturing	35 $\mu g/m^3$ 15.0 $\mu g/m^3$	24 hours Annual
$PM_{0.1}$	Residential heating, road transport, manufacturing, agriculture, waste processing plants		
Polycyclic aromatic hydrocarbons (PAHs)	Incomplete combustion of organic material (wood burning, fossil fuel combustion, etc.)		
Sulfur dioxide (SO_2)	Sulfur-containing fossil fuel combustion (coal, lignite, petroleum coke, heavy fuel oil, heating oil, diesel),	75 ppb 0.14 ppm (365 $\mu g/m^3$) 0.030 ppm (80 $\mu g/m^3$)	1 hour 24 hours Annual

Table 1: Continued.

Pollutants	Main sources	NAAQS	
		Level	Average time
Toxic metals			
Antimony (Sb)	miscellaneous plastics manufacturing, petroleum products, and fabricated structural metal products, thermal power generation		
Arsenic (As)	Heavy fuel oil combustion		
Cadmium (Cd)	Waste incineration, heavy fuel oil, and biomass burning		
Chromium (Cr)	Production of glass, cement, ferrous metallurgy, and foundries		
Cobalt (Co)	Nuclear facilities, production of steel and alloys		
Copper (Cu)	Combustion and waste treatment, processes of ferrous and nonferrous metallurgy		
Lead (Pb)	Road transport, electric batteries production	0.15 $\mu g/m^3$	Rolling 3 Months
Mercury (Hg)	Coal and oil burning, chlorine production, incineration of household, and industrial waste		
Nickel (Ni)	Heavy fuel oil combustion		
Selenium (Se)	Glass production, heavy fuel oil combustion		
Vanadium (V)	Oil refineries, combustion of fossil fuels		
Zinc (Zn)	Coal and heavy fuel combustion, ferrous and nonferrous metallurgy, waste incineration		
Volatile organic compounds (VOCs)	Road transport, industrial processes involving the use (basic and fine chemicals, metal degreasing, paint application, printing, adhesives, rubber, etc.), or not of solvents (petroleum refining, use of CFCs, production of alcoholic beverages), household products		

The chemical composition of PM varies according to environmental parameters (weather, continental, and/or regional influences) as well as to size. Indeed, PM_{10} are fragments from other larger particles observed during localized pollution episodes in urban areas and that may also include some pollen, spore, and plant. PM_{10} come from several sources like road transport, industrial, or construction activities [6] (Table 1). The urban aerosol mainly contains fine and ultrafine particles which consist of a core of elemental carbon from fossil fuel combustion and termed soots. Some inorganic components (ammonium, chloride, sulfates, nitrates, and metals), organic compounds—such as alkan, alkanoic acid, aliphatic acid, quinone, and PAHs—and biological species are adsorbed onto this carbon core [7].

The size of particles is directly linked to their deleterious potential on health. Indeed, FP, UFP, and DEP are inhaled with the air, penetrate deeply into the respiratory tract, and are mainly deposited in tracheobronchial and alveolar regions [5]. Industrial air pollution was clearly related to increased mortality and morbidity from respiratory and cardiovascular origin during episodes of heavily polluted fog (smog) in London in 1952 and the Ruhr in 1985 associated with 4,000 to 120,000 premature deaths, respectively, and an 8% increase in daily mortality. In addition, exposure to $PM_{2.5}$, PM_{10}, SO_2, and black smoke has been shown as being responsible for asthma exacerbation in both adults and children [8]. The International Agency for Research on Cancer (IARC) classifies DEP as a possible carcinogen (Group 2A) [9]. Thus, epidemiological studies show that occupational exposure of truck drivers is associated with an increased incidence of lung cancer [10]. Based on the statistical model of an American study, the French Agency of Environmental and Occupational Health Safety (AFSSET) estimated in 2002 that 1117 lung cancer deaths were caused by $PM_{2.5}$ exposure, a fraction of 11% [11]. Unlike the short-term effects linked to an inflammatory response, impacts of PM on carcinogenesis come from prolonged exposure. When mucociliary and alveolar clearance functions are exceeded, PM persist into lungs, leading to thickening of bronchial walls, an airway remodeling characterized by the hyperplasia of goblet and smooth muscle cells and a subepithelial fibrosis, as has been demonstrated in asthma and COPD (Chronic Obstructive Pulmonary Disease) [12]. Thereby, PM may act directly on the respiratory epithelium causing a range of various deteriorations to the total desquamation. To overcome this, the self-renewal of stem cells is accelerated, but proliferation and differentiation processes may escape to control and these adult lung stem cells are now considered as lung tumor initiators (see for review [13]). Given that the modulation of apoptotic cell death is an essential step in tumor initiation and promotion, this paper will focus on the molecular mechanisms of both induction and resistance

to apoptosis with a particular attention to mitochondria, the main executor (or executioner) of apoptosis.

2. Apoptosis

Since the remarkable scientific advances on the knowledge of cell death process, necrosis is not anymore considered as the only consequence of exposure to the toxic air pollutants. Indeed, impacts on activation or repression of apoptosis are now better described [17] and numerous *in vitro* studies demonstrated the modulation of apoptosis by environmental air pollutants including heavy or transition metals [18–20], carbon monoxide [21], and nondioxin-like PCBs (polychlorinated biphenyls) [22]. Apoptosis is a programmed cell death defined by morphological alterations (for review see [23]) leading to the progressive condensation of the cell into apoptotic bodies containing organelles or cytoplasmic fragments that are rapidly recognized and engulfed by neighboring cells and macrophages. The late-morphological changes are accompanied by biological alterations, such as the modification of lipid composition of the plasma membrane and permeability [24, 25], or the activation of various enzymes (e.g., phospholipase A2, DNase II, the caspase-activated DNase (CAD) [26], endonuclease G (EndoG) [27], the apoptosis-inducing factor (AIF) [28, 29]) leading to DNA fragmentation. All these apoptotic features may require activation of specific proteases called Caspases—for "cysteinyl aspartate-cleaving protease"—a family of proteins containing at least fourteen members in mammalians, eight of them actively participate to the execution of apoptosis while the others are involved in inflammation [30]. Under normal conditions, initiator Caspases-2, -8, -9, and -10 are expressed as inactive zymogens with a large N-terminal prodomain required for their autocatalytic activation into a tetrameric enzyme. When activated, the initiator caspase activates some executioner Caspases-3, -6, and -7, which in turn cleave specific substrates thus modifying multiple cellular functions such as DNA repair (DNA-PK, U1–70 kD, PARP), chromatin condensation (inhibitor of Caspase-Activated DNAse), or cytoskeleton stability (α-Fodrin, Lamin A, Actin); for review see [31].

The apoptotic cell death and caspases activation are mainly elicited by extrinsic and intrinsic pathways which are initiated by death receptors and intracellular events leading to mitochondrial dysfunction, respectively. In the extrinsic pathway, ligands of death receptors belonging to "tumour necrosis factor receptor" family (TNFR) promote formation of the multimolecular complex termed DISC (death-inducing signalling complex) [32] through the recruitment of adaptor proteins FADD (Fas-associated protein with death domain) and/or TRADD (TNF-receptor-associated death domain protein) and the subsequent activation of Caspase-8 and executive caspases.

In the intrinsic pathway (also called "mitochondrial pathway"), stimuli from different intracellular pathways (e.g., withdrawal of growth factors, exposure to toxins, hypoxia, bacterial or viral infections, physical or chemical stressors) converge on mitochondrial alterations that are the point of no return in apoptotic cell death. As reviewed elsewhere, permeabilization of outer and inner mitochondrial membranes (MMP) constitutes the limited and controlled step of the executive phase of apoptosis [33, 34]. In healthy cells, inner membrane (IM) is impermeable to protons in order to maintain the H^+ gradient necessary to oxidative phosphorylations (OXPHOS) and mitochondrial transmembrane potential ($\Delta\Psi m$). In dying cells, the IM's permeability increases to solutes less than 1.5 kDa and leads to the permeability transition caused by the opening of permeability transition pore complex (PTP) [35]. Despite a lack of consensus on the exact composition of the PTP, several proteins directly or indirectly constitute this complex such as the outer membrane- (OM-) inserted voltage-dependent anion channel (VDAC), the IM-located ANT (adenine nucleotide translocator), and the cyclophilin D in the mitochondrial matrix [36]. There is four isoforms of ANT (ANT 1–4) and three isoforms of VDAC (VDAC 1–3) that have antagonist effects on apoptosis, since VDAC1, ANT1, and ANT3 are proapoptotic proteins, while VDAC2, ANT2, and ANT4 are able to protect cells from death. In addition, several regulators interact closely with the PTP core proteins, in particular Hexokinase II, the translocator protein (TSPO), and Creatine Kinase which interact with VDAC in cytosol, outer membrane, and intermembrane space (IMS), respectively [35, 37, 38]. PTP opening can also be modulated *via* chemical modifications of PTP partners or through interaction with several pro- or antiapoptotic proteins. Indeed, thiols oxidation of ANT protein or proapoptotic members of the Bcl-2 family (i.e., Bax, Bak, and t-Bid) are well-known inducers of MMP [39, 40]. Generally, PTP opening and subsequent MMP results in $\Delta\Psi m$ dissipation associated with superoxide anion's production, swelling of the mitochondrial matrix as a consequence of the massive entry of water and solutes, and the release of many proapoptotic proteins from the IMS to the cytoplasm including Cytochrome *c*, Smac/DIABLO, and Omi/HtrA2 which participate to the caspase-dependent apoptotic pathway [41, 42]. Released Cytochrome *c* participates to the formation of a multiprotein complex termed "apoptosome" *via* physical interaction with the adaptor molecule Apaf-1 (apoptosis protease activating factor 1), the executioner procaspase-9, and ATP/dATP [43–46]. Furthermore, a caspase-independent apoptotic pathway can be activated by mitochondrial proteins AIF and EndoG that migrate towards nucleus to perform DNA fragmentation [33].

This mitochondrial central step may be positively or negatively modulated by Bcl-2 family proteins which mainly act through regulation of OM's permeabilization and Cytochrome *c* release. The Bcl-2 family contains three groups corresponding to prosurvival proteins (Bcl-2, Bcl-x_L , Bcl-w, Mcl-1, and A1/Bfl1), proapoptotic effectors (Bax, Bak), and a third subfamily called BH3-only proteins (Bad, Bik, Hrk, Bid, Bim, Bmf, Noxa, and Puma) which modulates activation of both first groups. The upregulation of Bcl-2 or other antiapoptotic members and/or the downregulation of Bax/Bak proteins have been reported to impair MMP and apoptosis underlying their fundamental role in the state of life *versus* cell death [47]. Thereby, Strasser et al. proposed a new mechanistic model of how Bax and Bak could promote

MMP, directly or indirectly; for review see [48]. Otherwise, the antiapoptotic proteins of the Bcl-2 family might inhibit cell death and MMP through two additional mechanisms: (i) the potential interaction with Apaf-1 protein leading to diminution of apoptosome formation or (ii) the direct inhibition of mitochondrial permeabilization. However the fact that Bcl-x_L might neutralize Apaf-1's function is still controversial, since data from different studies showing an inhibitory interaction between Bcl-x_L, Caspase-9, and Apaf-1 [49, 50] were rapidly refuted [51, 52]. Bcl-2 and Bcl-x_L are related to some bacterial proteins and data demonstrated their ability to form pores into membranes, to prevent the proton efflux triggered by calcium or reactive oxygen species (ROS) and the maintenance of mitochondrial ADP/ATP exchange [53, 54]. Bcl-2 also prevents excessive ROS production and impacts of the subsequent oxidative stress [55].

As a source and a cellular target of ROS, mitochondria regulate glucose metabolism, differentiation, or cell death and might play an important role in tumorigenesis. Currently, a new emerging concept considers mitochondrion as a ROS-signaling integrator [56, 57]. Mitochondrion is the site of OXPHOS by which ATP is formed by coupling with the electrons' transfer from a donor (NADH or FADH2) to the final acceptor oxygen of the mitochondrial respiratory chain. However, about 2% of the electrons escape from sites in complex I and/or complex III to react directly with oxygen thus generating superoxide anion ($O_2^{\bullet -}$) [56]. This leakage of electrons may appear in hypoxia conditions but could also participate under physiological conditions to various signaling pathways, since $O_2^{\bullet -}$, H_2O_2 (hydrogen peroxide) and $HO^{\bullet}$ (hydroxyl radical) are considered as intracellular messengers. Mitochondria seem to be the most potent intracellular source of ROS since the mitochondrial matrix concentration of $O_2^{\bullet -}$ was estimated to be 5- to 10-fold higher than that in the cytosol [58]. The Mn-superoxide dismutase (SOD) localized in the mitochondrial matrix rapidly dismutes $O_2^{\bullet -}$ in H_2O_2 which in turn can be decomposed by Catalase or may interact with $O_2^{\bullet -}$ by the Haber-Weiss reaction, or with Fe^{2+} (or Cu^{+}) by the Fenton reaction, leading to the generation of $HO^{\bullet}$. The mitochondrial ROS generation can be responsible for activation of death pathways by inducing (i) nuclear and mitochondrial DNA damages (i.e., formation of 8-hydroxydeoxyguanosine) leading to p53-dependent cell death [59], (ii) activation of signaling pathways involving NF-κB, JNK, or p38 MAPK [60, 61], (iii) MMP increase and Ca^{2+}-induced PTP opening [39, 62], and (iv) Cytochrome *c* release by oxidation of the anionic phospholipid cardiolipin [63, 64]. Nevertheless, mitochondrial ROS can also participate to protection against apoptosis by activation of antioxidant systems such as GSH (L-γ-glutamyl-L-cysteinylglycine) and multiple GSH-linked antioxidant enzymes (i.e., Gluthatione Peroxidases 1 and 4 [65, 66], Glutaredoxin 2 [67, 68], Glutathione S-transferase α [69]).

3. Air Pollutants and Cytotoxic Cell Death

Apoptosis is one of the possible consequences of acute or chronic exposure to air pollutants and various toxicants—such as PM, metals, and pesticides—are capable to target mitochondria, directly or indirectly [70–72]. For instance, rotenone which is used as pesticide inhibits the mitochondrial complex I. In addition, other pesticides such as pentachlorophenol and 2,4-dinitrophenol (DNP) induce cytotoxicity by uncoupling ATP synthesis and the mitochondrial H^+ gradient. Indeed, the small lipophilic molecule DNP captures and carries protons out of the IMS leading to the H^+ accumulation into the matrix and the disruption of the pH/H^+ gradient [73].

Although many studies describe the ability of air pollutants to trigger some characteristic features of apoptosis, only few detailed mechanistic studies have been published, the majority focusing solely on the oxidative stress emergence as a result of the unbalance between ROS production and activation of antioxidant defenses. Indeed, publications dealing with the cytotoxic consequences of airborne particles showed an induction of apoptosis associated with cellular stress, ROS production [74], $\Delta\Psi$m drop, caspases activation [75], and DNA fragmentation [70, 76, 77]. The cell death often demonstrated in experiments performed with PM in normal human lung tissue or airway epithelial cells was a mitochondria-mediated apoptosis, characterized by a marked reduction of mitochondrial dehydrogenase activity and the cytoprotective effects of mitochondrial inhibitors (e.g., rotenone, DIDS) [78, 79]. Cytochrome *c* release, activation of caspases-9 and -3, and PARP-1 cleavage were also observed after exposure to urban and industrial $PM_{2.5}$ in correlation with the induction of an oxidative stress studied by formation of 8-hydroxy-2′-desoxyguanosine (8-OHdG) [78, 80]. Thus, short-term exposure studies performed in respiratory cells with high doses of PM or its components led to a consensus that health effects as well as cytotoxic impacts of particulate pollution mainly involve ROS production and oxidative stress (Figure 1) [81, 82].

Increased production of ROS was first clearly identified as the central step of the proinflammatory response (GM-CSF, IL-6, IL-8, TNF-α) induced upon exposure to air pollutants *via* ROS-sensitive transcription factors such as NF-κB and AP-1 [82, 85]. Actually, particulate pollutants are considered as potent oxidants, and the induced intrinsic pathway of apoptosis may be associated with oxidative stress generated from organic (i.e., PAHs, nitro-PAHs/ketones/quinones) as well as inorganic compounds adsorbed on the surface of particles [84, 86–88]. Thus, PAHs induced-apoptosis is mainly mediated *via* the mitochondrial pathway (Caspase-3 activation, AIF, and EndoG release) in a p53-dependent manner in hepatic cells and macrophages [89–91], even if the causal relationship between genotoxic effects of BaP and induction of apoptosis is not established for all cell types [92]. Organic components are able to mimic the apoptogenic impact of PM in various cell types through activation of the aryl hydrocarbon receptor (AhR). AhR is a cytoplasmic ligand-dependent transcription factor which translocates to the nucleus in order to bind specific Xenobiotic Responsive Elements in target genes promoters, leading to activation of phase I and II metabolizing enzymes and contributing to detoxification. For instance, phase I enzymes such as cytochrome P450 oxidase 1A1 produce

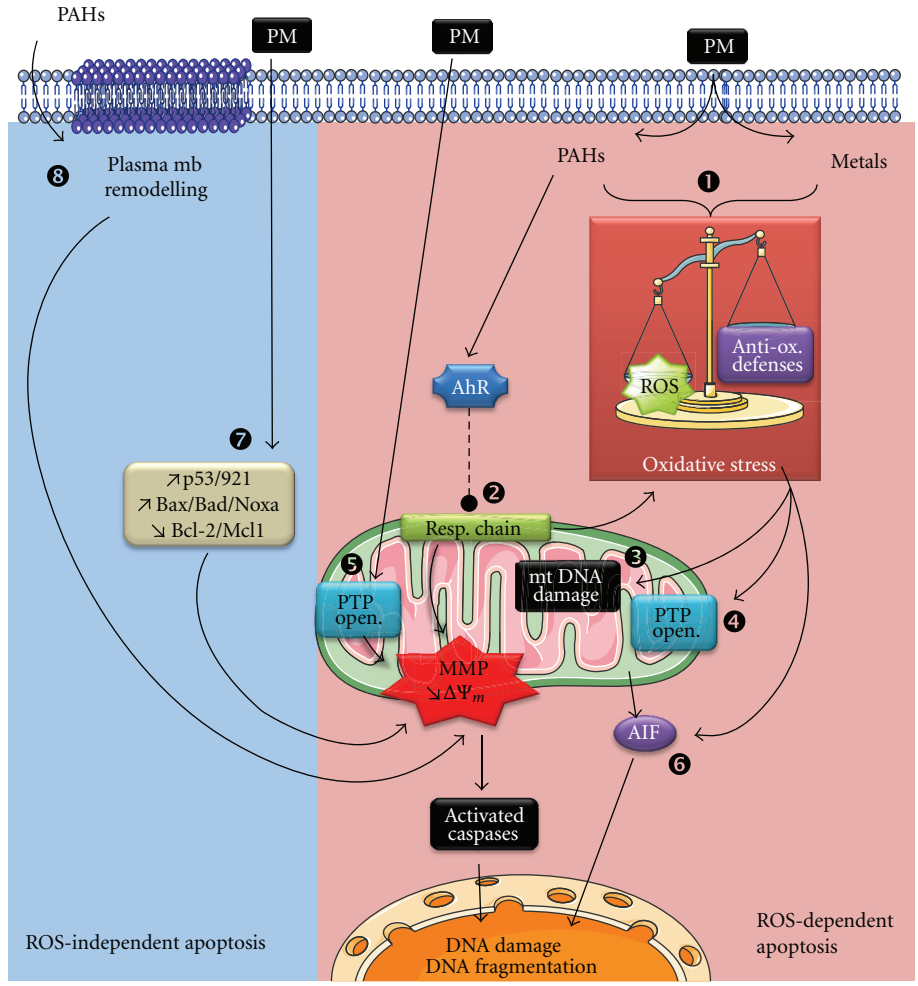


Figure 1: ROS-dependent and -independent apoptosis induced by particulate pollutants. Particulate matter (PM) or their compounds (PAHs and metals) provoke apoptotic cell death through ROS-dependent (pink zone) or ROS-independent (blue zone) pathways. Actually, particulate pollutants are considered as potent ROS generators from organic (i.e., PAHs) or metallic compounds (1) and leading to oxidative stress as the result of the unbalance between ROS production and activation of antioxidant defenses. Senft et al. demonstrated that AhR activation might regulate the mitochondrial respiratory chain function and induce production of $O_2^{\bullet -}$ and H_2O_2 from mitochondria ([83], (2)). As a consequence of oxidative stress, mitochondria are harmed by ROS that are responsible for damage of mitochondrial DNA (3), mitochondrial lipid peroxidation, and opening of PTP complex (PTP open. (4)). Mitochondrial membrane permeabilization (MMP) and PTP opening might also be a direct effect of diesel particles on isolated mitochondria ([84], (5)). As an additional pathway of the ROS-dependant apoptosis induced by air pollutants, the apoptogenic activity of AIF might be enhanced by xenobiotics, air pollutants, or their ROS derivatives (6). Some other ROS-independent signaling pathways have been identified such as the upregulation of proapoptotic proteins and/or the repression of prosurvival Bcl-2 family proteins (7). Recent publications also demonstrated a new mechanism of apoptosis triggered by PAHs through alterations of lipid rafts' composition and remodeling of the plasma membrane (8). Illustrations carried out thanks to Servier Medical Art.

H_2O_2 by metabolizing benzo(a)pyrene (BaP) [93] into a reactive intermediate (anti-7,8-dihydrodiol-9,10-epoxy-benzo(a)pyrene, BPDE) [94] known to trigger DNA damage and carcinogenesis [95]. *In vivo* studies performed in mice exposed to TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) showed an important oxidative response in correlation with the high affinity of the TCDD-AhR binding, suggesting a strong link between oxidative stress, Ah receptor, and its target genes CYP1A1 and 1A2 [96].

In this context, Senft et al. were interested to understand the involvement of AhR in mechanisms responsible for the dioxin-induced mitochondrial oxidative stress. These authors reported that liver mitochondria from mice exposed to TCDD (15 mg/kg) during three consecutive days showed a decrease of aconitase activity as a marker of mitochondrial ROS production, a mitochondrial generation of $O_2^{\bullet -}$ and H_2O_2 in the presence of succinate, as well as an activation of glutathione peroxidase-1 and glutathione reductases [83]. Interestingly, AhR knockout mice are protected from TCDD-induced ATP depletion, production of ROS, and the resultant oxidative stress response, suggesting that AhR might regulate the activity of mitochondrial electron transport

chain, especially the complexes III (stimulation) and IV (inhibition), and that the major source of the direct AhR-mediated oxidative stress response has a mitochondrial origin.

Furthermore, mitochondrion may be a site of ROS generation also in response to metallic environmental pollutants, as shown with cadmium or hexavalent chromium that trigger ROS production from the electron transfer chain as well as from the NADPH oxidase activity [97, 98]. In addition to organic compounds, heavy and transition metals such as vanadium, cadmium, mercury, lead, aluminum, titanium, chromium, iron, cobalt, nickel, copper, and zinc are often found in atmospheric pollutants and measured as adsorbed inorganic compounds on FP and UFP. Metals affect human health, especially when these toxicants compete with essential elements and thus modify many cellular processes [99]. Some metals are janus elements since they are both prominent inducers of ROS and essential cofactors for some antioxidant enzymes. As reviewed by Pulido and Parrish, transition metals promote apoptosis through ROS generation, mitochondria dysfunction, activation of MAPK, p53 and caspases, or downregulation of antiapoptotic proteins of Bcl-2 family [100]. Metals and the water-soluble fractions of PM are also known to cause inflammation and cancer mostly due to DNA damage as a consequence of ROS generation by Fenton reaction (see Section 2). Indeed, carcinogenic metals (i.e., As, Cd, Cr, Ni) promote apoptosis with DNA-base modifications, strand breaks and rearrangements [101]. Generation of ROS, decrease of intracellular GSH, accumulation of Ca^{2+}, loss of $\Delta\Psi m$, upregulation of Caspase-3, downregulation of Bcl-2, and deficiency of p53 protein led to arsenic-induced apoptosis [102, 103]. In case of cadmium, metallothionein expression determines the cell death fate (between apoptosis and necrosis), but Cd-induced apoptosis is due to inhibition of antioxidant enzymes, mitochondrial dysfunction [104], and PTP opening, probably through its interaction with thiol groups of ANT [105], whereas ROS and p53 contribute to apoptosis caused by chromium and selenium [106–108]. In addition, PM containing high levels of noncarcinogenic metals (i.e., cobalt, lead, iron, and zinc) were often shown to provoke ROS production (e.g., H_2O_2) leading to apoptosis through the mitochondrial pathway [109–113]. Under apoptotic conditions, zinc is also able to increase p53 expression and function probably by stabilizing this protein which contains a tightly bound zinc atom necessary for its DNA binding activity [114, 115].

As a consequence of oxidative stress, mitochondria are often damaged by ROS and experiments performed on HepG2 cells and liposomes clearly showed that $O_2^{\bullet -}$ alone seems to elicit apoptosis and a rapid and massive release of Cytochrome *c* independently of PTP opening but rather through a VDAC-dependent permeabilization of the outer mitochondrial membrane [116]. These results are in contradiction with those obtained by Xia et al. which performed, to the best of our knowledge, the only study of the prospective direct effect of Diesel particles on mouse liver-isolated mitochondria [84]. Xia et al. showed that the aromatic fraction of DEP can directly induce mitochondria swelling and depolarization leading to calcium overload in matrix [84]; this might be related to the massive decrease of the content in cardiolipin published earlier [117]. As mitochondrial swelling can be due to the long-lasting opening of PTP or the closure of VDAC, Xia et al. assessed the effect of the PTP inhibitor cyclosporine A (CsA) and demonstrated its ability to counteract DEP-induced swelling, suggesting that these organic compounds may directly promote PTP-mediated MMP [84]. In addition, oxidation of cardiolipin may be a crucial event of cell death triggered by xenobiotics as demonstrated by *in vivo* inhalation exposure to single-walled carbon nanotubes and *in vitro* experiments of LPS-induced apoptosis on pulmonary artery endothelial cells. Indeed, cardiolipin hydroperoxides and their hydroxy-derivatives are prominently accumulated in inflammatory, apoptotic, and oxidative stress conditions [118]. However, mitochondrial lipid peroxidation is not the only oxidative stress consequence responsible for mitochondria-driven apoptosis since $O_2^{\bullet -}$ and H_2O_2 can damage proteins as well as mitochondrial DNA. Mitochondrial DNA (mtDNA) is one of the main targets of mitochondrial ROS due to the close proximity of site production and the lack of protective histones. TCDD at low doses was shown to induce preferentially mitochondrial *versus* nuclear genotoxicity as assessed by 8-OHdG, reduction in mtDNA number, and increase in mtDNA deletions [119, 120].

As an additional pathway of the ROS-dependent apoptosis induced by air pollutants, the apoptogenic activity of AIF is controlled by the redox status as oxidized monomers have higher DNA affinity than NADH-reduced dimers [121]. Mitochondrial AIF is a NADH-dependent oxidoreductase containing a flavin adenine dinucleotide (FAD) and which is tethered to the inner membrane of mitochondria and participates to the caspase-independent pathway of apoptosis. However, a recent published data demonstrated that AIF has a quinone reductase activity that is around thousand-fold lower than Cytochrome P450 or NADPH : quinone oxidoreductase (NQO1) normally involved in the phases I and II of the xenobiotic detoxification processes [122]. This suggests that interaction of AIF with xenobiotics, air pollutants, or their ROS derivatives might promote its oxidized form and enhance its apoptogenic activity. Thereby, apoptosis induced by PM or wood smoke extracts on human alveolar macrophages and pulmonary artery endothelial cells was associated with AIF upregulation and its translocation to nucleus [123, 124]. In addition to the mitochondrial pathway, different studies also demonstrated that the extrinsic pathway of apoptosis (with TNF-α secretion, caspases-8 and -3 activation) is involved in the cytotoxic impacts of FP, UFP, and NP [125–127]. Data published in 2006 showed that apoptosis induced by fine particles in the lung epithelial cells has taken place in parallel with the induction of proliferation and that the two antagonistic phenomena appear to be induced by oxidative stress or EGFR [127, 128]. Finally, posttranslational oxidative modifications of proteins (i.e., nitrosylation, hydroxylation, glutathionylation of cystein residues) have been shown to promote aberrant activation of signal transduction cascades. Among these pathways, modulation of kinases ASK/JNK, Akt, or MAPK, or transcription factors Nrf2, NFκB, AP-1, and p53 was clearly

demonstrated following exposure to various environmental pollutants whose cellular effects are establishment of both an inflammatory and an apoptotic response [80, 129–132].

Through all examples cited previously, it is clear that cytotoxicity of air pollutants (PM, PAHs, metals, or herbicides) in various cells [70, 78, 133] mainly incriminates the excessive production of ROS capable of targeting mitochondria by different mechanisms (Figure 1) [83, 134]. At the same time as the ROS-dependent apoptosis, some other oxidative stress-independent signaling pathways have been identified. For instance, different PM were shown to upregulate the expression of potent regulators of the mitochondrial checkpoint such as p53 and its targets p21, Noxa, Bax, Bad, and Bim in parallel to the repression of Bcl-2 and Mcl-1 [70, 78, 125, 135]. Moreover, recent publications also demonstrated a new mechanism of apoptosis triggered by 1-Nitropyrene and BaP through lipid accumulation [136], alterations of plasma membrane microstructures, especially modulation of the Na^+/H^+ exchanger, inhibition of the gap junctional intercellular communication [137], and alteration of lipid rafts' composition [138]. These interesting outcomes emphasize the possible dialogue between plasma membrane alterations and cell death. Impacts on the plasma membrane remodeling might provide additional mechanistic explanations of how some chemicals exert their carcinogenic effect [25].

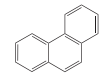
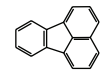
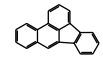
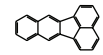
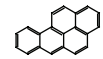
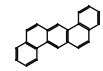
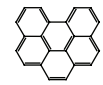
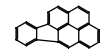
4. Cytoprotective Effects of Air Pollutants

Since mid-twentieth century, the incidence in lung cancer had rapidly increased and, in addition to cigarette smoke, indoor and outdoor air pollution was also questioned through epidemiological studies. The earliest studies underlined the higher incidence of lung cancer in urban *versus* rural areas, in relation to the nature or concentration of airborne particles. The IARC classified several air pollutants as human carcinogens (group 1, e.g., BaP, Chromium VI compounds, tobacco smoking), probably carcinogens (group 2A, e.g., dibenzo(a,h)anthracene, engine diesel exhaust, lead compounds), possibly carcinogens (group 2B, e.g., benzo(k)fluoranthene, gasoline exhaust, lead), not classifiable as to its carcinogenicity to humans (group 3, e.g., benzo(g,h,i)perylene, chromium III compounds), or probably not carcinogenic to humans (group 4) [9, 139, 140]. In particular, extracts of PM, BaP, and 1,6-dinitropyrene provoke DNA adducts, mutagenic effects in bacterial and mammalian cells, chromosomal damages, and cell transformation. Most of human cancers are carcinoma derived from epithelial cells, and in the case of lung cancers, the most common are squamous cell carcinoma (epidermoid carcinoma), large cell carcinoma, small cell carcinoma, and adenocarcinoma [141]. All cancerous cells have acquired six capabilities during the multistage process of tumorigenesis: (i) self-sufficiency in growth signals, (ii) insensitivity to growth-inhibitory signals leading to (iii) limitless replicative potential, (iv) evasion to apoptotic cell death, (v) sustained angiogenesis, and (vi) tissue invasion and metastasis [142]. During chronic exposure to air pollutants, acquisition of resistance towards apoptosis might be a significant step of the molecular mechanisms involved in the initiation and promotion of tumors as demonstrated by Teranishi et al. [143]. Unfortunately, too few toxicological studies have investigated this phenomenon of apoptosis resistance induced possibly by air pollutants and have tried to decipher in detail the underlying molecular mechanisms. In order to delineate mechanisms leading to lung cancers due to exposure to cooking oil fumes (COFs) and its main aldehyde component 2,4-decadienal (2,4-DDE), Hung et al. reported that these pollutants promote survival and proliferation of alveolar cancer cells A549 through increased expression of IAP1, IAP2, Survivin, and Cyclin D1 and the concomitant decrease of XIAP, Caspase-3, and p21 level [144].

As described previously, particulate pollutants or their components may induce cytotoxicity following high-concentrations exposure. Ferecatu et al. initiated studies to determine the specific effect of low doses of airborne particles on different bronchial epithelial cells (tumoral, immortalized, and primary cells) regarding induction or reduction of the apoptotic process. The authors demonstrated that $PM_{2.5}$ are not cytotoxic but rather trigger a resistance to mitochondrial apoptosis towards well-known cell death inducers (calcimycin (A23187), staurosporine, and oligomycin) [145]. The reduction of apoptosis observed after particle exposure is not related to the proinflammatory response and EGF pathway but is mediated by water-soluble as well as organic components such as heavy PAHs. Among all the water-soluble compounds of PM, a possible candidate responsible for the cytoprotective effect is zinc (Zn), already known to inhibit apoptosis and minimize the oxidative stress (e.g., lipid peroxidation) [146]. Zn may protect cells both directly by stabilizing lipids and proteins of cellular and organelles membranes and indirectly *via* the maintenance of glutathione levels [147]. The protective effect of Zn was also assigned to the reduction of DNA fragmentation, processing of procaspase-3 [148] and activation of cytoprotective signaling pathways (including Akt, ERK). In addition, some divalent transition metals (Mg^{2+}, Sr^{2+}, and Mn^{2+}) can competitively inhibit the calcium-induced PTP opening through a still-unknown mechanism, suggesting a possible direct and protective effect of metallic compounds on mitochondria [149].

Several studies have reported the cytoprotective effects of organic compounds of PM such as PAHs [145, 150] or their metabolites [143, 151], TCDD [152], non-Dioxin-like PCBs [153], and DEP [154]. The exact mechanisms of apoptosis inhibition are not fully understood but *in vitro* studies have shown the necessity of protein synthesis [155], p53 modulation [153, 156], and AhR activation [145]. Indeed, exposure of human bronchial epithelial cells for 4 hours with different PAHs, prior to A23187-treatment, showed a marked resistance to mitochondria-driven apoptosis only with PAHs containing at least five-aromatic rings, which are the most toxic and potent inducers of receptor Ah (Table 2) [157]. The need of protein synthesis and AhR activation might be linked to the transcription factor function of AhR that could either induce antiapoptotic genes or inhibit proapoptotic ones. This assumption is based on results which have shown

Table 2: The cytoprotective effect of $PM_{2.5}$ is related to PAHs with five-aromatics rings. Epithelial 16 HBE cells were pretreated during 4 hours with phenanthrene (124 nM), fluoranthene (268 nM), benzo(b)fluoranthene (333 nM), benzo(k)fluoranthene (333 nM), benzo(a)pyrene (270 nM), dibenzo(a,h)anthracene (35 nM), benzo(g,h,i)perylene (443 nM), and indeno(1,2,3-cd)pyrene (217 nM) prior to induction of apoptosis by A23187 (3 μM) for 20 supplementary hours. Results are mean ± SD ($n = 6$). Significance was calculated with Dunnett's test ($^*P < 0.01$ versus vehicule cyclohexane 1%). Percentages of DiOC low and PI high refer to cells showing either a drop of $\Delta\Psi$m or a permeabilization of the plasma membrane measured using DiOC and propidium iodide (PI) probes, respectively. Note that a 4 h vehicle pretreatment provides 93.00 ± 3.31% DiOC low and 96.97 ± 7.18% PI high of A23-induced apoptosis, respectively. Moreover, a 4 h $PM_{2.5}$ exposure (10 $\mu g/cm^2$) provides 64.33 ± 9.89% DiOC low and 39.45 ± 8.50% PI high of A23-induced apoptosis, respectively. The relative toxic potency of individual PAH compared to BaP is given as the toxic equivalency factor (TEF).

PAHs	TEF	Structure	A23187-induced apoptosis (% of control)	
			DiOC low	PI high
Phenanthrene	0.001–0.01		88.96 ± 5.00	94.43 ± 6.33
Fluoranthene	0.001–0.05		86.22 ± 7.13	87.37 ± 10.79
Benzo(b)fluoranthene	0.06–0.14		91.17 ± 3.62	99.17 ± 8.41
Benzo(k)fluoranthene	0.03–0.1		96.41 ± 6.56	104.09 ± 11.45
Benzo(a)pyrene	1		70.27 ± 5.60*	34.55 ± 3.68*
Dibenzo(a,h)anthracene	0.69–5		80.69 ± 1.26*	80.10 ± 5.37*
Benzo(g,h,i)perylene	0.01–0.03		75.49 ± 2.94*	75.91 ± 3.31*
Indeno(1,2,3-cd)pyrene	0.017–0.232		79.38 ± 4.43*	80.24 ± 10.38*

that AhR directly interacts with E2F1 leading to the reduction of E2F1-mediated proapoptotic genes such as *apaf-1* [158]. Moreover, the cytoprotective effect of AhR ligands is effective at the mitochondrial checkpoint of apoptosis by upregulating expression of antiapoptotic genes such as *bcl-2*, *bcl-x_L*, *mcl-1*, *agr2*, or *vdac2* [159–161].

Signaling pathways other than AhR were also found in the cytoprotective effects of air pollutants, especially protein kinases (e.g., Akt [162], ERK [163], JNK, PKA [164]), or the transcription factor Nrf2 (nuclear factor (erythroid-derived 2)-like 2). PI3K/Akt and AhR pathways seem to be interdependent for the cellular response to xenobiotics, since the presence of AhR is required for the cytoprotective function of Akt [165]. In addition, the antiapoptotic effect of DEP and BPDE (a BaP genotoxic metabolite) was linked to phosphorylation and activation of Akt [151], with the possible involvement of Thioredoxin-1 as demonstrated *in vivo* [166]. Furthermore, Akt promotes type II Hexokinase (HK II) phosphorylation and binding to the OM leading to the stabilization of PTP complex in closed conformation and the inhibition of Ca^{2+}-induced Cytochrome *c* release. Akt-dependent phosphorylation of mitochondrial HK II is further favored when glycogen synthase kinase 3β (GSK3β) is inactivated by Akt phosphorylation [167]. Finally, activation of NF-κB and AP-1 by exposure to airborne PM or cigarette smoke is well documented and admitted as being part of

inflammatory or proliferative response [168, 169], but their role in the modulation of apoptotic cell death remains unclear.

As recently reviewed, air pollutants are also inductors of Nrf2 which regulates the expression of phase II detoxifying enzymes as well as cytoprotective antioxidants [170]. Under normal conditions, Nrf2 is a cytoplasmic protein linked to its repressor Keap1 (Kelch-like ECH-associated protein) but exposure to low levels of electrophiles and ROS causes the nuclear translocation of Nrf2 and the subsequent expression of target genes containing antioxidant response elements (ARE). Indeed, numerous published studies have reported activation of Nfr2 after exposure of murine macrophages or human bronchial epithelial cells to DEP, PAHs, and UFP [81, 171, 172]. Among the antiapoptotic target proteins of Nfr2, two antioxidant enzymes are of particular interest because of their location and action on the mitochondria: Heme Oxygenase-1 (HO-1) and Glutathione S-transferase (GST) isoenzymes. Expression and activity of HO-1 are dramatically increased in mitochondrial fractions of human alveolar and bronchial cells exposed to cigarette smoke extract as an attempt to counteract its toxic effects, since the overexpression of HO-1 inhibits cell death and maintains ATP levels [173]. The cytoprotective mechanism of HO-1 involves its enzymatic reaction products such as biliverdin, carbon monoxide (CO), and ferrous iron [174]. In response to Fe^{2+} production, ferritin protein stability is increased and may protect cells from oxidative and Fas-induced apoptosis. However, biliverdin and free iron do not have any protective activity against oxidative stress-induced hepatic apoptosis, suggesting that CO may be the key molecule [175]. Indeed, CO protects against oxidative injuries and cell death, since in addition to limiting the translocation of Bcl-2 family proteins to mitochondria and the Cytochrome *c* release, Queiroga et al. proposed a new cytoprotective effect of CO directly on mitochondria [176]. They observed that a fifteen-minutes pretreatment with low doses of CO was able to prevent the calcium-induced swelling and depolarization of liver isolated mitochondria. Otherwise, GST enzymes are involved in detoxification of endogenous toxic metabolites, superoxide radicals, and xenobiotics. Cytosolic and mitochondrial GST (α, μ, π, and θ) were shown to be upregulated by numerous xenobiotics and some AhR ligands (TCDD, β-naphthoflavone) [177]. However, effect of mitochondrial GST on the direct control of this organelle is still subject of controversy: on the one hand, these enzymes are assumed to prevent cardiolipin oxidation and MMP [178], while on the other hand, Aniya's team demonstrated that the mitochondrial membrane-bound GST1 is activated by S-glutathionylation and contributes to Cytochrome *c* release through PTP opening [179].

ROS are possibly involved in tumor progression, metastasis, and multiple signaling pathways elicited by atmospheric xenobiotics. The correlation of intracellular oxidation—due to light-modified derivatives of BaP (BaP-1,6-dione, BaP-3,6-dione, BaP-4,5-dihydrodiol and 2-hydroxy-BaP-1,6-dione)—with the protection against serum withdrawal-induced apoptosis has clearly suggested that a certain dose of ROS enhances cell proliferation and survival [143].

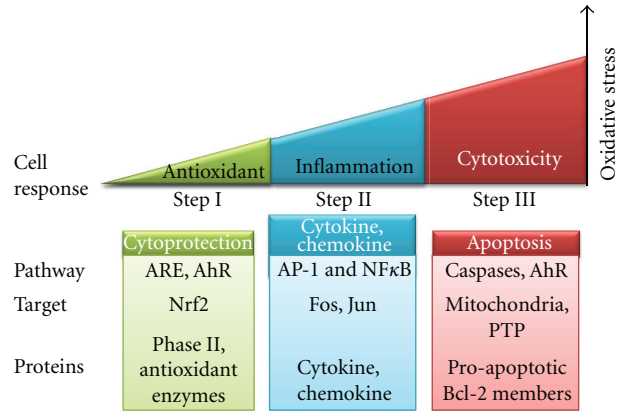


Figure 2: Hierarchical oxidative stress model in response to air pollutants. Low ROS production (Step I) induces activation of cellular antioxidant systems to restore redox homeostasis. If this protection is insufficient, the increased stress (Step II) triggers an inflammatory response through MAPK and NFκB pathways. At a final stage (Step III), all the defense systems are overwhelmed and high ROS levels lead to PTP opening and cell death by apoptosis. These responses depend on the pro/antioxidant balance which varies from one living being to another (adapted from [180]).

Under physiological conditions, cell viability is critically dependent on the maintenance of functional antioxidant systems especially in lung cells which are regularly exposed to air pollutants. GSH is one of the most abundant antioxidant found in the extracellular epithelial lining fluid and into respiratory cells from trachea to alveoli [181]. According to this preponderant role of GSH, administration of antioxidants, such as N-acetylcysteine (NAC), effectively prevents air pollutant-induced apoptosis [126, 171, 182] and *in vivo* exposure to dioxin increases levels of mitochondrial glutathione in wt, *cyp1a1* $^{-/-}$ and *cyp1a2* $^{-/-}$ mice, but not in *ahr* $^{-/-}$ mice [83].

5. Conclusion

In conclusion, impacts of air pollutants are mainly related to the redox status and the mitochondrial function of target cells. As described in this paper, proteins and signaling pathways involved in both cytoprotective and cytotoxic effects converge to the pro/antioxidant balance which determines the cellular response to environmental aggressions according to the hierarchical model proposed by Li et al. [180] (see Figure 2). Exposure to environmental pollutants may result in cellular disorders responsible for tissue damage and is therefore perceived as a cellular stress. A minor stress will induce a cellular response characterized by metabolic, morphological, or signaling alterations in order to deal with it. This phenomenon, termed adaptation, involves several processes such as hypertrophy, hyperplasia, or atrophy of the cells. Persistent exposures may also result in metaplasia (replacement of one by another cell type as is the case for the ciliated columnar cells that are replaced by squamous cells in the cigarette smokers' epithelium). In case of severe

injuries, adaptative processes are overwhelmed leading to cell death by necrosis, apoptosis, or autophagy. Air pollutants can directly affect the respiratory epithelium and cause transient damages (i.e., loss of cilia and tight junctions) until total desquamation. Thus, cellular adaptation allows tissue remodeling required for the repair/regeneration of the damaged lung epithelium.

In this context, the cytoprotective effects demonstrated for some xenobiotics might reflect the setting up of an adaptative mechanism converging to the inhibition of cell death deleterious for the lung tissue. However, as it was well described by Barouki [183], some adaptative mechanisms necessary to inhibit direct toxicity may have side effects that accumulate during repeated exposures. A plausible assumption for the exacerbation of lung cancers would be that resistance to apoptosis might occur into self-renewing stem cells known to be involved in physiological regeneration of the epithelium and suspected of being initiators of lung tumors [13].

Despite the latest significant advances, the specific mechanisms responsible for cytotoxic effects of air pollutants remain to be deciphered in more detail, mostly on the issues of chronic as well as multiple exposures to low concentrations of pollutants. Moreover, understanding the adaptive cytoprotective process is an important issue that should be considered in the risk assessment of air pollution.

Abbreviations

2,4-DDE: 2,4-decadienal
8-OHdG: 8-hydroxy-2′-desoxyguanosine
A23187: Calcium ionophore (calcimycin)
AFSSET: French Agency of Environmental and Occupational Health Safety
AhR: Aryl hydrocarbon receptor
AIF: Apoptosis-inducing factor
ANT: Adenine nucleotide translocator
Apaf-1: Apoptosis protease activating factor 1
ARE: Antioxidant response elements
As: Arsenic
BaP: Benzo(a)pyrene
BPDE: anti-7,8-dihydrodiol-9,10-epoxy-benzo(a)pyrene4
CAD: Caspase-activated DNase
Cd: Cadmium
CFCs: Chlorofluorocarbons
CH_4: Methane
CO: Carbon monoxide
Co: Cobalt
CO_2: Carbon dioxide
COF: Cooking oil fumes
COPD: Chronic obstructive pulmonary disease
Cr: Chromium
CsA: Cyclosporine A
Cu: Copper
DEP: Diesel exhaust particles
DIDS: 4,4-diisothiocyanatostilbene-2,2′disulfonic acid
DiOC: 3, 3 dihexyloxacarbocyanine iodide
DISC: Death-inducing signalling complex
DNP: 2,4-dinitrophenol
EGFR: Epidermal growth factor receptor
EndoG: Endonuclease G
EPA: Environmental Protection Agency
FAD: Flavin adenine dinucleotide
FADD: Fas-associated protein with death domain
FP: Fine particles
GSH: L-γ-glutamyl-L-cysteinylglycine
GSK3β: Glycogen synthase kinase 3β
GST: Glutathione S-transferase
H_2O_2: Hydrogen peroxide
H_2S: Hydrogen sulfide
Hg: Mercury
HK II: Hexokinase type II
$HO^{\bullet}$: Hydroxyl radical
HO-1: Heme oxygenase-1
IAP1: Inhibitors of apoptosis
IARC: International Agency for Research on Cancer
IM: Mitochondrial inner membrane
IMS: Mitochondrial intermembrane space
Keap1: Kelch-like ECH-associated protein
MMP: Mitochondrial membranes permeabilization
mtDNA: Mitochondrial DNA
N_2O: Nitrous oxide
NAAQS: National Ambient Air Quality Standards
NAC: N-acetylcysteine
Ni: Nickel
NO: Nitrogen monoxide
NO_2: Nitrogen dioxide
NOx: Nitrogen oxides
NP: Nanoparticles
NQO1: NADPH : quinone oxidoreductase
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
$O_2^{\bullet -}$: Superoxide anion
O_3: Ozone
OM: Mitochondrial outer membrane
OXPHOS: Oxidative phosphorylations
PAHs: Polycyclic aromatic hydrocarbons
PARP-1: Poly (ADP-ribose) polymerase 1
Pb: Lead
PCBs: Polychlorinated biphenyls
PCDDs: Polychlorinated dibenzo-p-dioxins
PCDFs: Polychlorinated dibenzofurans
PI: Propidium iodide
PM: Particulate matter
ppb: parts per billion
ppm: Parts per million
PTP: Permeability transition pore complex
ROS: Reactive oxygen species
Sb: Antimony
Se: Selenium
SO_2: Sulfur dioxide
SOD: Mn-superoxide dismutase
TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF: Toxic equivalency factor

TNFR: Tumour necrosis factor receptor
TRADD: TNF-receptor-associated death domain protein
TSPO: Translocator protein
UFP: Ultrafine particles
V: Vanadium
VDAC: Voltage-dependent anion channel
VOC: Volatile organic compounds
XIAP: X-linked inhibitor of apoptosis protein
Zn: Zinc
$\Delta\Psi m$: Mitochondrial transmembrane potential.

Acknowledgments

Support for this paper was from the French Agence Nationale de la Recherche (ANR), Centre National de la Recherche Scientifique (CNRS), Université Paris Diderot-Paris 7 (Ph.D. grant of Mélanie Leroux), Région Ile de France, and DIM SEnT (postdoctoral grant of Aïda Bouharrour). The authors particularly thank Dr. Christophe Lemaire, Dr. Ioana Ferecatu, and Dr. Armelle Baeza-Squiban for their critical reading of the paper.

References

[1] C. A. Pope III, R. T. Burnett, M. J. Thun et al., "Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution," *JAMA*, vol. 287, no. 9, pp. 1132–1141, 2002.

[2] J. Lewtas, "Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects," *Mutation Research*, vol. 636, no. 1–3, pp. 95–133, 2007.

[3] P. Borm and K. K. Donaldson, "An Introduction to Particle Toxicology; from coal mining to nanotechnology," in *Particle Toxicology*, P. Borm and K. K. Donaldson, Eds., pp. 1–12, CRC Press, 2007.

[4] B. Ostro et al., *The Effects of Particulate Matter Sources on Daily Mortality: A Case-Crossover Study of Barcelona*, Environ Health Perspect, Barcelona, Spain, 2011.

[5] G. Oberdörster, E. Oberdörster, and J. Oberdörster, "Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles," *Environmental Health Perspectives*, vol. 113, no. 7, pp. 823–839, 2005.

[6] T. Jones and K. Bérubé, "Mineralogy an structure of pathogenic particles," in *Particle Toxicology*, P. Borm and K. K. Donaldson, Eds., pp. 13–45, CRC Press, 2007.

[7] F. Marano, S. Boland, V. Bonvallot, A. Baulig, and A. Baeza-Squiban, "Human airway epithelial cells in culture for studying the molecular mechanisms of the inflammatory response triggered by diesel exhaust particles," *Cell Biology and Toxicology*, vol. 18, no. 5, pp. 315–320, 2002.

[8] W. Roemer, G. Hoek, and B. Brunekreef, "Effect of ambient winter air pollution on respiratory health of children with chronic respiratory symptoms," *American Review of Respiratory Disease*, vol. 147, no. 1, pp. 118–124, 1993.

[9] International Agency for Research on Cancer (IARC), http://www.iarc.fr/.

[10] P. Boffetta, S. D. Stellman, and L. Garfinkel, "Diesel exhaust exposure and mortality among males in the American Cancer Society prospective study," *American Journal of Industrial Medicine*, vol. 14, no. 4, pp. 403–415, 1988.

[11] French Agency of Environmental and Occupational Health Safety (AFSSET), "Estimation de l'impact lié à l'exposition chronique aux particules fines sur l'espérance de vie," in *Impact Sanitaire de la Pollution Atmosphérique Urbaine*, 2005.

[12] "Particulate air pollutants and small airway remodeling," in *Particle Toxicology*, A. Churg, Ed.P. Borm and K. K. Donaldson, Eds., pp. 75–87, CRC Press, 2007.

[13] J. P. Sullivan, J. D. Minna, and J. W. Shay, "Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy," *Cancer and Metastasis Reviews*, vol. 29, no. 1, pp. 61–72, 2010.

[14] Environmental Protection Agency (EPA), http://epa.gov/air/criteria.html.

[15] Centre Interprofesionnel Technique d'Etudes de la Pollution Atmosphérique (CITEPA), http://www.citepa.org/pollution/sources.htm.

[16] "Sources of air pollution," in *Air Pollution and Health*, C. Holman, Ed.S. T. Holgate et al., Ed., pp. 115–148, Academic Press, 1999.

[17] S. Orrenius, P. Nicotera, and B. Zhivotovsky, "Cell death mechanisms and their implications in toxicology," *Toxicological Sciences*, vol. 119, no. 1, pp. 3–19, 2011.

[18] D. K. Dhawan and V. D. Chadha, "Zinc: a promising agent in dietary chemoprevention of cancer," *Indian Journal of Medical Research*, vol. 132, no. 12, pp. 676–682, 2010.

[19] G. Gobe and D. Crane, "Mitochondria, reactive oxygen species and cadmium toxicity in the kidney," *Toxicology Letters*, vol. 198, no. 1, pp. 49–55, 2010.

[20] J. M. Matés, J. A. Segura, F. J. Alonso, and J. Márquez, "Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms," *Free Radical Biology and Medicine*, vol. 49, no. 9, pp. 1328–1341, 2010.

[21] H. T. Chung, B. M. Choi, Y. G. Kwon, and Y. M. Kim, "Interactive relations between nitric oxide (NO) and carbon monoxide (CO): heme oxygenase-1/CO pathway is a key modulator in NO-mediated antiapoptosis and anti-inflammation," *Methods in Enzymology*, vol. 441, pp. 329–338, 2008.

[22] M. C. Ferrante, G. Mattace Raso, E. Esposito et al., "Effects of non-dioxin-like polychlorinated biphenyl congeners (PCB 101, PCB 153 and PCB 180) alone or mixed on J774A.1 macrophage cell line: modification of apoptotic pathway," *Toxicology Letters*, vol. 202, no. 1, pp. 61–68, 2011.

[23] G. Kroemer, L. Galluzzi, P. Vandenabeele et al., "Classification of cell death: recommendations of the nomenclature committee on cell death 2009," *Cell Death and Differentiation*, vol. 16, no. 1, pp. 3–11, 2009.

[24] D. Wlodkowic, W. Telford, J. Skommer, and Z. Darzynkiewicz, "Apoptosis and beyond: cytometry in studies of programmed cell death," *Methods in Cell Biology*, vol. 103, pp. 55–98, 2011.

[25] X. Tekpli, J. A. Holme, O. Sergent, and D. Lagadic-Gossmann, "Importance of plasma membrane dynamics in chemical-induced carcinogenesis," *Recent Patents on Anti-Cancer Drug Discovery*, vol. 6, no. 3, pp. 347–353, 2011.

[26] D. Chen, R. A. Stetler, G. Cao et al., "Characterization of the rat DNA fragmentation factor 35/inhibitor of caspase-activated DNase (short form): the endogenous inhibitor of caspase-dependent DNA fragmentation in neuronal

apoptosis," *The Journal of Biological Chemistry*, vol. 275, no. 49, pp. 38508–38517, 2000.

[27] Y. Ishihara and N. Shimamoto, "Involvement of endonuclease G in nucleosomal DNA fragmentation under sustained endogenous oxidative stress," *The Journal of Biological Chemistry*, vol. 281, no. 10, pp. 6726–6733, 2006.

[28] Y. Harihara, K. Sanjo, and Y. Idezuki, "A modified cuff technique for suprahepatic vena cava anastomosis in rat liver transplantation," *Transplantation*, vol. 53, no. 3, pp. 707–709, 1992.

[29] E. Norberg, S. Orrenius, and B. Zhivotovsky, "Mitochondrial regulation of cell death: processing of apoptosis-inducing factor (AIF)," *Biochemical and Biophysical Research Communications*, vol. 396, no. 1, pp. 95–100, 2010.

[30] T.-J. Fan, L.-H. Han, R.-S. Cong, and J. Liang, "Caspase family proteases and apoptosis," *Acta Biochimica et Biophysica Sinica*, vol. 37, no. 11, pp. 719–727, 2005.

[31] F. Q. Alenzi, M. Lotfy, and R. K. Wyse, "Swords of cell death: caspase activation and regulation," *Asian Pacific Journal of Cancer Prevention*, vol. 11, no. 2, pp. 271–280, 2010.

[32] M. Paulsen and O. Janssen, "Pro- and anti-apoptotic CD95 signaling in T cells," *Cell Communication and Signaling*, vol. 9, article 7, 2011.

[33] G. Kroemer, L. Galluzzi, and C. Brenner, "Mitochondrial membrane permeabilization in cell death," *Physiological Reviews*, vol. 87, no. 1, pp. 99–163, 2007.

[34] L. Galluzzi, N. Zamzami, T. De La Motte Rouge, C. Lemaire, C. Brenner, and G. Kroemer, "Methods for the assessment of mitochondrial membrane permeabilization in apoptosis," *Apoptosis*, vol. 12, no. 5, pp. 803–813, 2007.

[35] O. Sharaf El Dein, C. Gallerne, A. Deniaud, C. Brenner, and C. Lemaire, "Role of the permeability transition pore complex in lethal inter-organelle crosstalk," *Frontiers in Bioscience*, vol. 14, pp. 3465–3482, 2009.

[36] V. Shoshan-Barmatz and D. Ben-Hail, "VDAC, a multifunctional mitochondrial protein as a pharmacological target," *Mitochondrion*, vol. 12, no. 1, pp. 24–34, 2012.

[37] F. Verrier, B. Mignotte, G. Jan, and C. Brenner, "Study of PTPC composition during apoptosis for identification of viral protein target," *Annals of the New York Academy of Sciences*, vol. 1010, pp. 126–142, 2003.

[38] F. Verrier, A. Deniaud, M. LeBras et al., "Dynamic evolution of the adenine nucleotide translocase interactome during chemotherapy-induced apoptosis," *Oncogene*, vol. 23, no. 49, pp. 8049–8064, 2004.

[39] G. P. McStay, S. J. Clarke, and A. P. Halestrap, "Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore," *Biochemical Journal*, vol. 367, no. 2, pp. 541–548, 2002.

[40] P. Costantini, A. S. Belzacq, H. L. A. Vieira et al., "Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis," *Oncogene*, vol. 19, no. 2, pp. 307–314, 2000.

[41] A. Philchenkov, M. Zavelevich, T. J. Kroczak, and M. Los, "Caspases and cancer: mechanisms of inactivation and new treatment modalities," *Experimental Oncology*, vol. 26, no. 2, pp. 82–97, 2004.

[42] G. Martinez-Ruiz, V. Maldonado, G. Ceballos-Cancino, J. P. R. Grajeda, and J. Melendez-Zajgla, "Role of Smac/DIABLO in cancer progression," *Journal of Experimental and Clinical Cancer Research*, vol. 27, no. 1, article 48, 2008.

[43] D. Acehan, X. Jiang, D. G. Morgan, J. E. Heuser, X. Wang, and C. W. Akey, "Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation," *Molecular Cell*, vol. 9, no. 2, pp. 423–432, 2002.

[44] T. F. Reubold, S. Wohlgemuth, and S. Eschenburg, "A new model for the transition of APAF-1 from inactive monomer to caspase-activating apoptosome," *The Journal of Biological Chemistry*, vol. 284, no. 47, pp. 32717–32724, 2009.

[45] S. Qi, Y. Pang, Q. Hu et al., "Crystal structure of the caenorhabditis elegans apoptosome reveals an octameric assembly of CED-4," *Cell*, vol. 141, no. 3, pp. 446–457, 2010.

[46] X. Teng and J. M. Hardwick, "The apoptosome at high resolution," *Cell*, vol. 141, no. 3, pp. 402–404, 2010.

[47] K. Andreau, M. Castedo, J. L. Perfettini et al., "Preapoptotic chromatin condensation upstream of the mitochondrial checkpoint," *The Journal of Biological Chemistry*, vol. 279, no. 53, pp. 55937–55945, 2004.

[48] A. Strasser, S. Cory, and J. M. Adams, "Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases," *The EMBO Journal*, vol. 30, no. 18, pp. 3667–3683, 2011.

[49] Y. Hu, M. A. Benedict, D. Wu, N. Inohara, and G. Núñez, "Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 8, pp. 4386–4391, 1998.

[50] G. Pan, K. O'Rourke, and V. M. Dixit, "Caspase-9, Bcl-X(L), and Apaf-1 form a ternary complex," *The Journal of Biological Chemistry*, vol. 273, no. 10, pp. 5841–5845, 1998.

[51] S. Conus, T. Rosse, and C. Borner, "Failure of Bcl-2 family members to interact with Apaf-1 in normal and apoptotic cells," *Cell Death and Differentiation*, vol. 7, no. 10, pp. 947–954, 2000.

[52] G. Hausmann, L. A. O'Reilly, R. Van Driel et al., "Pro-apoptotic apoptosis protease-activating factor 1 (Apaf-1) has a cytoplasmic localization distinct from Bcl-2 or Bcl-x(L)," *Journal of Cell Biology*, vol. 149, no. 3, pp. 623–633, 2000.

[53] S. Shimizu, Y. Eguchi, W. Kamiike et al., "Bcl-2 prevents apoptotic mitochondrial dysfunction by regulating proton flux," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 4, pp. 1455–1459, 1998.

[54] M. G. Vander Heiden, N. S. Chandel, P. T. Schumacker, and C. B. Thompson, "Bcl-x(L) prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange," *Molecular Cell*, vol. 3, no. 2, pp. 159–167, 1999.

[55] M. Wierzbicka, "Morphology of gingival epithelium in the light of modern research," *Czasopismo Stomatologiczne*, vol. 28, no. 10, pp. 969–972, 1975.

[56] I. Al Ghouleh, N. K. H. Khoo, U. G. Knaus et al., "Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling," *Free Radical Biology and Medicine*, vol. 51, no. 7, pp. 1271–1288, 2011.

[57] V. C. Fogg, N. J. Lanning, and J. P. MacKeigan, "Mitochondria in cancer: at the crossroads of life and death," *Chinese Journal of Cancer*, vol. 30, no. 8, pp. 526–539, 2011.

[58] E. Cadenas and K. J. A. Davies, "Mitochondrial free radical generation, oxidative stress, and aging," *Free Radical Biology and Medicine*, vol. 29, no. 3-4, pp. 222–230, 2000.

[59] S. Macip, M. Igarashi, P. Berggren, J. Yu, S. W. Lee, and S. A. Aaronson, "Influence of induced reactive oxygen species

in p53-mediated cell fate decisions," *Molecular and Cellular Biology*, vol. 23, no. 23, pp. 8576–8585, 2003.

[60] S.-Y. Ho, W. Wu Jr., H.-W. Chiu et al., "Arsenic trioxide and radiation enhance apoptotic effects in HL-60 cells through increased ROS generation and regulation of JNK and p38 MAPK signaling pathways," *Chemico-Biological Interactions*, vol. 193, no. 2, pp. 162–171, 2011.

[61] B. M. Emerling, L. C. Platanias, E. Black, A. R. Nebreda, R. J. Davis, and N. S. Chandel, "Mitochondrial reactive oxygen species activation of p38 mitogen-activated protein kinase is required for hypoxia signaling," *Molecular and Cellular Biology*, vol. 25, no. 12, pp. 4853–4862, 2005.

[62] A. J. Kowaltowski, R. F. Castilho, and A. E. Vercesi, "Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca2+ is dependent on mitochondrial-generated reactive oxygen species," *FEBS Letters*, vol. 378, no. 2, pp. 150–152, 1996.

[63] G. Paradies, G. Petrosillo, M. Pistolese, and F. M. Ruggiero, "The effect of reactive oxygen species generated from the mitochondrial electron transport chain on the cytochrome c oxidase activity and on the cardiolipin content in bovine heart submitochondrial particles," *FEBS Letters*, vol. 466, no. 2-3, pp. 323–326, 2000.

[64] S. Orrenius, V. Gogvadze, and B. Zhivotovsky, "Mitochondrial oxidative stress: implications for cell death," *Annual Review of Pharmacology and Toxicology*, vol. 47, pp. 143–183, 2007.

[65] T. R. Pushpa-Rekha, A. L. Burdsall, L. M. Oleksa, G. M. Chisolm, and D. M. Driscoll, "Rat phospholipid-hydroperoxide glutathione peroxidase. cDNA cloning and identification of multiple transcription and translation start sites," *The Journal of Biological Chemistry*, vol. 270, no. 45, pp. 26993–26999, 1995.

[66] J. Legault, C. Carrier, P. Petrov, P. Renard, J. Remacle, and M. E. Mirault, "Mitochondrial GPx1 decreases induced but not basal oxidative damage to mtDNA in T47D cells," *Biochemical and Biophysical Research Communications*, vol. 272, no. 2, pp. 416–422, 2000.

[67] M. Lundberg, C. Johansson, J. Chandra et al., "Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms," *The Journal of Biological Chemistry*, vol. 276, no. 28, pp. 26269–26275, 2001.

[68] H. Wu, K. Xing, and M. F. Lou, "Glutaredoxin 2 prevents H2O2-induced cell apoptosis by protecting complex I activity in the mitochondria," *Biochimica et Biophysica Acta*, vol. 1797, no. 10, pp. 1705–1715, 2010.

[69] Y. Aniya and N. Imaizumi, "Mitochondrial glutathione transferases involving a new function for membrane permeability transition pore regulation," *Drug Metabolism Reviews*, vol. 43, no. 2, pp. 292–299, 2011.

[70] J. Zhang, A. J. Ghio, W. Chang, O. Kamdar, G. D. Rosen, and D. Upadhyay, "Bim mediates mitochondria-regulated particulate matter-induced apoptosis in alveolar epithelial cells," *FEBS Letters*, vol. 581, no. 22, pp. 4148–4152, 2007.

[71] W. Y. Wani, S. Gudup, A. Sunkaria et al., "Protective efficacy of mitochondrial targeted antioxidant MitoQ against dichlorvos induced oxidative stress and cell death in rat brain," *Neuropharmacology*, vol. 61, no. 8, pp. 1193–1201, 2011.

[72] R. Franco, R. Sánchez-Olea, E. M. Reyes-Reyes, and M. I. Panayiotidis, "Environmental toxicity, oxidative stress and apoptosis: menage a trois," *Mutation Research*, vol. 674, no. 1-2, pp. 3–22, 2009.

[73] V. N. Samartsev, O. V. Markova, I. P. Zeldi, and A. V. Smirnov, "Role of the ADP/ATP and aspartate/glutamate antiporters in the uncoupling effect of fatty acids, lauryl sulfate, and 2, 4-dinitrophenol in liver mitochondria," *Biochemistry*, vol. 64, no. 8, pp. 901–911, 1999.

[74] M. Gualtieri, P. Mantecca, F. Cetta, and M. Camatini, "Organic compounds in tire particle induce reactive oxygen species and heat-shock proteins in the human alveolar cell line A549," *Environment International*, vol. 34, no. 4, pp. 437–442, 2008.

[75] F. Farina, G. Sancini, P. Mantecca, D. Gallinotti, M. Camatini, and P. Palestini, "The acute toxic effects of particulate matter in mouse lung are related to size and season of collection," *Toxicology Letters*, vol. 202, no. 3, pp. 209–217, 2011.

[76] E. Alfaro-Moreno, L. Martínez, C. García-Cuellar et al., "Biologic effects induced in vitro by PM10 from three different zones of Mexico City," *Environmental Health Perspectives*, vol. 110, no. 7, pp. 715–720, 2002.

[77] J. H. Choi, J. S. Kim, Y. C. Kim, Y. S. Kim, N. H. Chung, and M. H. Cho, "Comparative study of PM2.5—and PM10—induced oxidative stress in rat lung epithelial cells," *Journal of Veterinary Science*, vol. 5, no. 1, pp. 11–18, 2004.

[78] O. Kamdar, W. Le, J. Zhang, A. J. Ghio, G. D. Rosen, and D. Upadhyay, "Air pollution induces enhanced mitochondrial oxidative stress in cystic fibrosis airway epithelium," *FEBS Letters*, vol. 582, no. 25-26, pp. 3601–3606, 2008.

[79] G. Garçon, Z. Dagher, F. Zerimech et al., "Dunkerque City air pollution particulate matter-induced cytotoxicity, oxidative stress and inflammation in human epithelial lung cells (L132) in culture," *Toxicology in Vitro*, vol. 20, no. 4, pp. 519–528, 2006.

[80] Z. Dagher, G. Garçon, S. Billet et al., "Role of nuclear factor-kappa B activation in the adverse effects induced by air pollution particulate matter (PM2.5) in human epithelial lung cells (L132) in culture," *Journal of Applied Toxicology*, vol. 27, no. 3, pp. 284–290, 2007.

[81] A. Baulig, M. Garlatti, V. Bonvallot et al., "Involvement of reactive oxygen species in the metabolic pathways triggered by diesel exhaust particles in human airway epithelial cells," *American Journal of Physiology*, vol. 285, no. 3, pp. L671–L679, 2003.

[82] M. Lodovici and E. Bigagli, "Oxidative stress and air pollution exposure," *Journal of Toxicology*, vol. 2011, Article ID 487074, 9 pages, 2011.

[83] A. P. Senft, T. P. Dalton, D. W. Nebert et al., "Mitochondrial reactive oxygen production is dependent on the aromatic hydrocarbon receptor," *Free Radical Biology and Medicine*, vol. 33, no. 9, pp. 1268–1278, 2002.

[84] T. Xia, P. Korge, J. N. Weiss et al., "Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity," *Environmental Health Perspectives*, vol. 112, no. 14, pp. 1347–1358, 2004.

[85] A. Baeza-Squiban, V. Bonvallot, S. Boland, and F. Marano, "Airborne particles evoke an inflammatory response in human airway epithelium. Activation of transcription factors," *Cell Biology and Toxicology*, vol. 15, no. 6, pp. 375–380, 1999.

[86] D. Upadhyay, V. Panduri, A. Ghio, and D. W. Kamp, "Particulate matter induces alveolar epithelial cell DNA damage and apoptosis role of free radicals and the mitochondria," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 2, pp. 180–187, 2003.

[87] C. J. Obot, M. T. Morandi, T. P. Beebe, R. F. Hamilton, and A. Holian, "Surface components of airborne particulate matter induce macrophage apoptosis through scavenger receptors," *Toxicology and Applied Pharmacology*, vol. 184, no. 2, pp. 98–106, 2002.

[88] H. Andersson, E. Piras, J. Demma, B. Hellman, and E. Brittebo, "Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells," *Toxicology*, vol. 262, no. 1, pp. 57–64, 2009.

[89] N. Asare, X. Tekpli, M. Rissel et al., "Signalling pathways involved in 1-nitropyrene (1-NP)-induced and 3-nitrofluoranthene (3-NF)-induced cell death in Hepa1c1c7 cells," *Mutagenesis*, vol. 24, no. 6, pp. 481–493, 2009.

[90] K. Chramostová, J. Vondráček, L. Šindlerová, B. Vojtěšek, A. Kozubík, and M. Machala, "Polycyclic aromatic hydrocarbons modulate cell proliferation in rat hepatic epithelial stem-like WB-F344 cells," *Toxicology and Applied Pharmacology*, vol. 196, no. 1, pp. 136–148, 2004.

[91] J. Van Grevenynghe, L. Sparfel, M. L. Vee et al., "Cytochrome P450-dependent toxicity of environmental polycyclic aromatic hydrocarbons towards human macrophages," *Biochemical and Biophysical Research Communications*, vol. 317, no. 3, pp. 708–716, 2004.

[92] E. Hrubá, L. Trilecová, S. Marvanová et al., "Genotoxic polycyclic aromatic hydrocarbons fail to induce the p53-dependent DNA damage response, apoptosis or cell-cycle arrest in human prostate carcinoma LNCaP cells," *Toxicology Letters*, vol. 197, no. 3, pp. 227–235, 2010.

[93] R. Barouki and Y. Morel, "Repression of cytochrome P450 1A1 gene expression by oxidative stress: mechanisms and biological implications," *Biochemical Pharmacology*, vol. 61, no. 5, pp. 511–516, 2001.

[94] E. Hodgson, "Phase 1 metabolism of toxicants and metabolic interactions," in *Molecular and Biochemical Toxicology*, R. C. Smart and E. Hodgson, Eds., pp. 173–203, John Wiley & Sons, 4th edition, 2008.

[95] A. H. Conney, R. L. Chang, D. M. Jerina, and S. J. C. Wei, "Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite," *Drug Metabolism Reviews*, vol. 26, no. 1-2, pp. 125–163, 1994.

[96] A. G. Smith, B. Clothier, S. Robinson et al., "Interaction between iron metabolism and 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice with variants of the Ahr gene: a hepatic oxidative mechanism," *Molecular Pharmacology*, vol. 53, no. 1, pp. 52–61, 1998.

[97] A. Cuypers, M. Plusquin, T. Remans et al., "Cadmium stress: an oxidative challenge," *BioMetals*, vol. 23, no. 5, pp. 927–940, 2010.

[98] X. Wang, Y.-O. Son, Q. Chang et al., "NADPH oxidase activation is required in reactive oxygen species generation and cell transformation induced by hexavalent chromium," *Toxicological Sciences*, vol. 123, no. 2, pp. 399–410, 2011.

[99] D. B. Buchwalter, "Metals," in *Molecular and Biochemical Toxicology*, R. C. Smart and E. Hodgson, Eds., pp. 413–439, John Wiley & Sons, 2008.

[100] M. D. Pulido and A. R. Parrish, "Metal-induced apoptosis: mechanisms," *Mutation Research*, vol. 533, no. 1-2, pp. 227–241, 2003.

[101] D. Beyersmann and A. Hartwig, "Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms," *Archives of Toxicology*, vol. 82, no. 8, pp. 493–512, 2008.

[102] S. V. S. Rana, "Metals and apoptosis: recent developments," *Journal of Trace Elements in Medicine and Biology*, vol. 22, no. 4, pp. 262–284, 2008.

[103] J. J. Li, Q. Tang, Y. Li et al., "Role of oxidative stress in the apoptosis of hepatocellular carcinoma induced by combination of arsenic trioxide and ascorbic acid," *Acta Pharmacologica Sinica*, vol. 27, no. 8, pp. 1078–1084, 2006.

[104] J. P. Bebear, V. Calas, and B. Lablache Combier, "Carbocysteine in catarrhal ORL diseases," *Revue de Laryngologie-Otologie-Rhinologie*, vol. 111, no. 5, pp. 499–501, 1990.

[105] M. Li, T. Xia, C. S. Jiang, L. J. Li, J. L. Fu, and Z. C. Zhou, "Cadmium directly induced the opening of membrane permeability pore of mitochondria which possibly involved in cadmium-triggered apoptosis," *Toxicology*, vol. 194, no. 1-2, pp. 19–33, 2003.

[106] J. Ye, S. Wang, S. S. Leonard et al., "Role of reactive oxygen species and p53 in chromium(VI)-induced apoptosis," *The Journal of Biological Chemistry*, vol. 274, no. 49, pp. 34974–34980, 1999.

[107] P. Russo, A. Catassi, A. Cesario et al., "Molecular mechanisms of hexavalent chromium-induced apoptosis in human bronchoalveolar cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 33, no. 6, pp. 589–600, 2005.

[108] R. Zhao, N. Xiang, F. E. Domann, and W. Zhong, "Expression of p53 enhances selenite-induced superoxide production and apoptosis in human prostate cancer cells," *Cancer Research*, vol. 66, no. 4, pp. 2296–2304, 2006.

[109] K. Donaldson, V. Stone, P. J. A. Borm et al., "Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM10)," *Free Radical Biology and Medicine*, vol. 34, no. 11, pp. 1369–1382, 2003.

[110] J. Araya, M. Maruyama, A. Inoue et al., "Inhibition of proteasome activity is involved in cobalt-induced apoptosis of human alveolar macrophages," *American Journal of Physiology*, vol. 283, no. 4, pp. L849–L858, 2002.

[111] H. Geng, Z. Meng, and Q. Zhang, "In vitro responses of rat alveolar macrophages to particle suspensions and water-soluble components of dust storm PM2.5," *Toxicology in Vitro*, vol. 20, no. 5, pp. 575–584, 2006.

[112] D. W. Kamp, V. Panduri, S. A. Weitzman, and N. Chandel, "Asbestos-induced alveolar epithelial cell apoptosis: role of mitochondrial dysfunction caused by iron-derived free radicals," *Molecular and Cellular Biochemistry*, vol. 234-235, pp. 153–160, 2002.

[113] M. Jiménez Del Río and C. Vélez-Pardo, "Transition metal-induced apoptosis in lymphocytes via hydroxyl radical generation, mitochondria dysfunction, and caspase-3 activation: an *in vitro* model for neurodegeneration," *Archives of Medical Research*, vol. 35, no. 3, pp. 185–193, 2004.

[114] M. Provinciali, A. Donnini, K. Argentati, G. Di Stasio, B. Bartozzi, and G. Bernardini, "Reactive oxygen species modulate Zn^{2+}-induced apoptosis in cancer cells," *Free Radical Biology and Medicine*, vol. 32, no. 5, pp. 431–445, 2002.

[115] Y. Cho, S. Gorina, P. D. Jeffrey, and N. P. Pavletich, "Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations," *Science*, vol. 265, no. 5170, pp. 346–355, 1994.

[116] M. Madesh and G. Hajnóczky, "VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release," *Journal of Cell Biology*, vol. 155, no. 6, pp. 1003–1015, 2001.

[117] T. S. Hiura, N. Li, R. Kaplan, M. Horwitz, J. C. Seagrave, and A. E. Nel, "The role of a mitochondrial pathway in the

induction of apoptosis by chemicals extracted from diesel exhaust particles," *Journal of Immunology*, vol. 165, no. 5, pp. 2703–2711, 2000.

[118] V. A. Tyurin, Y. Y. Tyurina, M.-Y. Jung et al., "Mass-spectrometric analysis of hydroperoxy- and hydroxy-derivatives of cardiolipin and phosphatidylserine in cells and tissues induced by pro-apoptotic and pro-inflammatory stimuli," *Journal of Chromatography B*, vol. 877, no. 26, pp. 2863–2872, 2009.

[119] D. Shen, T. P. Dalton, D. W. Nebert, and H. G. Shertzer, "Glutathione redox state regulates mitochondrial reactive oxygen production," *The Journal of Biological Chemistry*, vol. 280, no. 27, pp. 25305–25312, 2005.

[120] S. C. Chen, T. L. Liao, Y. H. Wei, C. R. Tzeng, and S. H. Kao, "Endocrine disruptor, dioxin (TCDD)-induced mitochondrial dysfunction and apoptosis in human trophoblast-like JAR cells," *Molecular Human Reproduction*, vol. 16, no. 5, Article ID gaq004, pp. 361–372, 2010.

[121] I. F. Sevrioukova, "Redox-linked conformational dynamics in apoptosis-inducing factor," *Journal of Molecular Biology*, vol. 390, no. 5, pp. 924–938, 2009.

[122] L. Misevičien, Z. Anusevičius, J. Šarlauskas, I. F. Sevrioukova, and N. Čnas, "Redox reactions of the FAD-containing apoptosis-inducing factor (AIF) with quinoidal xenobiotics: a mechanistic study," *Archives of Biochemistry and Biophysics*, vol. 512, no. 2, pp. 183–189, 2011.

[123] Y. C. T. Huang, Z. Li, S. D. Harder, and J. M. Soukup, "Apoptotic and inflammatory effects induced by different particles in human alveolar macrophages," *Inhalation Toxicology*, vol. 16, no. 14, pp. 863–878, 2004.

[124] P. L. Liu, Y. L. Chen, Y. H. Chen, S. J. Lin, and Y. R. Kou, "Wood smoke extract induces oxidative stress-mediated caspase-independent apoptosis in human lung endothelial cells: role of AIF and EndoG," *American Journal of Physiology*, vol. 289, no. 5, pp. L739–L749, 2005.

[125] Z. Dagher, G. Garçon, S. Billet et al., "Activation of different pathways of apoptosis by air pollution particulate matter (PM2.5) in human epithelial lung cells (L132) in culture," *Toxicology*, vol. 225, no. 1, pp. 12–24, 2006.

[126] S. Hussain, L. C. J. Thomassen, I. Ferecatu et al., "Carbon black and titanium dioxide nanoparticles elicit distinct apoptotic pathways in bronchial epithelial cells," *Particle and Fibre Toxicology*, vol. 7, article 10, 2010.

[127] U. Sydlik, K. Bierhals, M. Soufi, J. Abel, R. P. F. Schins, and K. Unfried, "Ultrafine carbon particles induce apoptosis and proliferation in rat lung epithelial cells via specific signaling pathways both using EGF-R," *American Journal of Physiology*, vol. 291, no. 4, pp. L725–L733, 2006.

[128] I. Abbas, G. Garçon, F. Saint-Georges et al., "Occurrence of molecular abnormalities of cell cycle in L132 cells after in vitro short-term exposure to air pollution PM2.5," *Chemico-Biological Interactions*, vol. 188, no. 3, pp. 558–565, 2010.

[129] S. Shishodia and B. B. Aggarwal, "Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates activation of cigarette smoke-induced nuclear factor (NF)-κB by suppressing activation of IκBα kinase in human non-small cell lung carcinoma: correlation with suppression of cyclin D1, COX-2, and matrix metalloproteinase-9," *Cancer Research*, vol. 64, no. 14, pp. 5004–5012, 2004.

[130] W. H. Kuo, J. H. Chen, H. H. Lin, B. C. Chen, J. D. Hsu, and C. J. Wang, "Induction of apoptosis in the lung tissue from rats exposed to cigarette smoke involves p38/JNK MAPK pathway," *Chemico-Biological Interactions*, vol. 155, no. 1-2, pp. 31–42, 2005.

[131] S. Soberanes, D. Urich, C. M. Baker et al., "Mitochondrial complex III-generated oxidants activate ASK1 and JNK to induce alveolar epithelial cell death following exposure to particulate matter air pollution," *The Journal of Biological Chemistry*, vol. 284, no. 4, pp. 2176–2186, 2009.

[132] B. Y. Chin, M. E. Choi, M. D. Burdick, R. M. Strieter, T. H. Risby, and A. M. K. Choi, "Induction of apoptosis by particulate matter: role of TNF-α and MAPK," *American Journal of Physiology*, vol. 275, no. 5, pp. L942–L949, 1998.

[133] F. Peixoto, J. A. F. Vicente, and V. M. C. Madeira, "Comparative effects of herbicide dicamba and related compound on plant mitochondrial bioenergetics," *Journal of Biochemical and Molecular Toxicology*, vol. 17, no. 3, pp. 185–192, 2003.

[134] I. O. Kurochkin, M. Etzkorn, D. Buchwalter, L. Leamy, and I. M. Sokolova, "Top-down control analysis of the cadmium effects on molluscan mitochondria and the mechanisms of cadmium-induced mitochondrial dysfunction," *American Journal of Physiology*, vol. 300, no. 1, pp. R21–R31, 2011.

[135] D. Urich, S. Soberanes, Z. Burgess et al., "Proapoptotic Noxa is required for particulate matterinduced cell death and lung inflammation," *The FASEB Journal*, vol. 23, no. 7, pp. 2055–2064, 2009.

[136] N. Podechard, X. Tekpli, D. Catheline et al., "Mechanisms involved in lipid accumulation and apoptosis induced by 1-nitropyrene in Hepa1c1c7 cells," *Toxicology Letters*, vol. 206, no. 3, pp. 289–299, 2011.

[137] X. Tekpli, E. Rivedal, M. Gorria et al., "The B[a]P-increased intercellular communication via translocation of connexin-43 into gap junctions reduces apoptosis," *Toxicology and Applied Pharmacology*, vol. 242, no. 2, pp. 231–240, 2010.

[138] X. Tekpli, M. Rissel, L. Huc et al., "Membrane remodeling, an early event in benzo[α]pyrene-induced apoptosis," *Toxicology and Applied Pharmacology*, vol. 243, no. 1, pp. 68–76, 2010.

[139] "Air pollution and lung cancer," in *Air Pollution and Health*, J. M. Samet and A. J. Cohen, Eds.S. T. Holgate et al., Ed., pp. 841–564, Academic Press, 1999.

[140] "Carcinogenic responses to air pollutants," in *Air Pollution and Health*, R. O. McClellan and T. E. Jackson, Eds.S. T. Holgate et al., Ed., pp. 382–413, Academic Press, 1999.

[141] "Carcinogenesis," in *Molecular and Biochemical Toxicology*, R. C. Smart, S. J. Ewing, and K. D. Loomis, Eds.R. C. Smart and E. Hodgson, Eds., pp. 537–586, John Wiley & Sons, 1999.

[142] D. Hanahan and R. A. Weinberg, "The hallmarks of cancer," *Cell*, vol. 100, no. 1, pp. 57–70, 2000.

[143] M. Teranishi, T. Toyooka, T. Ohura, S. Masuda, and Y. Ibuki, "Benzo[a]pyrene exposed to solar-simulated light inhibits apoptosis and augments carcinogenicity," *Chemico-Biological Interactions*, vol. 185, no. 1, pp. 4–11, 2010.

[144] H. S. Hung, W. J. Wu, Y. W. Cheng, T. C. Wu, K. L. Chang, and H. Lee, "Association of cooking oil fumes exposure with lung cancer: involvement of inhibitor of apoptosis proteins in cell survival and proliferation in vitro," *Mutation Research*, vol. 628, no. 2, pp. 107–116, 2007.

[145] I. Ferecatu, M. C. Borot, C. Bossard et al., "Polycyclic aromatic hydrocarbon components contribute to the mitochondria-antiapoptotic effect of fine particulate matter on human bronchial epithelial cells via the aryl hydrocarbon receptor," *Particle and Fibre Toxicology*, vol. 7, article 18, 2010.

[146] A. Q. Truong-Tran, J. Carter, R. E. Ruffin, and P. D. Zalewski, "The role of zinc in caspase activation and apoptotic cell death," *BioMetals*, vol. 14, no. 3-4, pp. 315–330, 2001.

[147] A. Szuster-Ciesielska, K. Plewka, J. Daniluk, and M. Kandefer-Szerszeń, "Zinc inhibits ethanol-induced HepG2

cell apoptosis," *Toxicology and Applied Pharmacology*, vol. 229, no. 1, pp. 1–9, 2008.

[148] J. E. Carter, A. Q. Truong-Tran, D. Grosser, L. Ho, R. E. Ruffin, and P. D. Zalewski, "Involvement of redox events in caspase activation in zinc-depleted airway epithelial cells," *Biochemical and Biophysical Research Communications*, vol. 297, no. 4, pp. 1062–1070, 2002.

[149] P. Bernardi, A. Krauskopf, E. Basso et al., "The mitochondrial permeability transition from in vitro artifact to disease target," *The FEBS Journal*, vol. 273, no. 10, pp. 2077–2099, 2006.

[150] C. E. Rexroad and A. M. Powell, "Effect of serum-free co-culture and synchrony of recipients on development of cultured sheep embryos to fetuses," *Journal of Animal Science*, vol. 69, no. 5, pp. 2066–2072, 1991.

[151] A. D. Burdick, I. D. Ivnitski-Steele, F. T. Lauer, and S. W. Burchiel, "PYK2 mediates anti-apoptotic AKT signaling in response to benzo[a]pyrene diol epoxide in mammary epithelial cells," *Carcinogenesis*, vol. 27, no. 11, pp. 2331–2340, 2006.

[152] M. Chopra, A. M. Dharmarajan, G. Meiss, and D. Schrenk, "Inhibition of UV-C light-induced apoptosis in liver cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin," *Toxicological Sciences*, vol. 111, no. 1, pp. 49–63, 2009.

[153] L. Al-Anati, J. Högberg, and U. Stenius, "Non-dioxin-like PCBs interact with benzo[a]pyrene-induced p53-responses and inhibit apoptosis," *Toxicology and Applied Pharmacology*, vol. 249, no. 2, pp. 166–177, 2010.

[154] H. Bayram, K. Ito, R. Issa, M. Ito, M. Sukkar, and K. F. Chung, "Regulation of human lung epithelial cell numbers by diesel exhaust particles," *European Respiratory Journal*, vol. 27, no. 4, pp. 705–713, 2006.

[155] M. Chopra, M. Gährs, M. Haben, C. Michels, and D. Schrenk, "Inhibition of apoptosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin depends on protein biosynthesis," *Cell Biology and Toxicology*, vol. 26, no. 4, pp. 391–401, 2010.

[156] J. J. Mukherjee, S. Kumar, R. Gocinski, and J. Williams, "Phenolic fraction of tobacco smoke inhibits BPDE-induced apoptosis response and potentiates cell transformation: role of attenuation of p53 response," *Chemical Research in Toxicology*, vol. 24, no. 5, pp. 698–705, 2011.

[157] M. Hailwood, D. King, and E. Leoz, "Ambient air pollution by polycyclic aromatic hydrocarbons PAH," in *Position Paper Annexes*, European Commission, 2001.

[158] J. L. Marlowe, Y. Fan, X. Chang et al., "The aryl hydrocarbon receptor binds to E2F1 and inhibits E2F1-induced apoptosis," *Molecular Biology of the Cell*, vol. 19, no. 8, pp. 3263–3271, 2008.

[159] C. F. A. Vogel, W. Li, E. Sciullo et al., "Pathogenesis of aryl hydrocarbon receptor-mediated development of lymphoma is associated with increased cyclooxygenase-2 expression," *American Journal of Pathology*, vol. 171, no. 5, pp. 1538–1548, 2007.

[160] A. Ambolet-Camoit, L. C. Bui, S. Pierre et al., "2,3,7,8-Tetrachlorodibenzo-p-dioxin counteracts the p53 response to a genotoxicant by upregulating expression of the metastasis marker AGR2 in the hepatocarcinoma cell line HepG2," *Toxicological Sciences*, vol. 115, no. 2, pp. 501–512, 2010.

[161] H. Sarioglu, S. Brandner, M. Haberger et al., "Analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced proteome changes in 5L rat hepatoma cells reveals novel targets of dioxin action including the mitochondrial apoptosis regulator VDAC2," *Molecular and Cellular Proteomics*, vol. 7, no. 2, pp. 394–410, 2008.

[162] E. A. Ostrakhovitch, M. R. Lordnejad, F. Schliess, H. Sies, and L.-O. Klotz, "Copper ions strongly activate the phosphoinositide-3-kinase/Akt pathway independent of the generation of reactive oxygen species," *Archives of Biochemistry and Biophysics*, vol. 397, no. 2, pp. 232–239, 2002.

[163] J. J. Mukherjee, S. K. Gupta, and S. Kumar, "Inhibition of benzopyrene-diol-epoxide (BPDE)-induced bax and caspase-9 by cadmium: role of mitogen activated protein kinase," *Mutation Research*, vol. 661, no. 1-2, pp. 41–46, 2009.

[164] N. Agopyan, J. Head, S. Yu, and S. A. Simon, "TRPV1 receptors mediate particulate matter-induced apoptosis," *American Journal of Physiology*, vol. 286, no. 3, pp. L563–L572, 2004.

[165] R. Wu, L. Zhang, M. S. Hoagland, and H. I. Swanson, "Lack of the aryl hydrocarbon receptor leads to impaired activation of AKT/protein kinase B and enhanced sensitivity to apoptosis induced via the intrinsic pathway," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 1, pp. 448–457, 2007.

[166] M. Kaimul Ahsan, H. Nakamura, M. Tanito, K. Yamada, H. Utsumi, and J. Yodoi, "Thioredoxin-1 suppresses lung injury and apoptosis induced by diesel exhaust particles (DEP) by scavenging reactive oxygen species and by inhibiting DEP-induced downregulation of Akt," *Free Radical Biology and Medicine*, vol. 39, no. 12, pp. 1549–1559, 2005.

[167] A. Rasola, M. Sciacovelli, B. Pantic, and P. Bernardi, "Signal transduction to the permeability transition pore," *FEBS Letters*, vol. 584, no. 10, pp. 1989–1996, 2010.

[168] A. Baeza-Squiban, V. Bonvallot, S. Boland, and F. Marano, "Diesel exhaust particles increase NF-κB DNA binding activity and c-fos proto-oncogene expression in human bronchial epithelial cells," *Toxicology in Vitro*, vol. 13, no. 4-5, pp. 817–822, 1999.

[169] S. Shishodia, P. Potdar, C. G. Gairola, and B. B. Aggarwal, "Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-κB activation through inhibition of IκBα kinase in human lung epithelial cells: correlation with suppression of COX-2, MMP-9 and cyclin D1," *Carcinogenesis*, vol. 24, no. 7, pp. 1269–1279, 2003.

[170] V. Rubio, M. Valverde, and E. Rojas, "Effects of atmospheric pollutants on the Nrf2 survival pathway," *Environmental Science and Pollution Research*, vol. 17, no. 2, pp. 369–382, 2010.

[171] N. Li, M. I. Venkatesan, A. Miguel et al., "Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element," *Journal of Immunology*, vol. 165, no. 6, pp. 3393–3401, 2000.

[172] N. Li, J. Alam, M. I. Venkatesan et al., "Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals," *Journal of Immunology*, vol. 173, no. 5, pp. 3467–3481, 2004.

[173] D. J. Slebos, S. W. Ryter, M. Van Der Toorn et al., "Mitochondrial localization and function of heme oxygenase-1 in cigarette smoke-induced cell death," *American Journal of Respiratory Cell and Molecular Biology*, vol. 36, no. 4, pp. 409–417, 2007.

[174] D. Morse and A. M. K. Choi, "Heme oxygenase-1: from bench to bedside," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 6, pp. 660–670, 2005.

[175] B. M. Choi, H. O. Pae, Y. M. Kim, and H. T. Chung, "Nitric oxide-mediated cytoprotection of hepatocytes from

glucose deprivation-induced cytotoxicity: involvement of heme oxygenase-1," *Hepatology*, vol. 37, no. 4, pp. 810–823, 2003.

[176] C. S. F. Queiroga, A. S. Almeida, P. M. Alves, C. Brenner, and H. L. A. Vieira, "Carbon monoxide prevents hepatic mitochondrial membrane permeabilization," *BMC Cell Biology*, vol. 12, article 10, 2011.

[177] L. G. Higgins and J. D. Hayes, "Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents," *Drug Metabolism Reviews*, vol. 43, no. 2, pp. 92–137, 2011.

[178] H. Raza, "Dual localization of glutathione S-transferase in the cytosol and mitochondria: implications in oxidative stress, toxicity and disease," *The FEBS Journal*, vol. 278, no. 22, Article ID 10.1111/j.1742-4658.2011.08358.x, pp. 4243–4251, 2011.

[179] K. K. Lee, M. Shimoji, Q. S. Hossain, H. Sunakawa, and Y. Aniya, "Novel function of glutathione transferase in rat liver mitochondrial membrane: role for cytochrome c release from mitochondria," *Toxicology and Applied Pharmacology*, vol. 232, no. 1, pp. 109–118, 2008.

[180] N. Li, M. Hao, R. F. Phalen, W. C. Hinds, and A. E. Nel, "Particulate air pollutants and asthma: a paradigm for the role of oxidative stress in PM-induced adverse health effects," *Clinical Immunology*, vol. 109, no. 3, pp. 250–265, 2003.

[181] "Particle-mediated extracellular oxidative stress in the lung," in *Particle Toxicology*, F. J. Kelly and I. S. Mudway, Eds.K. Donaldson and P. Borm, Eds., pp. 89–117, CRC Press, 2007.

[182] N. Li, M. Wang, T. D. Oberley, J. M. Sempf, and A. E. Nel, "Comparison of the pro-oxidative and proinflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages," *Journal of Immunology*, vol. 169, no. 8, pp. 4531–4541, 2002.

[183] R. Barouki, "Linking long-term toxicity of xeno-chemicals with short-term biological adaptation," *Biochimie*, vol. 92, no. 9, pp. 1222–1226, 2010.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 749845, 9 pages
doi:10.1155/2012/749845

Review Article

Carbon Monoxide Targeting Mitochondria

Cláudia S. F. Queiroga,[1,2,3] Ana S. Almeida,[1,2] and Helena L. A. Vieira[1,2,3]

[1] *Instituto de Biologia Experimental e Tecnológica (IBET), Apartado 12, 2781-901 Oeiras, Portugal*
[2] *Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*
[3] *CEDOC, Faculdade de Ciência Médicas, Universidade Nova de Lisboa, 1169-056 Lisboa, Portugal*

Correspondence should be addressed to Helena L. A. Vieira, helena.vieira@fcm.unl.pt

Received 11 November 2011; Accepted 9 January 2012

Academic Editor: Catherine Brenner

Mitochondria present two key roles on cellular functioning: (i) cell metabolism, being the main cellular source of energy and (ii) modulation of cell death, by mitochondrial membrane permeabilization. Carbon monoxide (CO) is an endogenously produced gaseoustransmitter, which presents several biological functions and is involved in maintaining cell homeostasis and cytoprotection. Herein, mitochondrion is approached as the main cellular target of carbon monoxide (CO). In this paper, two main perspectives concerning CO modulation of mitochondrial functioning are evaluated. First, the role of CO on cellular metabolism, in particular oxidative phosphorylation, is discussed, namely, on: cytochrome *c* oxidase activity, mitochondrial respiration, oxygen consumption, mitochondrial biogenesis, and general cellular energetic status. Second, the mitochondrial pathways involved in cell death inhibition by CO are assessed, in particular the control of mitochondrial membrane permeabilization.

1. Introduction

Carbon monoxide (CO) is a colorless and odorless small molecule, widely known as a lethal gas and as a toxic air pollutant. CO toxicity was disclosed in 1912 by Douglas [1]; its high affinity for haemglobin, forming carboxyhaemglobin, compromises oxygen delivery in tissues and subsequently causes lethality. Several decades later, CO was found as an endogenous generated gas in humans [2, 3]. However, only in the late sixties, haem oxygenase (HO) was characterized as the enzyme responsible for haem cleavage, with the release of CO, free iron (Fe^{2+}) and biliverdin [4, 5].

There are two genetically distinct isozymes for HO: an inducible form haem-oxygenase-1 (HO-1) and a constitutively expressed form haem oxygenase-2 (HO-2). HO-1 occurs mainly in spleen, liver or bone marrow, and tissues that degrade senescent red blood cells; under conditions of haemolysis its activity dramatically increases. Higher levels of HO-2 occur mainly in testes and central nervous system [6]. Increase expression of HO-1 is associated with biological responses to several sources of stress, namely, oxidative stress, hypoxia, hyperoxia, misfolded protein response, hyperthermia, tumour promoter, ultraviolet radiation, and so forth. Concomitant with the increasing importance of HO activity in biological systems, CO is largely recognized as a homeostatic and cytoprotective molecule [7, 8]. Stimulation of endogenously generated CO and/or low doses of applied CO have shown to exert remarkable beneficial biological effects in many tissues: anti-inflammatory, antiapoptotic, antiproliferative and antiatherogenic. Three main areas of potential therapeutic applications have been extensively studied: cardiovascular diseases, inflammatory disorders, and organ transplantation [7], including the creation of several patents [9]. In the moment there are two clinical trials phase II on CO gas inhalation-based therapy: for treating patients with intestinal paralysis after colon surgery, for prevention of postoperative ileus (NCT01050712), and for the improvement of tolerability in patients receiving kidney transplants (NCT00531856).

The use of CO for therapeutic purposes presents two main advantages: (i) it is an endogenous product and the organism is fully adapted to and (ii) CO is not metabolized

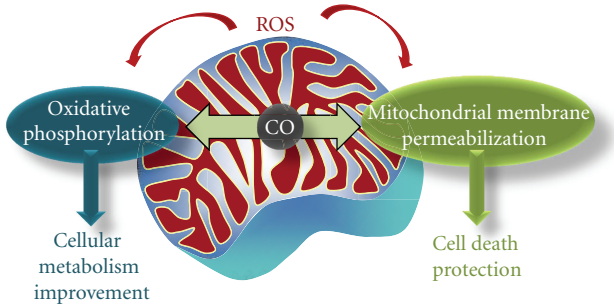


Figure 1: Two main aspects are involved in CO's cytoprotective role targeting mitochondria: modulation of cell metabolism by improvement of oxidative phosphorylation and inhibition of cell death by preventing mitochondrial membrane permeabilization.

and reversibly binds to its molecular targets, which makes the pharmacokinetic much simpler. Due to its therapeutic potential, large efforts have been initiated in the last years to develop new ways to deliver this gas to specific tissues and organs. The main example is CO-releasing molecules (CORMs). CORMs are organic and organometallic compounds, able to deliver CO in a timely and tissue-specific manner, permitting a significant reduction in carboxyhaemglobin formation and toxicity [7, 9, 10]. In this paper, the carbon monoxide influence on cellular and tissue homeostasis by its direct action on mitochondria is emphasised, in particular on two aspects: cell metabolism and cell death control (Figure 1).

2. Chemistry of Carbon Monoxide

In biological systems, CO binds almost exclusively to transition metals, namely, iron, manganese, vanadium, cobalt, tungsten, copper, nickel, and molybdenum, which are present in structural and functional proteins [10]. The metal centre can interact with ligands (usually gases: CO, NO, and O_2) modifying protein activity. The number of molecules targeted by CO in mammals is very limited; the majority are haem-containing proteins, whose function is regulated by the iron of this prosthetic group. Iron is involved in the regulation of protein function by being part of haem structure. In contrast to NO, that can bind to Fe^{3+} and Fe^{2+}, CO is only able to accept electrons from Fe^{2+}, which promotes a selectivity of CO-targeted haem proteins [10, 11]. Carbon monoxide presents high affinity for binding to haemoglobin and myoglobin, which competes with oxygen and compromises its delivery into tissues, causing hypoxia. Another member of globin superfamily is neuroglobin (Ngb), which is predominantly expressed in neurons and confers neuroprotection against hypoxic-ischemic injury [12]. Although the exact Ngb role is yet to be disclosed, its possible function includes O_2 storage and transport or detoxification of ROS and NO. Furthermore, CO binding to the haem centre of Ngb leads to conformational changes and cellular signalling [13].

CO also activates soluble guanylate cyclase (sGC) and nitric oxide synthase (NOS), but higher levels of CO are usually required and its physiological role is not yet clarified. Finally, the last mitochondrial electron transport chain complex, cytochrome c oxidase appears as another potential binding target for CO *in vivo* [14].

Despite the biological functions associated to CO *in vivo* and the existence of several proteins capable for binding CO *in vitro*; it is still a matter of discussion: the actual physiological target of CO.

3. Carbon Monoxide and Cytochrome *c* Oxidase

Mitochondrion regulates cell metabolism, being the main energy source, responsible for most of cellular ATP production *via* oxidative phosphorylation. Mitochondrial membrane permeabilization is also tightly involved in cell death modulation and participates in signal transduction cascades, since several signaling factors are released from mitochondrial inter-membrane space. In 1970, it was first claimed that CO's toxicity could be derived from its targeting and binding to the mitochondrial haem-protein cytochrome *c* oxidase (COX) [15]. Cytochrome *c* oxidase is the final electron acceptor of the mitochondrial electron transport chain, catalysing the oxidation of ferrocytochrome *c* by gaseous oxygen.

Binding of CO to COX (cytochrome a, a3) was followed in rat brain by reflectance spectrophotometry. In rat exposed to CO (up to around 70% of carboxyhaemglobin levels in the blood) cytochrome a, a3 absorption (605 nm) increased in the parietal cortex, but no data on COX enzymatic activity were revealed [16, 17]. 90 minutes after ending CO exposure, cytochrome oxidation state improved to 80% of control under normobaric oxygen exposure and recovered completely under hyperbaric oxygen exposure [17]. It is worthy of note that 70% of carboxyhaemglobin levels is very high and exceeds the limit of toxicity. Usually, the used CO concentrations for animal experiments (~250–500 ppm) reach around 10 to 20% of carboxyhaemglobin levels.

In isolated mitochondria from human muscle, CO partially prevented COX activity at 50, 100, and 500 ppm, while no effect was obtained in respiratory complexes I, II, or III [18]. In mouse leukaemic monocyte macrophage cell line, 1 h of CO exposition (250 ppm) decreased COX activity, which was assessed in permeabilized cells [19]. In contrast, cytochrome *c* oxidase presented a two-step response to low concentrations of CO saturated solutions (10 μM) in isolated mitochondria from mouse liver [20] and from rat brain [21]. In the first minutes (5 to 10) COX activity was partially prevented, while after 30 minutes its activity increased. Furthermore, COX-specific activity also increased whenever intact astrocytes were treated with 50 μM of CO after 3 or 24 h [21].

These apparent controversial data can be due to different tested CO concentrations, period of gas exposure, and the time point for assessing cytochrome c oxidase activity. Still, CO effect on COX activity is also dependent on the system

oxygen concentration. Indeed, CO competes with oxygen for binding to cytochrome *c* oxidase. However, the relative CO-O_2 affinity (M^*) is about 220 for haemoglobin and 20–25 for myoglobin while it is close to unit for cytochrome *c* oxidase [22]. Thus, the acute toxicity triggered by exogenous CO is mostly due to its high affinity for haemoglobin, limiting tissue oxygenation. Accordingly, CO capacity to bind to COX is highly dependent on oxygen concentration [14, 23]. Thus, another factor that might influence the distinct COX activity responses to CO found in the literature is the presence of oxygen concentration.

Accordingly, Fukuda et al. have shown that activation of hypoxia-inducing factor-1 (HIF-1) was involved in the regulation of cytochrome *c* oxidase subunits for optimizing the efficiency of mitochondrial respiration [24] and in macrophages CO-activated HIF-1 without increasing the rate of glycolysis [25]. Thus, one can speculate that CO improves COX activity by activation of HIF-1.

Finally, unlike NO and H_2S that are readily metabolized by oxidative processes within COX, CO oxidation is too slow to be physiologically relevant as a substrate for COX [14].

4. Carbon Monoxide and Mitochondrial Respiration

An indirect way for assessing CO effect on mitochondria and on COX activity is by following cellular oxygen consumption. Under normoxia (21% of oxygen in gaseous phase), exogenous CO application or endogenous CO (generated by overexpression of HO-1 or by lipopolysaccharide induced HO-1 expression) slightly inhibited cell respiration, but it is not clear the physiological importance of this inhibition. In contrast, endogenous or exogenous CO considerably decreased cellular respiration under hypoxia conditions (1% of oxygen) [23]. Thus, tissue hypoxia and CO appeared to have a synergistic effect on COX inhibition. It can be speculated that CO significantly inhibits COX only when tissue oxygen delivery is already compromised by the presence of high levels of carboxy-haemoglobin and carboxymyoglobin. Furthermore, under low O_2 concentration the electron transport chain is in a more reduced state, which is a more favourable state for CO to bind, since CO has the ability for binding to reduced iron [10, 26].

In isolated mitochondria from kidney, respiratory control index was assessed immediately after adding three different CO-releasing molecules (CORM-2, CORM-3, and CORM-A1) at 10, 50, and 100 μM. Oxygen consumption was measured in the presence (state 3) or absence (state 4) of ADP; the ratio between state 3 and 4 is the respiratory control index (RCR) and indicates the tightness of the coupling between respiration and phosphorylation. At this early time point CO decreased respiratory control index [27]. In contrast, during reperfusion, after kidney cold storage, CO is released by CORM-3 or CORM-A1 increased renal mitochondrial respiration, improving its function [28].

Iacono et al. have claimed that CO limits excessive mitochondrial ROS production and avoids oxidative stress by inducing a mild-uncoupling state [29]. This hypothesis was based on the following data. First, low concentrations (up to 20 μM) of CORM-3 weaken the coupling between ATP production and respiration. In the absence of exogenous ADP (state 4), CO-treated heart-isolated mitochondria presented an increase on oxygen consumption. In this situation, complex II appears to be the target of CO since inhibition of complex II (malonate addition) reverted the CO-induced augmentation of oxygen consumption. Second, in the presence of ADP (state 3), 100 μM of CORM-3 decreased oxygen consumption, which was claimed to be due to complex IV inhibition. Third, CORM-3 at 20 or 100 μM decreased mitochondrial membrane potential ($\Delta\Psi m$). This decrease was prevented by the addition of inhibitors for uncoupling respiration proteins (UCP) and for adenine nucleotide transporter (ANT), indicating that CO might open UCP and/or ANT for providing a mild uncoupling state. Taken all together, CO-induced cytoprotection was correlated with a mitochondrial mild uncoupling stimulation, which decreases excessive and toxic mitochondrial ROS production [29]. However, all data were obtained using isolated heart mitochondria and the actual physiological role remains to be disclosed. Still, only an early-response of CO was assessed, thus further studies are necessary to clarify the CO late response on mitochondrial mild uncoupling.

In a distinct experimental approach, astrocytes isolated from cortex have received one brief exposition to CO (by addition of CO-saturated solution at 50 μM final concentration from which the gas diffuses rapidly) and cell-specific oxygen consumption was assessed during 36 h. Indeed, brief exposition to CO increased cellular oxygen consumption in intact astrocytes, which was justified by an improvement of mitochondrial respiratory chain and oxidative metabolism [21].

Accordingly to the two-step time response of cytochrome *c* oxidase activity to CO; whereas it was observed an early or late response corresponding to a decrease or an increase on COX activity, respectively [20, 21]. Lancel et al. have also found that mitochondrial respiration has two distinct responses to CO depending on its concentration [30]. In heart-isolated mitochondria, 0.5 and 1 μM of CORM-3 increased respiratory control ratio (RCR) and mitochondrial transmembrane potential ($\Delta\Psi m$), while at 5 and 10 μM there is a decrease in RCR and $\Delta\Psi m$ [30]. Once again, experimental conditions (concentration or period of exposure) might change the tight mitochondrial balance and modulation of oxidative metabolism, namely, the influence of CO on COX activity, mitochondrial respiration, or cellular oxygen consumption.

5. Carbon Monoxide and Mitochondrial Biogenesis

CO effect on mitochondria is not limited to organelle functioning but also involves modulation of their population. In fact, mitochondrial biogenesis stimulation appears to be one of the CO biological functions in several models.

In the heart and in isolated cardiomyocytes, CO triggered mitochondrial biogenesis, which was activated by the expression of nuclear respiratory factor 1 (NRF-1), binding to mitochondrial transcription factor 3 (TFAM-3) [31]. CO activation of mitochondrial biogenesis involved both guanylate cyclase and prosurvival kinase Akt/PKB and occurred in a hydrogen-peroxide-(H_2O_2-) dependent manner. Cell transfection with mitochondrial-targeted catalase, which scavenges mitochondrial H_2O_2, prevented CO-induced mitochondrial DNA replication [31]. Likewise, heam oxygenase modulates cardiac mitochondrial biogenesis via gene expression and nuclear translocation of nuclear factor erythroid-2-related factor 2 (Nrf2), which upregulates expression of nuclear respiratory factor 1 (NRF-1) [32]. Furthermore, the same authors have shown that CO/haem oxygenase system prevented murine doxorubicin cardiomyopathy by reversing mitochondrial biogenesis inhibition [33]. In another pathological model, peritonitis-induced sepsis, carbon monoxide can rescue mice from death by providing energetic metabolic support *via* activating mitochondrial biogenesis [30]. CORM-3 treatment activated mitochondrial biogenesis and induced an increase on RCR, $\Delta\Psi m$, mitochondrial H_2O_2 concentration, and mitochondrial DNA level. However, none great effect on pro- or anti-inflammatory markers (TNF-α or IL-10) was observed, only after 48 h a slight increase on IL-10 was found [30]. More recently, in another rodent model of sepsis, induction of HO-1 coupled activation of mitochondrial biogenesis to anti-inflammatory cytokine expression, such as IL-10 or sIL1-Ra [34]. A boost of CO exposure, using saturated solutions, also activated mitochondrial biogenesis in astrocytes from rat cortex [21]. Finally, in human skeletal muscle exposition to low concentrations of CO (1 h/day at 100 ppm during 5 days) stimulated mitochondrial biogenesis involving regulation of the mitochondrial DNA transcriptome [35].

Therefore, activation of mitochondrial biogenesis is another biological function conferred by carbon monoxide for the improvement of cell metabolism and for providing cytoprotection.

6. Carbon Monoxide, Metabolism, and Energy Status

Based on the facts that CO increases mitochondrial population, modulates mitochondrial respiration, and can regulate mitochondrial respiratory complexes, it is not surprising the great influence of CO on cellular metabolism and energetic status.

Carbon monoxide improved cardiac energy in a model of ischemia and reperfusion in pigs, preventing edema and apoptosis [36]. Pigs were ventilated for 2 h with 250 ppm CO and then subjected to cardiopulmonary bypass. CO treatment had no effect on ATP/ADP ratio before ischemia, but after reperfusion CO resulted in higher levels of ATP. Likewise, phosphocreatine, a high-energy phosphate reserve in the cell, which facilitates intracellular high-energy phosphate transport, slightly decreased with CO treatment before ischemia. Yet, after reperfusion, the phosphocreatine levels in CO-treated hearts were significantly higher than in nontreated animals. Thus, CO improved energetic status and prevented cardiac tissue damage by edema and cell death [36]. Likewise, in a similar model of heart ischemia in pigs, CO was administrated at concentrations with the goal to obtain up to 5% of carboxyhaemglobin in the blood. In CO-treated animals the lactate production/glucose consumption ratio decreased, meaning that higher amounts of pyruvate entered and were metabolized by TCA cycle and a decrease on glycolytic metabolism was observed [37]. In human hepatocytes or primary culture of mouse hepatocytes, exogenous or heam-oxygenase-1-derived CO stimulated ATP generation [38, 39]. In this model, activation of soluble guanylyl cyclase (sGC) was the proposed pathway for ATP generation increase, and the strength of p38 MAPK activation was correlated with the availability of ATP. Furthermore, 1 h of CO inhalation (at low concentrations: 100 to 500 ppm) enhanced sGC activity and ATP generation in mouse liver, improving the survival in mice death after initiation of fulminant hepatitis [38]. Finally, in primary culture of astrocytes, CO treatment also stimulated ATP generation by improving oxidative metabolism [21].

7. Carbon Monoxide and Cytoprotection

In 2000 Brouard et al. demonstrated for the first time the antiapoptotic property of the system HO-1/CO in endothelial cells, whose mechanism of action was dependent on the activation of the p38 mitogen-activated protein kinase (MAPK) signaling transduction pathway [40]. Since then and in many different models of lung, brain, smooth muscle cells, liver or endothelial cells low concentrations of exogenous CO, or haem oxygenase-derived CO have shown to confer resistance against cell death [6–8].

In hepatocytes, CO prevented TNF-α-induced apoptosis via inhibiting caspase-8 [41] or CO prevented oxidative stress-induced apoptosis by inhibition of p54 JNK isoform [42]. Likewise, CO-induced hepatocyte NF-kappaB activation and apoptosis protection presenting reactive oxygen species (ROSs) generation as signaling molecules [43]. Low levels of exogenous CO attenuated anoxia-reoxygenation-induced lung endothelial cell apoptosis via activation of p38 MAPK and STAT 3, which prevented caspase-3 activation [44, 45]. Additionally, CO protected against hyperoxia-induced endothelial cell apoptosis by preventing excessive ROS formation, bid activation, mitochondrial translocation of Bax, cytochrome *c* release, and caspase-9/3 activation [46]. In neuronal primary cultures, CO prevented glutamate-induced apoptosis through ROS generation and activation of sGC and inducible nitric oxide synthase (iNOS) [47]. Carbon monoxide blocks apoptosis in vascular smooth muscle cells, in part, by activating the cGMP signaling pathway [48, 49].

Thus, cytoprotection, namely, prevention of apoptosis, jointly with anti-inflammatory properties, are the two most explored biological functions of CO.

8. Role of Mitochondria and ROS Signalling on Costimulated Cytoprotection

Despite the existence of a long list of publications demonstrating cytoprotection of CO, the role of mitochondria on CO-induced cell death modulation is still poorly explored. First evidence showing mitochondria involvement on CO mode of action raised from the crucial role of ROS as signalling molecules, which were generated at mitochondrial level. Experimental approaches using respiratory deficient ρ^0 cells revealed the importance of mitochondrial generated ROS for CO to prevent inflammation in macrophages [19, 25] and to inhibit cell death in hepatocytes [43]. Furthermore, antioxidant addition has also indicated the importance of ROS signalling in several models, by reversing biological functions of CO, such as: (i) cardioprotection *via* inhibition of L-type Ca^{2+} channels [50], (ii) inhibition of apoptosis in astrocytes and neurons [47, 51], (iii) antiproliferative effect in airway smooth muscle cells [52], and (iv) induction of mitochondrial biogenesis in cardiomyocytes [31]. Thus, low levels of ROS appear as important signalling molecules in CO biology. It is worthy of note that although presenting ROS as essential signalling molecules, CO also limits mitochondrial oxidative stress and excessive ROS generation, for instance by inducing a mild uncoupling effect [29]. How CO controls the tight balance between generation of low signalling and high toxic levels of ROS is still a matter of debate.

Several hypotheses exist in the literature for the mode CO generates signalling ROS. Under physiological conditions, mitochondria continuously produce low levels of anion superoxide (O_2^-) as a byproduct of oxidative phosphorylation since 1–3% of the consumed oxygen is incompletely reduced to O_2^-. Anion superoxide is rapidly converted into hydrogen peroxide (H_2O_2) by the superoxide dismutase present in the mitochondrial matrix (Figure 2(a)) [53]. Interestingly, hydrogen peroxide is much more stable than anion superoxide, is capable of diffusing through biological membranes [54] and is a potent signalling molecule [55]. A growing body of evidence suggests that ROSs are physiologically generated at the level of complexes I and III of the mitochondrial respiratory chain. The most accepted hypothesis for CO to generate mitochondrial ROS is supported by CO capacity of (partially and/or reversely) inhibiting cytochrome c oxidase (complex IV), leading to electron accumulation at complex III level, which facilitates anion superoxide generation (Figure 2(b)) [56]. Still, one can also speculate that CO induces mitochondrial ROS generation by accelerating mitochondrial respiration and oxidative phosphorylation, increasing the amount of oxygen that is not totally reduced into water (Figure 2(c)). The exact mitochondrial target and mode of action for CO to accelerate mitochondrial respiration and functioning is still a matter of debate: complex IV is the strongest candidate [56], but complex II also seems to be involved [29]. Furthermore, CO also stimulates mitochondrial biogenesis, which can cause augmentation of ROS by increased mitochondrial population.

9. Mitochondrial Membrane Permeabilization and Carbon Monoxide

Apoptosis occurs via two distinct pathways: an extrinsic pathway (relying on cell surface membrane receptors) and an intrinsic pathway, which is triggered by several conditions of intracellular stress, leading to mitochondrial membrane permeabilization (MMP). In many models, MMP induces (i) mitochondrial transmembrane potential dissipation, (ii) respiratory chain uncoupling, (iii) ROS overproduction, (iv) ATP synthesis arrest, and (v) the release of several death-regulating molecules (activating proteases and nucleases), making the cell death process irreversible [57]. Despite the vast amount of publications concerning cell death prevention by CO, very few data are available for CO's direct implication on MMP control.

In 2006 Piantadosi et al. performed an interesting study on mitochondrial permeability pore transition, oxidative stress, and carbon monoxide. Rats were continuously exposed to 50 ppm of CO for 1, 3, or 7 days. At day 1 and 3, CO has increased a prooxidative state at liver mitochondria, which were more sensitive to Ca^{2+} for opening a pore on mitochondrial membrane. While at day 7, continuously exposure to CO prevented MMP in liver mitochondria by increasing expression of antioxidant enzymes: haem oxygenase-1 (HO-1) and manganese superoxide dismutase (SOD-2) [58]. Thus, CO-induced preconditioning generates an anti-oxidant state, which promotes liver resistance against MMP.

The direct role of CO on targeting mitochondria was evaluated in isolated nonsynaptic mitochondria from rat cortex. MMP was induced by Ca^{2+} and carboxyatractyloside treatment followed by the assessment of: (i) loss of mitochondrial potential, (ii) the opening of a ~800 Da pore through the inner membrane, (iii) swelling, and (iv) cytochrome *c* release [51]. CO inhibition of these four events was reversed by the addition of an antioxidant, β-carotene, indicating that ROS are important signalling molecules at mitochondrial level. Moreover, CO induced slight increase in mitochondrial-oxidized glutathione, which triggered ANT glutathionylation and enhanced its ATP/ADP translocation activity through the inner membrane. Thus, CO directly prevented MMP and its consequent astrocytic cell death and accelerated ATP/ADP transport through mitochondria. Accordingly, upregulation of HO-1 (by overexpression or cobalt protoporphyrin addition) is associated with an increase on mitochondrial transport carriers (carnitine, deoxynucleotide, and ATP/ADP carriers) and cytochrome *c* oxidase activities in experimental diabetes [59]. In isolated liver mitochondria from mouse, pretreatment with CO also inhibited mitochondrial swelling, depolarization, and the opening of a pore through the inner membrane, in a ROS-dependent manner [20]. In addition, cytochrome c oxidase transiently responded to low concentrations of CO by decreasing its activity in the first 5 minutes after treatment, while later on there was an increase of COX activity detected up to 30 minutes [20].

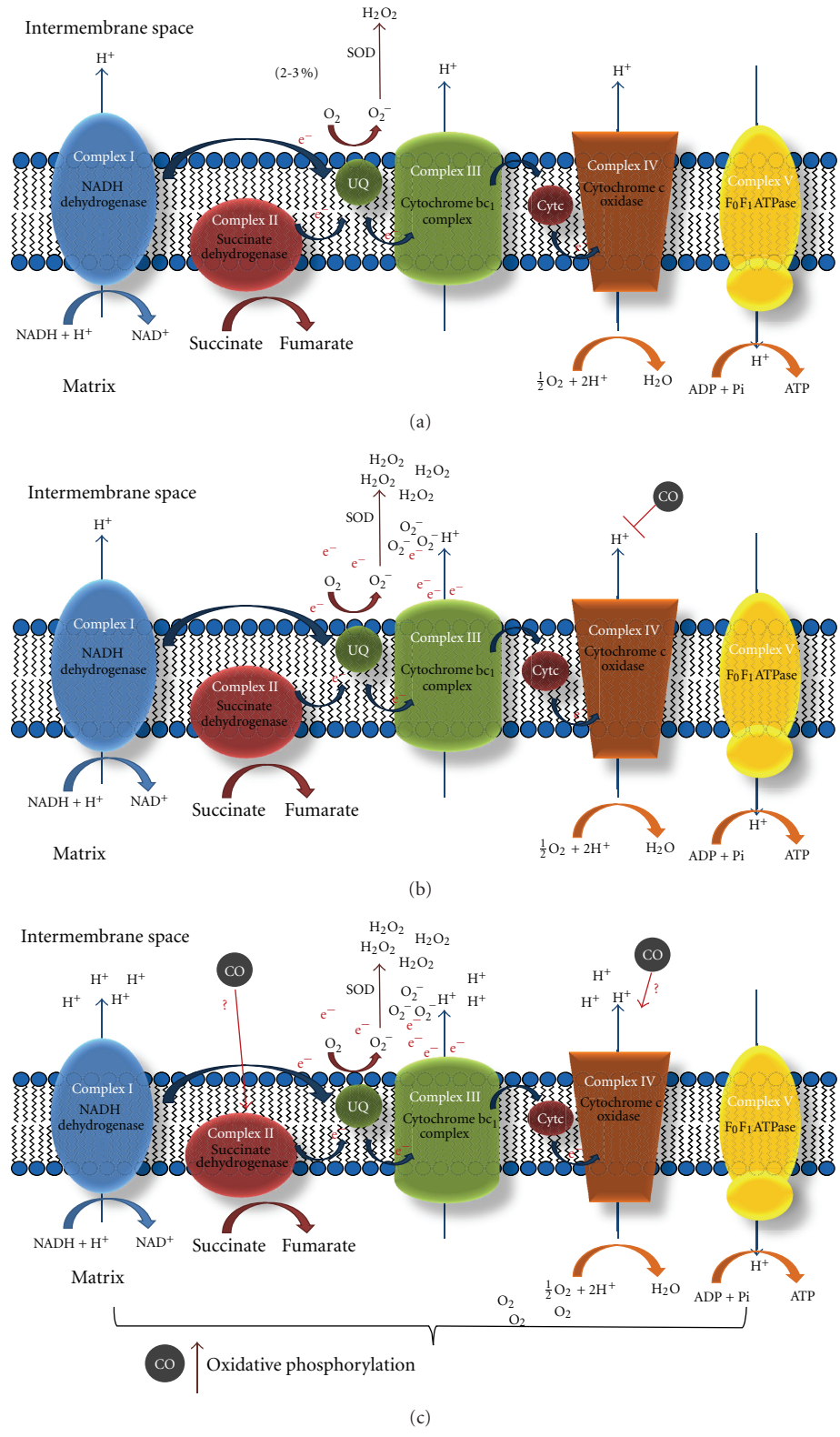


Figure 2: Proposed models for CO action on mitochondria. (a) Under physiological conditions, 1–3% of the consumed oxygen is incompletely reduced to anion superoxide (O_2^-), which is rapidly converted into hydrogen peroxide (H_2O_2) by the superoxide dismutase present in the mitochondrial matrix. (b) The most accepted hypothesis for CO to generate mitochondrial ROS is based on partially and/or reversely inhibition of cytochrome *c* oxidase (complex IV), leading to electron accumulation at complex III level, which facilitates anion superoxide generation. (c) Since low doses of CO also improve mitochondrial respiration, it can be speculated that CO induces mitochondrial ROS generation because oxidative phosphorylation is accelerated. The exact mitochondrial target is not fully understood, but complexes II and IV are strong candidates.

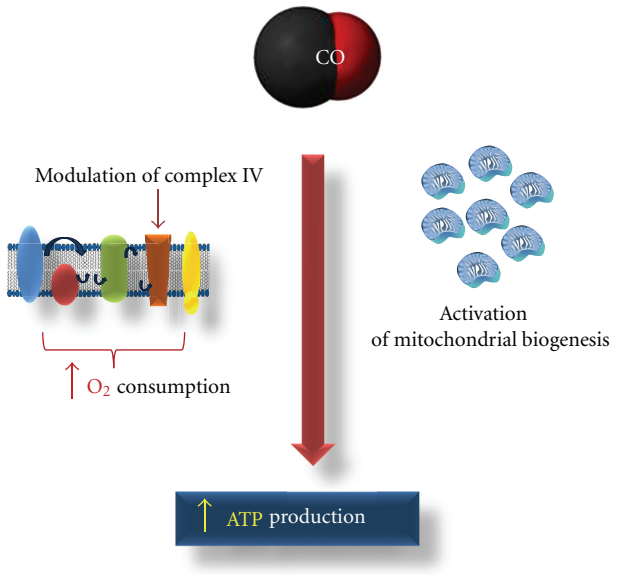


Figure 3: CO controls mitochondrial functioning and improves cellular energetic state (increased ATP generation) by two main ways: enhance of oxidative phosphorylation and induction of mitochondrial biogenesis.

It is worth of note that CO stimulates the antiapoptotic protein Bcl-2 expression in lung and cerebral ischemia models [60, 61], and Bcl-2 can translocate into mitochondrial membranes for preventing their permeabilization and cell death. Thus, CO is also capable for preventing MMP by indirectly acting on Bcl-2 expression levels and subcellular localization.

10. Final Remarks

"The dose makes the poison", carbon monoxide, a simple and small molecule, known to be toxic, presents beneficial pleiotropic effects. Low concentrations of CO are able to activate distinct endogenous cell defence mechanisms: antiapoptosis, anti-inflammation, antiproliferation, metabolism improvement, cardioprotection, and so forth. By preconditioning the cells CO is a cytoprotective factor, and mitochondria appear as the main cellular targets. In addition, CO is an endogenous gaseoustransmitter, which is physiologically generated in response to several types of stress.

Diverse, and apparently controversial, data are available in the literature concerning CO model of action on mitochondria, in particular on cytochrome c oxidase activity and on oxygen consumption. One can speculate that CO biological activity might depend on two main factors: period of CO exposure and gas concentration, giving rise to distinct responses. Furthermore, different CO sources increase the system complexity and do not facilitate data comparison. For instance, CO can be applied by different modes: (i) one single burst of CO (CO-saturated solutions fast gas diffusion), (ii) gas exposition (continuously application during the period of exposition), and (iii) CO-releasing molecules. Depending on the used CORM and its specific molecular characteristics, these molecules can be slow or fast CO releasers or can differently respond to tissues or to a physiological situation, such as an increase on oxidative stress.

In conclusion, CO controls mitochondrial functioning and oxidative metabolism, improving cellular energetic state, by modulation: COX activity, oxygen consumption, mitochondrial biogenesis, and ROS generation (Figure 3). Additionally, CO also prevents cell death: (i) by directly targeting mitochondria and inhibiting mitochondrial membrane permeabilization (MMP), (ii) by increasing antiapoptotic gene expression, such as Bcl-2, which also prevents MMP, or (iii) by interacting with the apoptosis-inducing cytochrome c-cardiolipin complex and inhibiting caspase activation [62].

The future in CO research field lays on the disclosure of the cross-talk between cell death and cell metabolism modulation (Figure 1). Mitochondria are the key organelle involved in the control of both cellular events. Thus, searching for the physiological mitochondrial target of CO and the biochemical and cellular mechanisms involved is crucial for the development of this gaseoustransmitter as a novel therapeutic agent.

Abbreviations

ANT: Adenosine nucleotide translocase
ATP: Adenosine triphosphate
O_2^- Anion superoxide
CO: Carbon monoxide
CORM: Carbon monoxide releasing molecules
COX: Cytochrome *c* oxidase or complex IV
HIF-1: Hypoxia-inducing factor-1
HO: Haem oxygenase
H_2O_2: Hydrogen peroxide
MMP: Mitochondrial membrane permeabilization
NRF-1: Nuclear respiratory factor 1
Nrf-2: Nuclear factor erythroid-2 related factor 2
ppm: Parts per million
RCR: Respiratory control index
ROS: Reactive oxygen species
sGC: Guanylate cyclase
TFAM: Mitochondrial transcription factor 3
UCP: Uncoupling respiration proteins
$\Delta\Psi m$: Mitochondrial membrane potential.

Acknowledgments

This work was supported by the Portuguese Fundação para a Ciência e Tecnologia project PTDC/SAU-NEU/098747/2008, HLAV's SFRH/BPD/27125/2006, and CSFQ's SFRH/BD/43387/2008 fellowships.

References

[1] C. G. Douglas, "The laws of combination of haemoglobin with carbon monoxide and oxygen," *The Journal of Physiology*, vol. 44, pp. 275–304, 1912.

[2] R. F. Coburn, W. S. Blakemore, and R. E. Forster, "Endogenous carbon monoxide production in man," *The Journal of Clinical Investigation*, vol. 42, pp. 1172–1178, 1963.

[3] D. J. Slebos, S. W. Ryter, and A. M. K. Choi, "Heme oxygenase-1 and carbon monoxide in pulmonary medicine," *Respiratory Research*, vol. 4, 2003.

[4] R. Tenhunen, H. S. Marver, and R. Schmid, "The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 61, no. 2, pp. 748–755, 1968.

[5] R. Tenhunen, H. S. Marver, and R. Schmid, "Microsomal heme oxygenase. Characterization of the enzyme," *Journal of Biological Chemistry*, vol. 244, no. 23, pp. 6388–6394, 1969.

[6] S. W. Ryter, J. Alam, and A. M. K. Choi, "Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications," *Physiological Reviews*, vol. 86, no. 2, pp. 583–650, 2006.

[7] R. Motterlini and L. E. Otterbein, "The therapeutic potential of carbon monoxide," *Nature Reviews Drug Discovery*, vol. 9, no. 9, pp. 728–743, 2010.

[8] M. P. Soares and F. H. Bach, "Heme oxygenase-1: from biology to therapeutic potential," *Trends in Molecular Medicine*, vol. 15, no. 2, pp. 50–58, 2009.

[9] G. L. Bannenberg and H. L. A. Vieira, "Therapeutic applications of the gaseous mediators carbon monoxide and hydrogen sulfide," *Expert Opinion on Therapeutic Patents*, vol. 19, no. 5, pp. 663–682, 2009.

[10] J. Boczkowski, J. J. Poderoso, and R. Motterlini, "CO-metal interaction: vital signaling from a lethal gas," *Trends in Biochemical Sciences*, vol. 31, no. 11, pp. 614–621, 2006.

[11] G. P. Roberts, H. Youn, and R. L. Kerby, "CO-Sensing mechanisms," *Microbiology and Molecular Biology Reviews*, vol. 68, no. 3, pp. 453–473, 2004.

[12] D. A. Greenberg, K. Jin, and A. A. Khan, "Neuroglobin: an endogenous neuroprotectant," *Current Opinion in Pharmacology*, vol. 8, no. 1, pp. 20–24, 2008.

[13] K. Nienhaus and G. U. Nienhaus, "Searching for neuroglobin's role in the brain," *IUBMB Life*, vol. 59, no. 8-9, pp. 490–497, 2007.

[14] C. E. Cooper and G. C. Brown, "The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance," *Journal of Bioenergetics and Biomembranes*, vol. 40, no. 5, pp. 533–539, 2008.

[15] B. Chance, M. Erecinska, and M. Wagner, "Mitochondrial responses to carbon monoxide toxicity," *Annals of the New York Academy of Sciences*, vol. 174, no. 1, pp. 193–204, 1970.

[16] S. D. Brown and C. A. Piantadosi, "In vivo binding of carbon monoxide to cytochrome c oxidase in rat brain," *Journal of Applied Physiology*, vol. 68, no. 2, pp. 604–610, 1990.

[17] S. D. Brown and C. A. Piantadosi, "Recovery of energy metabolism in rat brain after carbon monoxide hypoxia," *Journal of Clinical Investigation*, vol. 89, no. 2, pp. 666–672, 1992.

[18] J. R. Alonso, F. Cardellach, S. López, J. Casademont, and Ò. Miró, "Carbon monoxide specifically inhibits cytochrome C oxidase of human mitochondrial respiratory chain," *Pharmacology and Toxicology*, vol. 93, no. 3, pp. 142–146, 2003.

[19] B. S. Zuckerbraun, B. Y. Chin, M. Bilban et al., "Carbon monoxide signals via inhibition of cytochrome c oxidase and generation of mitochondrial reactive oxygen species," *FASEB Journal*, vol. 21, no. 4, pp. 1099–1106, 2007.

[20] C. S. F. Queiroga, A. S. Almeida, C. Martel, C. Brenner, P. M. Alves, and H. L. A. Vieira, "Carbon monoxide prevents hepatic mitochondrial membrane permeabilization," *BMC Cell Biology* , vol. 12, article 10, 2010.

[21] A. S. Almeida, "Carbon monoxide modulates apoptosis by reinforcing oxidative metabolism in astrocytes: role of Bcl-2," *Journal of Biological Chemistry*. In press.

[22] P. Haab, "The effect of carbon monoxide on respiration," *Experientia*, vol. 46, no. 11-12, pp. 1202–1206, 1990.

[23] G. D'Amico, F. Lam, T. Hagen, and S. Moncada, "Inhibition of cellular respiration by endogenously produced carbon monoxide," *Journal of Cell Science*, vol. 119, no. 11, pp. 2291–2298, 2006.

[24] R. Fukuda, H. Zhang, J. W. Kim, L. Shimoda, C. V. Dang, and G. Semenza, "HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells," *Cell*, vol. 129, no. 1, pp. 111–122, 2007.

[25] B. Y. Chin, G. Jiang, B. Wegiel et al., "Hypoxia-inducible factor 1α stabilization by carbon monoxide results in cytoprotective preconditioning," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 12, pp. 5109–5114, 2007.

[26] M. Palacios-Callender, M. Quintero, V. S. Hollis, R. J. Springe, and S. Moncada, "Endogenous NO regulates superioxide production at low oxygen concentrations by modifying the redox state of cytochrome c oxidase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 20, pp. 7630–7635, 2004.

[27] A. Sandouka, E. Balogun, R. Foresti et al., "Carbon monoxide-releasing molecules (CO-RMs) modulate respiration in isolated mitochondria," *Cellular and Molecular Biology*, vol. 51, no. 4, pp. 425–432, 2005.

[28] A. Sandouka, B. J. Fuller, B. E. Mann, C. J. Green, R. Foresti, and R. Motterlini, "Treatment with CO-RMs during cold storage improves renal function at reperfusion," *Kidney International*, vol. 69, no. 2, pp. 239–247, 2006.

[29] L. Lo Iacono, J. Boczkowski, R. Zini et al., "A carbon monoxide-releasing molecule (CORM-3) uncouples mitochondrial respiration and modulates the production of reactive oxygen species," *Free Radical Biology and Medicine*, vol. 50, no. 11, pp. 1556–1564, 2011.

[30] S. Lancel, S. M. Hassoun, R. Favory, B. Decoster, R. Motterlini, and R. Neviere, "Carbon monoxide rescues mice from lethal sepsis by supporting mitochondrial energetic metabolism and activating mitochondrial biogenesis," *Journal of Pharmacology and Experimental Therapeutics*, vol. 329, no. 2, pp. 641–648, 2009.

[31] H. B. Suliman, M. S. Carraway, L. G. Tatro, and C. A. Piantadosi, "A new activating role for CO in cardiac mitochondrial biogenesis," *Journal of Cell Science*, vol. 120, no. 2, pp. 299–308, 2007.

[32] C. A. Piantadosi, M. S. Carraway, A. Babiker, and H. B. Suliman, "Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via nrf2-mediated transcriptional control of nuclear respiratory factor-1," *Circulation Research*, vol. 103, no. 11, pp. 1232–1240, 2008.

[33] H. B. Suliman, M. S. Carraway, A. S. Ali, C. M. Reynolds, K. E. Welty-Wolf, and C. A. Piantadosi, "The CO/HO system reverses inhibition of mitochondrial biogenesis and prevents murine doxorubicin cardiomyopathy," *Journal of Clinical Investigation*, vol. 117, no. 12, pp. 3730–3741, 2007.

[34] C. A. Piantadosi, C. M. Withers, R. R. Bartz et al., "Heme oxygenase-1 couples activation of mitochondrial biogenesis to anti-inflammatory cytokine expression," *Journal of Biological Chemistry*, vol. 286, no. 18, pp. 16374–16385, 2011.

[35] M. A. Rhodes, M. S. Carraway, C. A. Piantadosi et al., "Carbon monoxide, skeletal muscle oxidative stress, and mitochondrial

biogenesis in humans," *American Journal of Physiology, Heart and Circulatory Physiology*, vol. 297, no. 1, pp. H392–H399, 2009.

[36] M. Lavitrano, R. T. Smolenski, A. Musumeci et al., "Carbon monoxide improves cardiac energetics and safeguards the heart during reperfusion after cardiopulmonary bypass in pigs," *FASEB Journal*, vol. 18, no. 10, pp. 1093–1095, 2004.

[37] K. Ahlstrom, B. Biber, A. Åberg et al., "Metabolic responses in ischemic myocardium after inhalation of carbon monoxide," *Acta Anaesthesiologica Scandinavica*, vol. 53, no. 8, pp. 1036–1042, 2009.

[38] T. Y. Tsui, A. Obed, Y. T. Siu et al., "Carbon monoxide inhalation rescues mice from fulminant hepatitis through improving hepatic energy metabolism," *Shock*, vol. 27, no. 2, pp. 165–171, 2007.

[39] T. Y. Tsui, Y. T. Siu, H. J. Schlitt, and S. T. Fan, "Heme oxygenase-1-derived carbon monoxide stimulates adenosine triphosphate generation in human hepatocyte," *Biochemical and Biophysical Research Communications*, vol. 336, no. 3, pp. 898–902, 2005.

[40] S. Brouard, L. E. Otterbein, J. Anrather et al., "Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis," *Journal of Experimental Medicine*, vol. 192, no. 7, pp. 1015–1025, 2000.

[41] H. S. Kim, P. A. Loughran, P. K. Kim, T. R. Billiar, and B. S. Zuckerbraun, "Carbon monoxide protects hepatocytes from TNF-α/Actinomycin D by inhibition of the caspase-8-mediated apoptotic pathway," *Biochemical and Biophysical Research Communications*, vol. 344, no. 4, pp. 1172–1178, 2006.

[42] L. C. de la Rosa, T. E. Vrenken, R. A. Hannivoort et al., "Carbon monoxide blocks oxidative stress-induced hepatocyte apoptosis via inhibition of the p54 JNK isoform," *Free Radical Biology and Medicine*, vol. 44, no. 7, pp. 1323–1333, 2008.

[43] H. S. Kim, P. A. Loughran, J. Rao, T. R. Billiar, and B. S. Zuckerbraun, "Carbon monoxide activates NF-κB via ROS generation and Akt pathways to protect against cell death of hepatocytes," *American Journal of Physiology, Gastrointestinal and Liver Physiology*, vol. 295, no. 1, pp. G146–G152, 2008.

[44] X. Zhang, P. Shan, J. Alam, X. Y. Fu, and P. J. Lee, "Carbon monoxide differentially modulates STAT1 and STAT3 and inhibits apoptosis via a phosphatidylinositol 3-kinase/Akt and p38 kinase-dependent STAT3 pathway during anoxia-reoxygenation injury," *Journal of Biological Chemistry*, vol. 280, no. 10, pp. 8714–8721, 2005.

[45] X. Zhang, P. Shan, L. E. Otterbein et al., "Carbon monoxide inhibition of apoptosis during ischemia-reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase pathway and involves caspase 3," *Journal of Biological Chemistry*, vol. 278, no. 2, pp. 1248–1258, 2003.

[46] X. Wang, Y. Wang, H. P. Kim, K. Nakahira, S. W. Ryter, and A. M. K. Choi, "Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation," *Journal of Biological Chemistry*, vol. 282, no. 3, pp. 1718–1726, 2007.

[47] H. L. A. Vieira, C. S. F. Queiroga, and P. M. Alves, "Preconditioning induced by carbon monoxide provides neuronal protection against apoptosis," *Journal of Neurochemistry*, vol. 107, no. 2, pp. 375–384, 2008.

[48] X. M. Liu, G. B. Chapman, K. J. Peyton, A. I. Schafer, and W. Durante, "Carbon monoxide inhibits apoptosis in vascular smooth muscle cells," *Cardiovascular Research*, vol. 55, no. 2, pp. 396–405, 2002.

[49] X. M. Liu, G. B. Chapman, K. J. Peyton, A. I. Schafer, and W. Durante, "Antiapoptotic action of carbon monoxide on cultured vascular smooth muscle cells," *Experimental Biology and Medicine*, vol. 228, no. 5, pp. 572–575, 2003.

[50] J. L. Scragg, M. L. Dallas, J. A. Wilkinson, G. Varadi, and C. Peers, "Carbon monoxide inhibits L-type Ca^{2+} channels via redox modulation of key cysteine residues by mitochondrial reactive oxygen species," *Journal of Biological Chemistry*, vol. 283, no. 36, pp. 24412–24419, 2008.

[51] C. S. F. Queiroga, A. S. Almeida, C. Martel, C. Brenner, P. M. Alves, and H. L. A. Vieira, "Glutathionylation of adenine nucleotide translocase induced by carbon monoxide prevents mitochondrial membrane permeabilization and apoptosis," *Journal of Biological Chemistry*, vol. 285, no. 22, pp. 17077–17088, 2010.

[52] C. Taillé, J. El-Benna, S. Lanone, J. Boczkowski, and R. Motterlini, "Mitochondrial respiratory chain and NAD(P)H oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle," *Journal of Biological Chemistry*, vol. 280, no. 27, pp. 25350–25360, 2005.

[53] H. Nohl, L. Gille, and K. Staniek, "Intracellular generation of reactive oxygen species by mitochondria," *Biochemical Pharmacology*, vol. 69, no. 5, pp. 719–723, 2005.

[54] T. P. Cash, Y. Pan, and M. C. Simon, "Reactive oxygen species and cellular oxygen sensing," *Free Radical Biology and Medicine*, vol. 43, no. 9, pp. 1219–1225, 2007.

[55] E. A. Veal, A. M. Day, and B. A. Morgan, "Hydrogen peroxide sensing and signaling," *Molecular Cell*, vol. 26, no. 1, pp. 1–14, 2007.

[56] M. Bilban, A. Haschemi, B. Wegiel, B. Y. Chin, O. Wagner, and L. E. Otterbein, "Heme oxygenase and carbon monoxide initiate homeostatic signaling," *Journal of Molecular Medicine*, vol. 86, no. 3, pp. 267–279, 2008.

[57] G. Kroemer, L. Galluzzi, and C. Brenner, "Mitochondrial membrane permeabilization in cell death," *Physiological Reviews*, vol. 87, no. 1, pp. 99–163, 2007.

[58] C. A. Piantadosi, M. S. Carraway, and H. B. Suliman, "Carbon monoxide, oxidative stress, and mitochondrial permeability pore transition," *Free Radical Biology and Medicine*, vol. 40, no. 8, pp. 1332–1339, 2006.

[59] M. A. Di Noia, S. Van Driesche, F. Palmieri et al., "Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome C oxidase activity in experimental diabetes," *Journal of Biological Chemistry*, vol. 281, no. 23, pp. 15687–15693, 2006.

[60] N. Panahian, M. Yoshiura, and M. D. Maines, "Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice," *Journal of Neurochemistry*, vol. 72, no. 3, pp. 1187–1203, 1999.

[61] X. Zhang, P. Shan, J. Alam, R. J. Davis, R. A. Flavell, and P. J. Lee, "Carbon monoxide modulates Fas/Fas ligand, caspases, and Bcl-2 family proteins via the p38α mitogen-activated protein kinase pathway during ischemia-reperfusion lung injury," *Journal of Biological Chemistry*, vol. 278, no. 24, pp. 22061–22070, 2003.

[62] S. M. Kapetanaki, G. Silkstone, I. Husu, U. Liebl, M. T. Wilson, and M. H. Vos, "Interaction of carbon monoxide with the apoptosis-inducing cytochrome c-cardiolipin complex," *Biochemistry*, vol. 48, no. 7, pp. 1613–1619, 2009.

Hindawi Publishing Corporation
Biochemistry Research International
Volume 2012, Article ID 951539, 12 pages
doi:10.1155/2012/951539

Review Article

Mitochondria Death/Survival Signaling Pathways in Cardiotoxicity Induced by Anthracyclines and Anticancer-Targeted Therapies

David Montaigne, Christopher Hurt, and Remi Neviere

Department of Physiology (EA4484), Faculty of Medicine, University of Lille 1, Place de Verdun, 59045 Lille, France

Correspondence should be addressed to Remi Neviere, rneviere@univ-lille2.fr

Received 16 November 2011; Revised 4 January 2012; Accepted 9 January 2012

Academic Editor: Catherine Brenner

Anthracyclines remain the cornerstone of treatment in many malignancies but these agents have a cumulative dose relationship with cardiotoxicity. Development of cardiomyopathy and congestive heart failure induced by anthracyclines are typically dose-dependent, irreversible, and cumulative. Although past studies of cardiotoxicity have focused on anthracyclines, more recently interest has turned to anticancer drugs that target many proteins kinases, such as tyrosine kinases. An attractive model to explain the mechanism of this cardiotoxicity could be myocyte loss through cell death pathways. Inhibition of mitochondrial transition permeability is a valuable tool to prevent doxorubicin-induced cardiotoxicity. In response to anthracycline treatment, activation of several protein kinases, neuregulin/ErbB2 signaling, and transcriptional factors modify mitochondrial functions that determine cell death or survival through the modulation of mitochondrial membrane permeability. Cellular response to anthracyclines is also modulated by a myriad of transcriptional factors that influence cell fate. Several novel targeted chemotherapeutic agents have been associated with a small but worrying risk of left ventricular dysfunction. Agents such as trastuzumab and tyrosine kinase inhibitors can lead to cardiotoxicity that is fundamentally different from that caused by anthracyclines, whereas biological effects converge to the mitochondria as a critical target.

1. Introduction

Cardiotoxicity is a term often used to describe a broad range of adverse effects on heart function induced by therapeutic molecules. These effects may either emerge early in preclinical studies or become apparent latter in the clinical setting after the drug has already been licensed for clinical use. The use of several chemotherapeutics for the treatment of cancer is associated with a risk of cardiovascular complications [1–3]. They present as a defect in cardiac function that can be either symptomatic or not. The classic example of this issue is the use of anthracyclines such as doxorubicin, which is commonly prescribed to treat hematological malignancies and solid tumors [4, 5].

Potential cardiovascular toxicities linked to anticancer drugs include increases of QT duration, arrhythmias, and myocardial ischemia (antimetabolite compounds) [6], hypertension and thromboembolic complications (antiangiogenic agents) [7], and myocardial dysfunction [1]. The latter, variable in severity, can be reversible or not and can occur during treatment or later on. For example, the clinical use of anthracyclines such as doxorubicin is hampered by the development of cardiomyopathy and congestive heart failure, which are typically dose-dependent and cumulative. While acute cardiotoxicity occurs, the most troublesome form manifests late after treatment and is characterized by structural changes of the human heart [8], leading to decreases in the left ventricle wall thickness and myocardium mass, as well as reduced ventricular compliance [9]. Unlike acute toxicity, the delayed manifestation of anthracycline use often presents as symptomatic heart failure and is considered largely irreversible [1, 5, 10].

Although past studies of cardiotoxicity have focused on anthracyclines, more recently interest has turned to

anticancer drugs that target many proteins kinases, such as tyrosine kinases [2]. Targeted therapeutics, particularly those that inhibit the activity of protein kinases that are mutated and/or overexpressed in cancer, have revolutionized the treatment of some cancers and improved survival in many others [11, 12]. Unexpected cardiotoxicity induced by targeted drugs has been related to the existence of numerous parallels between signaling pathways that drive tumorigenesis and those that regulate survival of cardiomyocytes [12–15]. For example, on-target heart toxicity of trastuzumab, a monoclonal antibody against the ErbB2 receptor [16] revealed that human epidermal growth factor receptor 2 signaling also interfered with survival pathway in cardiomyocyte, a terminally differentiated cell [16, 17]. At this point, it can be hypothesized that mitochondrial dysfunction and ATP depletion are the main contributors to targeted therapy-induced cardiac toxicity [15].

An attractive model to explain the mechanism of this cardiotoxicity could be myocyte loss through cell death pathways [18, 19]. Given the limited regenerative capacity of the heart, cumulative toxicity may be explained by the progressive increase of cardiac cell loss. Cardiac cell stress (specifically oxidative stress induced by anthracyclines and many kinase inhibitors) activates apoptosis and necrosis via a mitochondrial pathway [1, 10]. As mitochondria are a central component of intrinsic apoptotic and necrotic pathways, mitochondrial "effects" of anticancer drugs are to be an expected outcome of adverse interactions between the drug and cells [20].

2. Overview of Anthracycline Cardiotoxicity

2.1. Clinical Picture of Cardiac Toxicity. Anthracycline-induced cardiotoxicity has been categorized into acute, early-onset chronic progressive and late-onset chronic progressive forms [21]. Acute cardiotoxicity occurs during or shortly after drug infusion and includes nonspecific EKG changes and arrhythmias, which may be accompanied in some patients by heart failure and pericarditis-myocarditis syndrome [9]. These complications are typically reversible, not dose-dependent and do not preclude further anthracycline use. Single cases of acute cardiac failure and sudden death were also reported [9, 22]. The subacute cardiac toxicity occurs within a few weeks, clinically resembles myocarditis (with edema and thickening of the left ventricle LV walls), and is associated with 60% mortality [22]. Acute cardiotoxicity occur in 1% of patients, whereas the subacute form occurs in 1.4–2% of patients [9]. Clinically the most significant effect of anthracyclines is chronic cardiac toxicity that may lead to LV dysfunction and congestive heart failure [23, 24]. Late-onset chronic progressive cardiotoxicity usually appears at least one year after completion of therapy and manifests clinically in 1.6–5% of patients [24]. Late-onset chronic progressive may not become evident until 10 to 20 years after the first dose of cancer treatment. The prognosis in anthracycline-related heart failure is poor, with 50% 2-year mortality in untreated established LV dysfunction [24, 25]. The most important risk factor for late cardiac toxicity is the cumulative anthracycline dose [25].

It is believed that each anthracycline dose causes structural changes to cardiomyocytes, which ultimately lead to cardiomyocyte death. These defects are balanced by compensatory mechanisms until a certain threshold, above which ventricular remodeling common to multiple forms of cardiac injury is triggered. The estimated risk of chronic heart failure for doxorubicin dose greater than 400 mg/m^2 ranges from 3% to 5%, for 550 mg/m^2 from 7% to 26%, and for 700 mg/m^2 from 18% to 48% [1, 10]. In clinical practice, however, it seems wise to estimate the risk as being in the upper limit of the given ranges and adapt maximum cumulative doses accordingly [26]. Despite a considerable variability in individual dose-response relationship for cardiac toxicity, the maximum lifetime cumulative dose for doxorubicin is 400 to 550 mg/m^2 [1, 10]. The long-term follow-up data of patients treated with adjuvant anthracycline-based regimens demonstrated increased incidence of symptomatic and asymptomatic left ventricular dysfunction and decrease in the mean LVEF value, suggesting that the incidence and severity of postanthracycline heart damage increases with time [26]. Overall, these data stress the importance of cardiac follow-up well beyond the treatment period.

2.2. Proposed Pathophysiology. Despite a remarkably extensive literature on many aspects of cardiotoxicity, a single unifying theory for the deleterious effects of anthracyclines on the heart is still lacking. Anthracycline-induced myocardial damage has long been regarded as occurring primarily through the generation of reactive oxygen species and free radicals [27]. This oxidative stress model is supported by many studies showing ROS formation, especially in the setting of increased intracellular iron levels, in response to anthracycline treatment [27, 28]. Recent studies, however, have suggested that the ROS model is inadequate to account for all features of anthracycline cardiotoxicity [27]. Indeed, there is strong evidence that anthracycline cardiotoxicity stems from (at least partially) ROS-independent mechanisms, such as cardiomyocyte apoptosis or necrosis, disruption of normal sarcomere structure and altered energetics impairing the cardiac cell ability to generate adequate contraction [18, 29–31]. Recent studies have highlighted the effects of anthracyclines on compensatory prosurvival mechanisms, such as neuregulin/heregulin-Erb/HER2 and cell salvage kinase pathways, which may modulate the development of heart failure [32, 33]. Figure 1 provides a summary of the different signaling pathways involved in anthracycline-induced cardiotoxicity.

2.3. Oxidative Stress Pathway and Iron Hypothesis. The chemical structure of anthracyclines is complex: these drugs are composed of an aglycone and a sugar. The aglycone consists of a tetracyclic ring with adjacent quinone-hydroquinone moieties. Quinone moieties have toxicological importance because of their involvement in both reductive and oxidative biotransformation leading to highly reactive species involved in cardiotoxicity [27, 34].

One-electron reduction of the tetracyclic ring of anthracyclines leads to the formation of a semiquinone free radical. This radical is relatively stable in an anoxic environment,

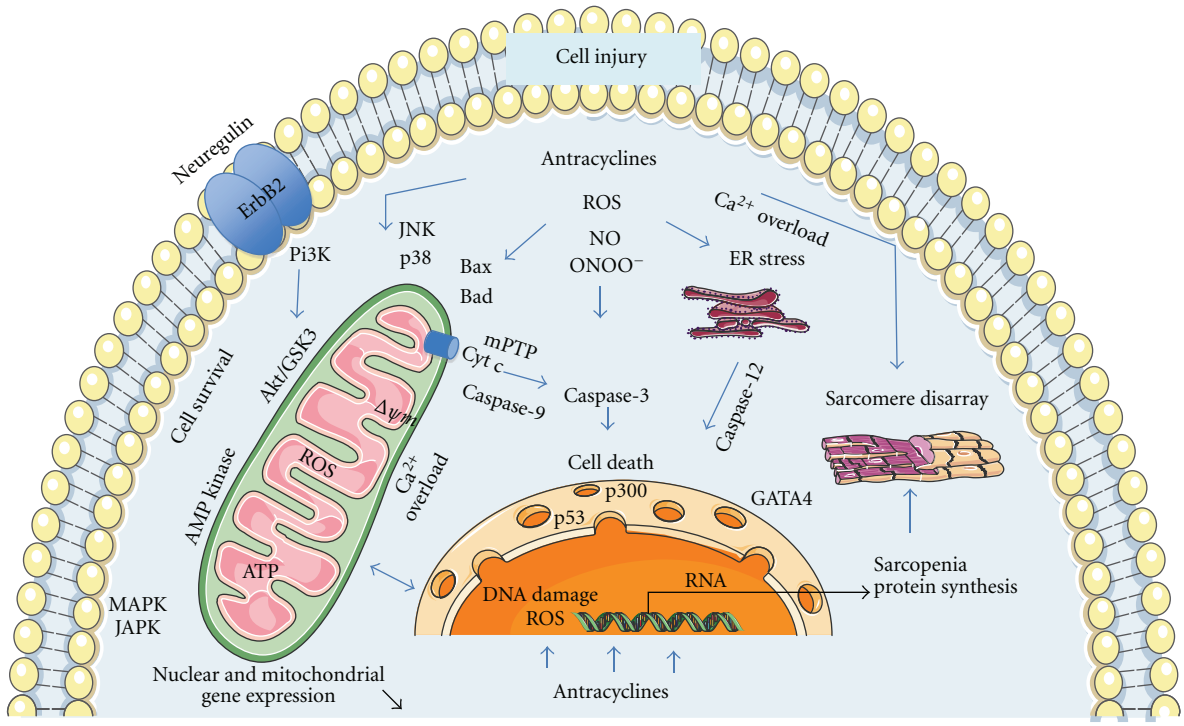


Figure 1: Potential signaling pathways involved in anthracycline-induced cardiomyocyte injury. Anthracycline-induced cell death is balanced by intracellular survival signaling which is linked to neuregulin/ErbB2 and Akt activation. The suggested principal mechanism of anthracycline damage is via generation of reactive oxygen species ROS by iron-anthracycline complexes, leading to lipid peroxidation and membrane damage. Oxidative stress (ROS, nitric oxide NO, and peroxynitrite ONOO–) causes activation of kinase pathways (mitogen-activated protein kinase MAPK, stress-activated protein kinase SAPK, c-Jun N-terminal kinases JNK) modulating response to anthracyclines and linking to apoptotic pathway. In mitochondria, ROS and calcium overload lead to the release of cytochrome c (cyt c) from mitochondria into cytoplasm, via mitochondrial permeability transition pore opening (mPTP), which results in membrane potential dissipation (delta psi m), activation of caspases and apoptosis. Other putative mechanisms include damage to nuclear DNA, disruption of sarcomeric protein, suppression of transcription factors (GATA-4, p300, p53) that regulate cell survival and sarcomeric protein synthesis, and disturbance of energy metabolism.

but under normoxic conditions, its unpaired electron is donated to oxygen, forming superoxide radicals. Suitable flavoproteins such as complex I catalyze the formation of reduced semiquinone radicals by accepting electrons from NADH or NADPH and donating them to anthracyclines. This sequence of reactions, known as "redox cycling," can be highly damaging, because a relatively small amount of drug is sufficient for the formation of numerous superoxide radicals. The redox cycling of anthracyclines has been described in cytoplasm, mitochondria, and sarcoplasmic reticulum [27]. The first targets of anthracycline-mediated free-radical damage are various cellular membranes, which are rich in lipids prone to peroxidation. This radical damage results in production of many stable and highly toxic aldehydes, which further attack macromolecular targets. Although formation of ROS is induced by the quinone moiety of anthracyclines, oxidative stress can also occur via induction of nitric oxide synthase, leading to superoxide anion, nitric oxide and peroxynitrite formation [18, 27].

Promotion of myocardial oxidative stress remains by far the most frequently proposed mechanisms of anthracycline-induced cardiotoxicity [27]. Production of ROS that follows nuclear binding of the drug results in injuries to DNA as well as to cell membranes and mitochondria. ROS production is involved in a vast variety of cardiotoxicity inducing mechanisms, including impaired expression of cardiac proteins, disruption of cellular and mitochondrial calcium homeostasis, induction of mitochondrial DNA lesions, disruption of mitochondrial bioenergetics and ATP transfer systems, and degradation of myofilament and cytoskeleton proteins [18, 29–31]. Involvement of oxidative stress in the pathogenesis of anthracycline cardiotoxicity has been supported by several approaches, that is, isolated cardiac cells displaying perimitochondrial ROS production in response to anthracycline exposition, cultured cell and animal models showing that antioxidant prevented anthracycline-induced cardiotoxicity, and resistance of transgenic mice overexpressing the mitochondrial manganese-superoxide dismutase to anthracycline-induced cardiotoxicity [35].

Anthracyclines may promote the formation of ROS through redox cycling of their aglycones as well as their anthracycline-iron complexes. Indeed, unless adequately sequestered within the cells, iron can dramatically promote ROS production by the Fenton and Haber-Weiss reactions and the formation of reactive anthracycline-iron complexes [27, 34]. Cellular iron (Fe) level is tightly regulated by the

transferring receptor and storage regulating ferritin, both of which are, themselves regulated at the posttranscriptional level by interactions of Fe-regulatory-protein (IRP-1) with specific motifs iron-responsive-elements (IREs) in target genes. Doxorubicin and its metabolites can disrupt the Fe-S cluster of cytoplasmic aconitase and inhibit IRP-1. In doxorubicin-treated cardiomyocytes, increased IRP-1 inhibition leads to intracellular Fe accumulation causing increased oxidative stress [36].

Consistent results suggesting the involvement of oxidative and nitrosative oxidant stresses in anthracycline-induced cardiotoxicity provide a rationale for cardioprotection with antioxidants in humans [27, 37]. Unfortunately, use of different antioxidant agents have failed to provide protection in both preclinical experiments and clinical studies [3]. First generation antioxidant molecules such as N-acetylcystein, vitamins D, E have been investigated on the basis of some protective effects observed in animal models [27]. None of these antioxidant approaches has yet shown consistent efficiency. The only compound found to reduce long-term cardiac dysfunction is dexrazoxane, although whether its efficiency is related to its antioxidant properties or other mechanisms remains under debate [3]. For example, underlying mechanisms of dexrazoxane include prevention of iron accumulation, which is implicated in increased ROS production and anthracycline-induced cardiotoxicity.

2.4. Mitochondrial Apoptosis and Necrosis Pathway. Cardiac side effects of anthracyclines involve two main mechanisms, which interact with each other, oxidative stress and apoptosis [18, 27]. Most of the cellular events induced by ROS generation contribute to cardiomyocyte death, which has been shown to be a primary mechanism for anthracycline-induced cardiomyopathy. Indeed, cardiac myocyte loss following activation of both apoptotic and necrotic pathways provide an attractive explanation for anthracycline-induced cardiotoxicity [18, 20, 38]. Studies in animals have demonstrated that apoptotic cell death occurs after in vivo exposure to anthracyclines [38]. Experimental cell cultures have also shown that anthracyclines induce both apoptotic and necrotic cell death [38]. Evidence of mitochondrial injuries and hallmarks of apoptosis have been found in endomyocardial biopsies of patients treated with anthracyclines [8, 39]. Overall, cardiac myocyte death following anthracycline administration typically presents with biochemical features of apoptosis and the morphological aspect of cell necrosis [18, 20, 38].

Anthracycline-induced apoptosis in the heart appears to involve a mitochondrial pathway, which requires Bax, cytochrome c and caspase-3 [20]. Typically, anthracycline treatment increases mitochondrial oxidative stress and disrupts intracellular calcium levels [40, 41]. Increased intracellular calcium, favored by calcium flux aberrations, eventually raises mitochondrial calcium levels. Above a certain threshold, this calcium overload triggers permeability transition of the mitochondria, resulting in the dissipation of transmembrane potential, as well as mitochondrial swelling and increased permeability of its outer membrane to apoptotic factors such as cytochrome c [20]. In the cytosol, cytochrome c forms a complex with the adaptor protein apoptosis protease activator protein-1 (Apaf-1), dATP, and caspase-9, so-called apoptosome, which in turn activates caspase-9. The intrinsic pathway converges then to the downstream executioner caspases [20].

The current hypothesis is that necrotic and some forms of apoptotic cell death involve prolonged opening of a large conductance pore in the mitochondria, known as the mitochondrial permeability transition pore mPTP [36]. In its fully open state, the mPTP has been reported to allow unrestricted movement of solutes of <1.5 kDa. The activation of the mPTP in isolated mitochondria has been shown to lead to mitochondrial swelling, which is commonly used as an assay for mPTP opening. In spite of the great recent interest concerning mPTP and its apparent importance in cell death, its molecular identity is unknown. It has been proposed that the mPTP is formed through conformational change in the association of the adenine nucleotide translocator (ANT) with the voltage dependent anion channel (VDAC) contact sites between the inner and outer mitochondrial membranes [42, 43]. Cyclophilin D is thought to regulate the opening of the pore via its interactions with ANT. These interactions are inhibited by cyclosporine A supporting the idea that cyclophilin D play a role in pore opening [42, 43]. Recent studies have shown that genetic ablation of either ANT or VDAC isoforms did not result in the absence of mPTP, suggesting that neither of these proteins is an obligatory component [43]. In contrat, ablation of cyclophilin D reduces ischemia-reperfusion-induced cell death, suggesting a role for cyclophilin in the mPTP [44, 45]. Growing evidence suggests that the phosphate carrier is a critical component of the mPTP and that interaction between ANT and the phosphate carrier can modulate mPTP opening [45].

For many years, it has been put forward that the mPTP contributed mainly to apoptotic cell death as a protagonist of mitochondrial permeabilization. Recent data suggests, however, that an increase in mitochondrial membrane permeabilization is one of the key events in both apoptotic and necrotic cell death [43, 44]. This information is important since necrosis occurs in many forms of adult human heart injuries, including the cardiotoxic effects of anticancer drugs. Indeed, in the mid-2000s, new experimental studies suggested that mPTP did not initiate apoptosis and that this complex instead played a central role in necrosis, especially in the heart. In this line of reasoning, it has been shown that cyclosporine A (CsA), a known inhibitor of the mPTP, can reduce the occurrence of cardiac and brain cell necrosis during ischemia reperfusion injury. Studies of cyclophilin-D-deficient mice have also provided consistent evidence that the mPTP plays a crucial role in cell necrosis [46, 47]. In these mice, mPTP was still functional but cyclophilin-D ablation increased the amount of calcium required for mPTP opening and abolished the sensitivity to CsA [46, 48]. Cyclophilin-D-deficient mice had increased resistance against necrotic stimuli such as calcium overload, whereas these animals still died in response to treatments with classical apoptotic inducers such as staurosporine or etoposide. Overall, this data indicates that mPTP opening is chiefly involved in cardiac cell necrosis rather than in triggering cytochrome c release during early apoptosis.

2.5. Alternative Types of Cell Death: Oncosis and Autophagy. Features of cell oncosis, which is typically associated in cardiomyocytes with mitochondrial and cytoplasmic swelling, coagulated sarcomere and early rupture of the plasma membrane [49] have been described in anthracycline-induced cardiac cell damage [50]. As mentioned above, recent studies have shown that this form of cell death can be well controlled and programmed through mPTP-dependent mechanisms. The rationale is that increased ROS leads to mitochondrial calcium overloading, promotes mPTP opening, causes mitochondrial swelling and ATP depletion, and hence triggers necrotic cell death [20]. Autophagy has evolved as a conserving process that uses bulk degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles. In the heart, autophagy is important for the turnover of organelles at low basal levels under normal conditions and it is upregulated in response to stresses such as ischemia/reperfusion and in cardiovascular diseases such as heart failure. Recent evidence suggests that autophagic cell death may play a significant role in the myocardial dysfunction induced by doxorubicin [51]. Overall, it could be stated that in anthracyclin cardiotoxicity, mitochondria is the crossroad for apoptosis, necrosis and autophagy processes, which may converge in dying cells in response to different pathways including ROS production, calcium overload, and DNA lesions.

3. Mitochondria-Related Survival/Death Pathways

If apoptotic and necrotic cell death are central to the feature of anthracycline-induced cardiotoxicity, then the underlying mechanisms in play are worth exploring, as they may lead to cytoprotective strategies. Likewise, better understanding of activation of cell survival pathways in response to anthracycline exposition may also provide valuable knowledge that would help in the development of new cytoprotective strategies.

3.1. Mitochondrial Permeability Transition. Bioenergetic failure, enzyme inhibitions, lipid peroxidations, induction of membrane disorders as well as the initiation of oxidative stress are being attributed to the accumulation of anthracyclines at or inside mitochondria. From heart tissue perfused with anthracyclines two distinct cellular sites of drug accumulation were the nuclei and mitochondria, which become labeled with the drug [52, 53]. Hence, it has been commonly proposed that deleterious signals related to anthracycline exposure converge to the mitochondria to favor mPTP-mediated cell death [54]. In addition, if this hypothesis is correct, an understanding cardioprotective mechanisms is intimately linked to an understanding of the mechanisms by which mitochondria regulate cell death.

Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration can be demonstrated by using cardiac mitochondria isolated from doxorubicin-treated animals [40, 41]. For example, activation of the selective cyclosporine- (CsA-)sensitive calcium channel of cardiac mitochondria by doxorubicin occurs both in vitro [55] and in cardiac mitochondria isolated in rats having undergone chronic in vivo treatment with doxorubicin [56]. In the latter protocol of exposition (2 mg/kg/week doxorubicin treatment for 13 weeks), isolated mitochondria have a lower respiratory control ratio and exhibit an enhanced CsA-sensitive release of mitochondrial calcium. Associated with this was a calcium-induced loss of membrane potential, which may be inhibited by either cyclosporine A or ruthenium red. Further experiments have demonstrated that doxorubicin treatment in vivo causes a dose-dependent and irreversible interference of mitochondrial calcium transport and calcium-dependent regulation of membrane potential indicative of an induction of the mPTP and of an increased sensitivity to calcium-induced loss of cell viability [57, 58]. Implication of the mPTP in the cardiotoxicity of doxorubicin has been explored in cyclophilin-D-deficient mice, cyclophilin-D being a mitochondrial matrix peptidyl-prolyl isomerase known to modulated mitochondrial transition pore opening. The result that cyclophilin-D deficiency in mice inhibited doxorubicin-induced cardiomyocyte necrosis and heart failure suggests that mPTP is involved in doxorubicin-induced cardiotoxicity [48]. This contention is also supported by animal studies showing prevention of doxorubicin cardiotoxicity by in vivo CsA or FK506 treatment [59, 60]. In human atrial trabeculae, our group also demonstrated that cyclosporine A prevented doxorubicin-induced mitochondrial dysfunction and impaired contractile performance induced by doxorubicin [61]. These findings reinforce the rationale that mPTP is involved in the development of doxorubicin cardiotoxicity in the human myocardium.

3.2. Survival Protein Kinase Signaling. Accumulating evidence indicates that several protein kinases (i.e., Akt, PKCs, EKR, GSK-3b, hexokinase) receive extra mitochondrial signals and modify mitochondrial proteins that determine cell death or survival, such as the mPTP [62]. Activities of some of these kinases are mutually regulated, and phosphorylation of GSK-3β and hexokinase in mitochondria appears to directly modify the mPTP, elevating its opening threshold [62]. Doxorubicin may induce inhibition of Akt phosphorylation, which increases active glycogen synthase kinase-3b (GSK-3b) [63]. GSK-3b is a protein kinase linked to the regulation of a variety of cellular functions within the myocardium, including glycogen metabolism, gene expression and cellular survival [62]. GSK-3b phosphorylation, and, therefore inhibition, could confer cardioprotection through its potential mitochondrial effects on the mPTP. Strategies that prevent GSK-3b activation via upstream kinase activation have been shown to be protective against doxorubicin treatment. For example, pretreatment with various therapeutic molecules (erythropoietin, thrombopoietin, CO/HO1) can protect the myocardium against doxorubicin-induced impaired heart function and cardiomyocyte apoptosis by activating Pi3k-Akt cell survival pathways [63–65]. In contrast, upregulation of Ser/Thr phosphatase PP1 by doxorubicin may be involved in the Akt dephosphorylation, resulting in executioner caspase activation and cell death [66].

Cellular stress and specifically oxidative stress has been shown to activate mitogen/stress activated protein kinases (MAPKs and SAPKs) that appear to be important in determining cell fate. MAPKs and SAPKs pathways modulate the response of the heart to anthracycline exposure [67] and have also been proposed as cellular mediators linking anthracyclines to the apoptotic cell death pathway [18, 30]. Under treatment with anthracyclines that significantly induces myocyte apoptosis in the primary cultures of neonatal mouse cardiomyocytes, p38 MAPK is dramatically activated. That p38 MAPK may be involved at least in part in the anthracycline-induced myocyte apoptosis is demonstrated by two important observations. First, the time-course analysis revealed that p38 MAPK activation typically precedes the onset of apoptosis. Second, application of inhibitors of p38 MAPK significantly inhibits anthracycline-induced myocyte apoptosis [68]. Although most studies have focused on the ERK member of the MAPKs, other members of the MAPKs/SAPKs family have been associated with cardioprotection through the modulation of the mPTP. For example, JNK and p38 are activated by doxorubicin and linked mPTP to cardiac myocyte apoptosis [69]. Strategies reducing activation of the MPAKs/SAPKs pathways are typically protective against doxorubicin cardiotoxicity.

3.3. Genomic Analyses and Cellular Energetic Deficits. Doxorubicin typically causes selective downregulation of many nuclear genes that encode for proteins with mitochondrial function [70, 71]. The depressive effect on the expression of genes that comprise the mitochondrial proteome is persistent and can be observed weeks after prolonged administration of doxorubicin [70]. Previous studies suggest that a prominent feature of doxorubicin-induced cardiotoxicity is a profound alteration in the abundance of transcripts related to energy metabolism and mitochondrial performance. Consistent evidence suggests that cellular energy deficits related to decrease in fatty acid and glucose oxidation could play a critical role in the development of the cardiomyopathy induced by anthracyclines [29]. For example, oxidation of the long-chain fatty acid palmitate is inhibited by doxorubicin within minutes in isolated cardiomyocyte preparation, as well as in chronic situation in which cardiomyocytes are isolated from doxorubicin-treated rats. In these studies, impairment of carnitine palmitoyl transferase I and depletion of its substrate l-carnitine by doxorubicin was demonstrated [72]. Reduction in fatty acid oxidation is not accompanied by upregulation of glucose utilization as a compensatory response [73]. Rather, doxorubicin-induced cardiomyopathy is associated with a decreased utilization of both fatty acids and glucose substrates, which has been related to the effects of doxorubicin on cellular glucose supply [74] and the impairment of phosphofructokinase, the rate-limiting enzyme of glycolysis [75].

Furthermore, the selective effects of doxorubicin on suppression of mitochondria gene expression is accompanied by a coordinate and adaptive response of energy-sensing molecules [76], such as AMP-activated protein kinase (AMPK), hypoxia-inducible factor 1 (HIF1), nuclear respiratory factors (Nrf) and proliferator-activated receptor gamma coactivator1 (PGC1) [77]. Proteomic analyses revealed consistent changes in proteins involved in mitochondria energy production, energy channeling and mitochondrial antioxidant protection [78, 79]. Overall, this information is in line with doxorubicin-induced mitochondrial defects at different stages of cardiac energy metabolism, including reduction of oxidative capacity, changes in the profile of energy substrate utilization, disruption of energy transfer systems such as mitochondrial CK, and AMPK-dependent energy signaling pathways.

3.4. Anthracyclines Induce Sarcomere Functional and Structural Changes. Functional and structural changes to cardiomyocyte sarcomeres have been observed in both experimental experiments and in endomyocardial biopsies of patients treated with anthracyclines. Loss of myofibrils, disarray of myofibrils, swelling of mitochondria and dilation of the sarcoplasmic reticulum were observed [80, 81]. Breakdown of sarcomeres typically involved early-onset degradation of the giant myofilament protein, titin. As titin maintains sarcomere integrity, its accelerated degradation via calpain proteolytic activity in response to doxorubicin can rapidly lead to sarcomere disorganization and progressive cardiomyocyte contractile dysfunction [82].

Several lines of evidence indicate that an abnormal calcium handling of myocardial cells may explain, at least in part, the cardiac dysfunction seen in doxorubicin-induced cardiomyopathy. For example, doxorubicin has been shown to inhibit the gene transcription of the sarcoplasmic reticulum Ca2+-ATPase [83] and to activate cardiac calcium release channels (ryanodine receptors) [84]. A decrease of sarcoplasmic reticulum calcium load and hence calcium-induced calcium release has been observed with doxorubicin in isolated guinea pig ventricular myocytes [85]. The mechanism by which doxorubicin affects calcium homeostasis of cardiac myocytes has not been fully defined but may involve an iron-catalyzed direct effect of doxorubicin, doxorubicin-induced formation of reactive oxygen intermediates [83], and conversion of doxorubicin to the toxic alcohol metabolites [86].

3.5. Transcriptional Factors. Several lines of evidence suggest that progressive anthracycline-induced cardiac injury results from effects on myocyte differentiation programs thereby impeding myocyte survival and the cardiac adaptative response. Genes with anthracycline inhibited expression include genes encoding, transcriptional factors [71, 73]. For example, anthracyclines can disrupt expression and activity of the transcription factor GATA-4 [87]. Transcriptional factor GATA-4 is a member of a zinc finger transcriptional factor family that is critical for regulating differentiation, sarcomere synthesis and survival signaling. GATA-4 is expressed in the heart and regulates several specific cardiac genes, including antiapoptotic genes, making it a key regulator of heart development. This important survival factor is rapidly depleted in response to doxorubicin treatment [64, 88]. Anthracyclines downregulate GATA-4 expression in cardiac myocytes and upregulation of GATA-4 can suppress doxorubicin-induced myocyte apoptosis and drug-induced

cardiotoxicity [88, 89]. These prosurvival effects have been linked to the effects of GATA-4 on the upstream activator of the antiapoptotic gene Bcl-Xl. Since the overexpression of GATA-4 can attenuate the incidence of apoptosis induced by anthracyclines, GATA-4 may serve as an antiapoptotic factor in the heart. Moreover, GATA-4 also regulates expression of several cardiac specific genes that are involved in sarcomere synthesis, such as cardiac troponin C and I and myosin light chain-3 [90]. Hence, one potential mechanism by which anthracycline may induced myocardial dysfunction is via suppression of sarcomere protein expression and sarcopenia in response to GATA-4 reduction [91].

In addition to GATA-4, the cardiac ankyrin repeat protein transcriptional regulator CARP and the transcriptional coactivating factor p300 have been implicated in the cardiotoxicity of anthracyclines. CARP is rapidly degraded in myocytes after anthracycline exposure [91, 92]. Suppression of CARP expression using short-interference RNA is sufficient to induce myofibrillar disarray and cell dysfunction [93]. Similar to CARP, p300 is degraded after doxorubicin exposure through p38 kinases alpha and beta and is associated with apoptosis in neonatal cardiomyocytes [93, 94]. In these experiments, restoration of p300 inhibited doxorubicin-induced cell death.

Doxorubicin treatment has been associated with increased expression and activation of p53 tumor suppressor protein, which activates the intrinsic mitochondrial apoptotic pathway [95]. Consistently, p53-knockout mice and adult mouse hearts expressing cardiac myocyte-restricted dominant-interfering p53 are partially protected against doxorubicin-induced cell death and myocardial dysfunction [96]. In addition, activation of p53 may also mediate anthracycline-induced cardiotoxicity through other pathways independent of cardiomyocyte apoptosis. For example, p53-mediated inhibition of mammalian target of rapamycin signaling (mTOR) may contribute to the cardiac mass reduction and myocardial dysfunction observed in doxorubicin-treated mice [96]. Hence, acute doxorubicin-induced toxicity could result from p53-dependent modulation of mTOR activity. It may be thus of considerable interest to determine whether upstream effectors that activate mTOR pathway would be cardioprotective against doxorubicin-induced cardiac toxicity.

3.6. Neuregulin/ErbB2 Cardioprotective Program. Unexpected cardiac side effect of ErbB2 antagonists, such as trastuzumab, has sparked great scientific efforts to elucidate the role of Neuregulin/ErbB2 pathway in cardiomyocyte functional and structural integrity [16, 17, 97]. Before this observation, ErbB2 signaling was only recognized as being indispensable to normal fetal cardiac development. Subsequent studies have demonstrated that stimulation of the ErbB2 signaling by ErbB-receptor ligands improves cardiomyocyte function and survival in the heart [97].

The first evidence regarding the protective effects of the ErbB2 signaling in the adult heart came from clinical trials in breast cancer patients using trastuzumab, a monoclonal antibody that blocks the ErbB2 receptor [1, 2]. The incidence of clinical heart failure increased five-fold in patients treated concurrently with chemotherapy drug doxorubicin and trastuzumab compared to those treated with doxorubicin alone [1]. Mostly based on the analogy between ErbB2 knockout-induced cardiomyopathy and trastuzumab-induced heart failure, many studies have concluded that trastuzumab causes heart failure by blocking the physiological actions of ErbB2 in the heart [32, 33]. The synergistic increase of heart failure incidence has been related to the fact that ErbB2 expression is upregulated following doxorubicin administration, while trastuzumab inhibits the ErbB2 downstream pathways, which is essential for cell repair, survival, and function. Thus, if trastuzumab inhibits the ErbB2 cardioprotective pathways during a vulnerable period after anthracycline injury, the anthracycline damage could be augmented, resulting in increased cell death [2].

Overall, these results suggest that ErbB2 inhibition result in mitochondrial apoptotic signaling in cardiomyocytes leading to increased cell loss in the heart. Therefore, up-regulation of the cardiac neuregulin/ErbB2 pathway may be one strategy to limit myocardial anthracycline injury. Experimental work on both animals and humans has demonstrated that exercise is a potent activator of neuregulin release with subsequent activation of ErbB2 activation [98]. The fact that exercise protects against calcium-induced cardiac mitochondrial permeability transition and reduces cell death following doxorubicin administration could be related to the neuregulin/ErbB2 survival pathway activation [99].

3.7. Lessons from Targeted Chemotherapy. Several novel "targeted" agents have been associated with a small but worrisome risk of heart dysfunction [2, 12–14]. These agents include the tyrosine kinase inhibitors sunitinib, lapatinib, and imatinib, which are members of a growing class of targeted chemotherapy agents. Clues as to the nature of the cardiotoxicity due to these agents are beginning to emerge that point to the mitochondria. To date, the only approved kinase inhibitor that is clearly associated with clinical cardiotoxicity is sunitinib, whereas the extent of imatinib-induced cardiotoxicity is still under scrutiny [12].

On-target cardiac toxicity is inherent to kinase inhibition and quickly becomes apparent since many of the pathways that regulate cancer cell survival also regulate essential processes in cardiomyocytes, including contractile function and survival [14, 17]. The ATP binding pocket represents the key region of the kinase targeted by most inhibitors. Conservation of that ATP binding pocket among kinases means that inhibitors can also inhibit unintended kinases, and if any of these kinases serve important functions in the heart, off-target cardiotoxicity can occur. An additional issue is that targeting of kinases will achieve entire pathway targeting. For example, the inhibition of multiple components of the Pi3kinase/Akt pathway is a viable strategy for cancers, but this pathway also maintains cardiomyocyte homeostasis and protects cardiomyocytes from death [100]. Eventually, kinase inhibitors could mediate toxicity through the inhibition of non-kinase targets, such an enzyme requiring ATP to perform its function [12, 13].

To illustrate the complexities inherent in identifying mechanisms of kinase inhibitor-induced cardiac toxicity, suni-

tinib-induced cardiac injury will be discussed. Cardiac dysfunction was first related to systemic hypertension secondary to VEGFR2 and PDGFRbeta inhibition by sunitinib [101]. As several patients developed cardiotoxicity in absence of hypertension, additional mechanisms were explored. In this study, endomyocardial biopsy samples from two patients who presented with profound sunitinib-induced heart failure were obtained. Abnormal histopathological changes included marked mitochondrial swelling, which could indicate mPTP and energetic failure [101, 102]. In cultured cardiomyocytes, the same mitochondrial abnormalities were observed and were associated with apoptotic cell death [101, 102]. Studies in cardiomyocytes have confirmed that energy compromise was involved in sunitinib-induced cardiac toxicity but surprisingly was not associated with activation of AMP kinase, the master fuel control of the cell [102]. Lack of response to this energy loss was the result of direct inhibition of AMP kinase by sunitinib, so-called off-target toxicity. AMP kinase inhibition has also been reported in isolated heart exposed to doxorubicin [73], whereas recent studies demonstrated that AMPK gene expression and enzyme activities were acutely increased [71]. These results are important since AMPK is implicated in many survival pathways, including Pi3k/Akt/mTOR axis [103].

Likewise, imatinib causes a modest but consistent decline in left ventricular function, which was associated with loss of myocardial mass and increased cell death [104]. Studies in cardiomyocytes showed that imatinib leads to significant mitochondrial dysfunction with mitochondria swelling, mitochondrial membrane potential collapse followed by cytochrome c release and energetic failure [15, 104]. This process was associated with a cell death that has the biochemical features of apoptosis and the morphological aspect of cell necrosis. Mitochondria isolated from hearts of mice treated with imatinib showed enhanced calcium-induced swelling and mitochondrial permeability transition mPTP. As is the case with trastuzumab, mitochondrial dysfunction plays a central role in the cardiotoxic response to imatinib, but the mechanism seems to be the induction of endoplasmic reticulum ER stress by the drug [15, 104]. ER stress in response to imatinib exposition has been related to the downstream activation of the c-Jun N-terminal kinase (JNK) family of stress MAPKs [105]. Similarly, ER stress-mediated apoptotic pathway has also been reported to mediate cardiac cell death induced by doxorubicin [20]. In this case, caspase 12, which resides in the ER, is an essential caspase to initiate ER-mediated apoptosis that is activated in doxorubicin-treated hearts [106].

The Pi3k/Akt/mTOR pathway more than any other epitomizes the similarity between cancer cell signaling and survival signaling in cardiac cells [54]. The cautionary note is that Pi3k/Akt/mTOR pathway is also critical for cardiomyocyte integrity and survival. Hence, inhibition of multiple components of the pathway would jeopardize cardiomyocyte integrity and survival [100]. Conversely, activation of this signaling cascade, together with other Akt-activated molecules (such as GSK-3β, mTOR and p70S6 kinase), elicits prosurvival and cardiovascular protective effects, which are mediated by inhibition of opening of the mPTP [62].

4. Conclusion

Cardiac dysfunction is the most severe side effects of anthracycline treatment. The major mechanism of anthracycline damage involves the generation of reactive oxygen species ROS by iron-anthracycline complexes, leading to lipid peroxidation and membrane damage. In mitochondria, ROS and calcium overload lead to mitochondrial permeability transition pore opening (mPTP), which is associated with the release of cytochrome c (cyt c) from mitochondria into cytoplasm and cell death. Cardiac myocyte loss following activation of both apoptotic and necrotic pathways provide an attractive explanation for anthracycline-induced cardiotoxicity. There is evidence that the stressed cardiac myocyte survival may rely on both growth and survival pathways that are altered in the anthracycline-exposed myocardium. Activation of ErbB2 and Pi3k/Akt/mTOR pathways represents a major adaptative mechanism for cardioprotection, which are altered by anthracyclines. The delicate balance between pro- and antiapoptosis signaling that relies on kinase-regulated pathways creates a cause for concern when one attempts to use anticancer molecules, that is, targeted therapies that can impair the coordinated function of this kinase network.

Abbreviations

(HER2 or EbrB2):	Human epidermal growth factor receptor 2
Pi3k:	Phosphatidylinositol-3-Kinase
Akt:	Serine/threonine protein kinase
GSK3:	Glycogen Synthase Kinase 3;
BCL:	B Cell Lymphoma
Bax:	(BCL)-associated X
ER stress:	Endoplasmic reticulum
GATA:	Family name is derived from their ability to bind to the consensus DNA sequence (A/T) GATA (A/G)
p53:	Tumor suppressor p53
p300:	Transcription factor p300
$\Delta\Psi$:	Mitochondrial membrane potential.

Acknowledgments

Authors thank Dr. Hurt for English editing skills, Dr. Marechal for his advice and the members of EA4484 research team for its support. The authors also thank university of Lille, France, for the financial support (Grant no. EA4484).

References

[1] T. Eschenhagen, T. Force, M. S. Ewer et al., "Cardiovascular side effects of cancer therapies: a position statement from the Heart Failure Association of the European Society of Cardiology," *European Journal of Heart Failure*, vol. 13, no. 1, pp. 1–10, 2011.

[2] M. S. Ewer and S. M. Ewer, "Cardiotoxicity of anticancer treatments: what the cardiologist needs to know," *Nature Reviews Cardiology*, vol. 7, no. 10, pp. 564–575, 2010.

[3] E. C. van Dalen, H. N. Caron, H. O. Dickinson, and L. C. Kremer, "Cardioprotective interventions for cancer patients

receiving anthracyclines," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD003917, 2005.

[4] A. L. A. Ferreira, L. S. Matsubara, and B. B. Matsubara, "Anthracycline-induced cardiotoxicity," *Cardiovascular and Hematological Agents in Medicinal Chemistry*, vol. 6, no. 4, pp. 278–281, 2008.

[5] J. J. Monsuez, J. C. Charniot, N. Vignat, and J. Y. Artigou, "Cardiac side-effects of cancer chemotherapy," *International Journal of Cardiology*, vol. 144, no. 1, pp. 3–15, 2010.

[6] M. W. Saif, M. M. Shah, and A. R. Shah, "Fluoropyrimidine-associated cardiotoxicity: revisited," *Expert Opinion on Drug Safety*, vol. 8, no. 2, pp. 191–202, 2009.

[7] S. M. Gressett and S. R. Shah, "Intricacies of bevacizumab-induced toxicities and their management," *Annals of Pharmacotherapy*, vol. 43, no. 3, pp. 490–501, 2009.

[8] M. T. Meinardi, W. T. A. Van Der Graaf, D. J. Van Veldhuisen, J. A. Gietema, E. G. E. De Vries, and D. T. Sleijfer, "Detection of anthracycline-induced cardiotoxicity," *Cancer Treatment Reviews*, vol. 25, no. 4, pp. 237–247, 1999.

[9] A. U. Buzdar, C. Marcus, T. L. Smith, and G. R. Blumenschein, "Early and delayed clinical cardiotoxicity of doxorubicin," *Cancer*, vol. 55, no. 12, pp. 2761–2765, 1985.

[10] E. T. Yeh and C. L. Bickford, "Cardiovascular complications of cancer therapy: incidence, pathogenesis, diagnosis, and management," *Journal of the American College of Cardiology*, vol. 53, no. 24, pp. 2231–2247, 2009.

[11] S. Aggarwal, "Targeted cancer therapies," *Nature Reviews Drug Discovery*, vol. 9, no. 6, pp. 427–428, 2010.

[12] T. Force and K. L. Kolaja, "Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes," *Nature Reviews Drug Discovery*, vol. 10, no. 2, pp. 111–126, 2011.

[13] T. Force, D. S. Krause, and R. A. Van Etten, "Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition," *Nature Reviews Cancer*, vol. 7, no. 5, pp. 332–344, 2007.

[14] H. Cheng and T. Force, "Molecular mechanisms of cardiovascular toxicity of targeted cancer therapeutics," *Circulation Research*, vol. 106, no. 1, pp. 21–34, 2010.

[15] M. H. Chen, R. Kerkelä, and T. Force, "Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics," *Circulation*, vol. 118, no. 1, pp. 84–95, 2008.

[16] S. A. Crone, Y. Y. Zhao, L. Fan et al., "ErbB2 is essential in the prevention of dilated cardiomyopathy," *Nature Medicine*, vol. 8, no. 5, pp. 459–465, 2002.

[17] G. W. De Keulenaer, K. Doggen, and K. Lemmens, "The vulnerability of the heart as a pluricellular paracrine organ: lessons from unexpected triggers of heart failure in targeted ErbB2 anticancer therapy," *Circulation Research*, vol. 106, no. 1, pp. 35–46, 2010.

[18] D. B. Sawyer, X. Peng, B. Chen, L. Pentassuglia, and C. C. Lim, "Mechanisms of anthracycline cardiac injury: can we identify strategies for cardioprotection?" *Progress in Cardiovascular Diseases*, vol. 53, no. 2, pp. 105–113, 2010.

[19] M. H. Chen, S. D. Colan, and L. Diller, "Cardiovascular disease: cause of morbidity and mortality in adult survivors of childhood cancers," *Circulation Research*, vol. 108, no. 5, pp. 619–628, 2011.

[20] Y. W. Zhang, J. Shi, Y. J. Li, and L. Wei, "Cardiomyocyte death in doxorubicin-induced cardiotoxicity," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 57, no. 6, pp. 435–445, 2009.

[21] M. Ryberg, D. Nielsen, G. Cortese, G. Nielsen, T. Skovsgaard, and P. K. Andersen, "New insight into epirubicin cardiac toxicity: competing risks analysis of 1097 breast cancer patients," *Journal of the National Cancer Institute*, vol. 100, no. 15, pp. 1058–1067, 2008.

[22] B. N. Bernaba, J. B. Chan, C. K. Lai, and M. C. Fishbein, "Pathology of late-onset anthracycline cardiomyopathy," *Cardiovascular Pathology*, vol. 19, no. 5, pp. 308–311, 2010.

[23] H. De Graaf, W. V. Dolsma, P. H. B. Willemse et al., "Cardiotoxicity from intensive chemotherapy combined with radiotherapy in breast cancer," *British Journal of Cancer*, vol. 76, no. 7, pp. 943–945, 1997.

[24] B. M. P. Aleman, A. W. Van Den Belt-Dusebout, M. L. De Bruin et al., "Late cardiotoxicity after treatment for Hodgkin lymphoma," *Blood*, vol. 109, no. 5, pp. 1878–1886, 2007.

[25] P. A. Ganz, M. A. Hussey, C. M. Moinpour et al., "Late cardiac effects of adjuvant chemotherapy in breast cancer survivors treated on Southwest Oncology Group protocol S8897," *Journal of Clinical Oncology*, vol. 26, no. 8, pp. 1223–1230, 2008.

[26] S. M. Swain, F. S. Whaley, and M. S. Ewer, "Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials," *Cancer*, vol. 97, no. 11, pp. 2869–2879, 2003.

[27] T. Šimůnek, M. Štěrba, O. Popelová, M. Adamcová, R. Hrdina, and V. Gerši, "Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron," *Pharmacological Reports*, vol. 61, no. 1, pp. 154–171, 2009.

[28] E. Raschi, V. Vasina, M. G. Ursino, G. Boriani, A. Martoni, and F. de Ponti, "Anticancer drugs and cardiotoxicity: insights and perspectives in the era of targeted therapy," *Pharmacology and Therapeutics*, vol. 125, no. 2, pp. 196–218, 2010.

[29] M. Tokarska-Schlattner, M. Zaugg, C. Zuppinger, T. Wallimann, and U. Schlattner, "New insights into doxorubicin-induced cardiotoxicity: the critical role of cellular energetics," *Journal of Molecular and Cellular Cardiology*, vol. 41, no. 3, pp. 389–405, 2006.

[30] B. Chen, X. Peng, L. Pentassuglia, C. C. Lim, and D. B. Sawyer, "Molecular and cellular mechanisms of anthracycline cardiotoxicity," *Cardiovascular Toxicology*, vol. 7, no. 2, pp. 114–121, 2007.

[31] V. A. Sardão, P. J. Oliveira, J. Holy, C. R. Oliveira, and K. B. Wallace, "Morphological alterations induced by doxorubicin on H9c2 myoblasts: nuclear, mitochondrial, and cytoskeletal targets," *Cell Biology and Toxicology*, vol. 25, no. 3, pp. 227–243, 2009.

[32] D. Rayson, D. Richel, S. Chia, C. Jackisch, S. van der Vegt, and T. Suter, "Anthracycline-trastuzumab regimens for HER2/neu-overexpressing breast cancer: current experience and future strategies," *Annals of Oncology*, vol. 19, no. 9, pp. 1530–1539, 2008.

[33] T. Horie, K. Ono, H. Nishi et al., "Acute doxorubicin cardiotoxicity is associated with miR-146a-induced inhibition of the neuregulin-ErbB pathway," *Cardiovascular Research*, vol. 87, no. 4, pp. 656–664, 2010.

[34] H. G. Keizer, H. M. Pinedo, G. J. Schuurhuis, and H. Joenje, "Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity," *Pharmacology and Therapeutics*, vol. 47, no. 2, pp. 219–231, 1990.

[35] L. Wojnowski, B. Kulle, M. Schirmer et al., "NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity," *Circulation*, vol. 112, no. 24, pp. 3754–3762, 2005.

[36] G. Minotti, R. Ronchi, E. Salvatorelli, P. Menna, and G. Cairo, "Doxorubicin irreversibly inactivates iron regulatory

proteins 1 and 2 in cardiomyocytes: evidence for distinct metabolic pathways and implications for iron-mediated cardiotoxicity of antitumor therapy," *Cancer Research*, vol. 61, no. 23, pp. 8422–8428, 2001.

[37] S. Fogli, P. Nieri, and M. C. Breschi, "The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage," *The FASEB Journal*, vol. 18, no. 6, pp. 664–675, 2004.

[38] G. Minotti, P. Menna, E. Salvatorelli, G. Cairo, and L. Gianni, "Anthracyclines: molecular advances and pharmacologie developments in antitumor activity and cardiotoxicity," *Pharmacological Reviews*, vol. 56, no. 2, pp. 185–229, 2004.

[39] R. A. Rowan, M. A. Masek, and M. E. Billingham, "Ultrastructural morphometric analysis of endomyocardial biopsies. Idiopathic dilated cardiomyopathy, anthracycline cardiotoxicity, and normal myocardium," *American Journal of Cardiovascular Pathology*, vol. 2, no. 2, pp. 137–144, 1988.

[40] J. M. Berthiaume and K. B. Wallace, "Adriamycin-induced oxidative mitochondrial cardiotoxicity," *Cell Biology and Toxicology*, vol. 23, no. 1, pp. 15–25, 2007.

[41] K. B. Wallace, "Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis," *Cardiovascular Toxicology*, vol. 7, no. 2, pp. 101–107, 2007.

[42] P. Bernardi, A. Krauskopf, E. Basso et al., "The mitochondrial permeability transition from in vitro artifact to disease target," *FEBS Journal*, vol. 273, no. 10, pp. 2077–2099, 2006.

[43] A. P. Halestrap, "What is the mitochondrial permeability transition pore?" *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 6, pp. 821–831, 2009.

[44] C. P. Baines, "The mitochondrial permeability transition pore and ischemia-reperfusion injury," *Basic Research in Cardiology*, vol. 104, no. 2, pp. 181–188, 2009.

[45] C. P. Baines, "The molecular composition of the mitochondrial permeability transition pore," *Journal of Molecular and Cellular Cardiology*, vol. 46, no. 6, pp. 850–857, 2009.

[46] T. Nakagawa, S. Shimizu, T. Watanabe et al., "Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death," *Nature*, vol. 434, no. 7033, pp. 652–658, 2005.

[47] A. C. Schinzel, O. Takeuchi, Z. Huang et al., "Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 34, pp. 12005–12010, 2005.

[48] H. Nakayama, X. Chen, C. P. Baines et al., "Ca^{2+}- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure," *The Journal of Clinical Investigation*, vol. 117, no. 9, pp. 2431–2444, 2007.

[49] E. Arbustini, A. Brega, and J. Narula, "Ultrastructural definition of apoptosis in heart failure," *Heart Failure Reviews*, vol. 13, no. 2, pp. 121–135, 2008.

[50] T. L'Ecuyer, Z. Allebban, R. Thomas, and R. Vander Heide, "Glutathione S-transferase overexpression protects against anthracycline-induced H9C2 cell death," *American Journal of Physiology*, vol. 286, no. 6, pp. H2057–H2064, 2004.

[51] L. Lu, W. Wu, J. Yan, X. Li, H. Yu, and X. Yu, "Adriamycin-induced autophagic cardiomyocyte death plays a pathogenic role in a rat model of heart failure," *International Journal of Cardiology*, vol. 134, no. 1, pp. 82–90, 2009.

[52] K. Nicolay, J. J. Fok, and W. Voorhout, "Cytofluorescence detection of adriamycin-mitochondria interactions in isolated, perfused rat heart," *Biochimica et Biophysica Acta*, vol. 887, no. 1, pp. 35–41, 1986.

[53] M. Tokarska-Schlattner, M. Zaugg, R. Da Silva et al., "Acute toxicity of doxorubicin on isolated perfused heart: response of kinases regulating energy supply," *American Journal of Physiology*, vol. 289, no. 1, pp. H37–H47, 2005.

[54] S. Javadov, J. C. Hunter, G. Barreto-Torres, and R. Parodi-Rullan, "Targeting the mitochondrial permeability transition: cardiac ischemia-reperfusion versus carcinogenesis," *Cellular Physiology and Biochemistry*, vol. 27, no. 3-4, pp. 179–190, 2011.

[55] L. E. Solem and K. B. Wallace, "Selective activation of the sodium-independent, cyclosporin A-sensitive calcium pore of cardiac mitochondria by doxorubicin," *Toxicology and Applied Pharmacology*, vol. 121, no. 1, pp. 50–57, 1993.

[56] L. E. Solem, T. R. Henry, and K. B. Wallace, "Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration," *Toxicology and Applied Pharmacology*, vol. 129, no. 2, pp. 214–222, 1994.

[57] L. E. Solem, L. J. Heller, K. B. Wallace, and K. B. Wallace, "Dose-dependent increase in sensitivity to calcium-induced mitochondrial dysfunction and cardiomyocyte cell injury by doxorubicin," *Journal of Molecular and Cellular Cardiology*, vol. 28, no. 5, pp. 1023–1032, 1996.

[58] S. Zhou, A. Starkov, M. K. Froberg, R. L. Leino, and K. B. Wallace, "Cumulative and irreversible cardiac mitochondrial dysfunction induced by doxorubicin," *Cancer Research*, vol. 61, no. 2, pp. 771–777, 2001.

[59] I. A. Al-Nasser, "In vivo prevention of adriamycin cardiotoxicity by cyclosporin A or FK506," *Toxicology*, vol. 131, no. 2-3, pp. 175–181, 1998.

[60] X. Marechal, D. Montaigne, C. Marciniak et al., "Doxorubicin-induced cardiac dysfunction is attenuated by ciclosporin treatment in mice through improvements in mitochondrial bioenergetics," *Clinical Science*, vol. 121, no. 9, pp. 405–413, 2011.

[61] D. Montaigne, X. Marechal, S. Preau et al., "Doxorubicin induces mitochondrial permeability transition and contractile dysfunction in the human myocardium," *Mitochondrion*, vol. 11, no. 1, pp. 22–26, 2011.

[62] M. Ovize, G. F. Baxter, F. Di Lisa et al., "Postconditioning and protection from reperfusion injury: where do we stand: position Paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology," *Cardiovascular Research*, vol. 87, no. 3, pp. 406–423, 2010.

[63] K. H. Kim, G. Y. Oudit, and P. H. Backx, "Erythropoietin protects against doxorubicin-induced cardiomyopathy via a phosphatidylinositol 3-kinase-dependent pathway," *Journal of Pharmacology and Experimental Therapeutics*, vol. 324, no. 1, pp. 160–169, 2008.

[64] H. B. Suliman, M. S. Carraway, A. S. Ali, C. M. Reynolds, K. E. Welty-Wolf, and C. A. Piantadosi, "The CO/HO system reverses inhibition of mitochondrial biogenesis and prevents murine doxorubicin cardiomyopathy," *The Journal of Clinical Investigation*, vol. 117, no. 12, pp. 3730–3741, 2007.

[65] K. Li, R. Y. T. Sung, Z. H. Wei et al., "Thrombopoietin protects against in vitro and in vivo cardiotoxicity induced by doxorubicin," *Circulation*, vol. 113, no. 18, pp. 2211–2220, 2006.

[66] G. C. Fan, X. Zhou, X. Wang et al., "Heat shock protein 20 interacting with phosphorylated akt reduces doxorubicin-triggered oxidative stress and cardiotoxicity," *Circulation Research*, vol. 103, no. 11, pp. 1270–1279, 2008.

[67] R. A. Thandavarayan, K. Watanabe, F. R. Sari et al., "Modulation of doxorubicin-induced cardiac dysfunction in dominant-negative p38α mitogen-activated protein kinase

mice," *Free Radical Biology and Medicine*, vol. 49, no. 9, pp. 1422–1431, 2010.

[68] M. Khan, S. Varadharaj, L. P. Ganesan et al., "C-phycocyanin protects against ischemia-reperfusion injury of heart through involvement of p38 MAPK and ERK signaling," *American Journal of Physiology*, vol. 290, no. 5, pp. H2136–H2145, 2006.

[69] E. Bartha, I. Solti, A. Szabo et al., "Regulation of kinase cascade activation and heat shock protein expression by poly(ADP-ribose) polymerase inhibition in doxorubicin-induced heart failure," *Journal of Cardiovascular Pharmacology*, vol. 58, no. 4, pp. 380–391, 2011.

[70] J. M. Berthiaume and K. B. Wallace, "Persistent alterations to the gene expression profile of the heart subsequent to chronic doxorubicin treatment," *Cardiovascular Toxicology*, vol. 7, no. 3, pp. 178–191, 2007.

[71] A. V. Pointon, T. M. Walker, K. M. Phillips et al., "Doxorubicin in vivo rapidly alters expression and translation of myocardial electron transport chain genes, leads to ATP loss and caspase 3 activation," *PloS One*, vol. 5, no. 9, Article ID e12733, 2010.

[72] S. Abdel-Aleem, M. M. El-Merzabani, M. Sayed-Ahmed, D. A. Taylor, and J. E. Lowe, "Acute and chronic effects of adriamycin on fatty acid oxidation in isolated cardiac myocytes," *Journal of Molecular and Cellular Cardiology*, vol. 29, no. 2, pp. 789–797, 1997.

[73] S. Wakasugi, A. J. Fischman, J. W. Babich et al., "Myocardial substrate utilization and left ventricular function in adriamycin cardiomyopathy," *Journal of Nuclear Medicine*, vol. 34, no. 9, pp. 1529–1535, 1993.

[74] S. Hrelia, D. Fiorentini, T. Maraldi et al., "Doxorubicin induces early lipid peroxidation associated with changes in glucose transport in cultured cardiomyocytes," *Biochimica et Biophysica Acta*, vol. 1567, pp. 150–156, 2002.

[75] R. Jeyaseelan, C. Poizat, H. Y. Wu, and L. Kedes, "Molecular mechanisms of doxorubicin-induced cardiomyopathy. Selective suppression of Reiske iron-sulfur protein, ADP/ATP translocase, and phosphofructokinase genes is associated with ATP depletion in rat cardiomyocytes," *The Journal of Biological Chemistry*, vol. 272, no. 9, pp. 5828–5832, 1997.

[76] K. L. Thompson, B. A. Rosenzweig, J. Zhang et al., "Early alterations in heart gene expression profiles associated with doxorubicin cardiotoxicity in rats," *Cancer Chemotherapy and Pharmacology*, vol. 66, no. 2, pp. 303–314, 2010.

[77] P. J. Fernandez-Marcos and J. Auwerx, "Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis," *American Journal of Clinical Nutrition*, vol. 93, no. 4, pp. 884S–890S, 2011.

[78] M. Štěrba, O. Popelová, J. Lenčo et al., "Proteomic insights into chronic anthracycline cardiotoxicity," *Journal of Molecular and Cellular Cardiology*, vol. 50, no. 5, pp. 849–862, 2011.

[79] S. N. Kumar, E. A. Konorev, D. Aggarwal, and B. Kalyanaraman, "Analysis of proteome changes in doxorubicin-treated adult rat cardiomyocyte," *Journal of Proteomics*, vol. 74, no. 5, pp. 683–697, 2011.

[80] R. L. Jones and D. W. Miles, "Use of endomyocardial biopsy to assess anthracycline-induced cardiotoxicity," *Lancet Oncology*, vol. 6, no. 2, p. 67, 2005.

[81] B. Mackay, M. S. Ewer, C. H. Carrasco, and R. S. Benjamin, "Assessment of anthracycline cardiomyopathy by endomyocardial biopsy," *Ultrastructural Pathology*, vol. 18, no. 1-2, pp. 203–211, 1994.

[82] C. C. Lim, C. Zuppinger, X. Guo et al., "Anthracyclines induce calpain-dependent titin proteolysis and necrosis in cardiomyocytes," *The Journal of Biological Chemistry*, vol. 279, no. 9, pp. 8290–8299, 2004.

[83] M. Arai, A. Yoguchi, T. Takizawa et al., "Mechanism of doxorubicin-induced inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase gene transcription," *Circulation Research*, vol. 86, no. 1, pp. 8–14, 2000.

[84] S. R. M. Holmberg and A. J. Williams, "Patterns of interaction between anthraquinone drugs and the calcium-release channel from cardiac sarcoplasmic reticulum," *Circulation Research*, vol. 67, no. 2, pp. 272–283, 1990.

[85] Y. X. Wang and M. Korth, "Effects of doxorubicin on excitation-contraction coupling in guinea pig ventricular myocardium," *Circulation Research*, vol. 76, no. 4, pp. 645–653, 1995.

[86] R. D. Olson, P. S. Mushlin, D. E. Brenner et al., "Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 10, pp. 3585–3589, 1988.

[87] Y. Kim, A. G. Ma, K. Kitta et al., "Anthracycline-induced suppression of GATA-4 transcription factor: implication in the regulation of cardiac myocyte apoptosis," *Molecular Pharmacology*, vol. 63, no. 2, pp. 368–377, 2003.

[88] A. Aries, P. Paradis, C. Lefebvre, R. J. Schwartz, and M. Nemer, "Essential role of GATA-4 in cell survival and drug-induced cardiotoxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 18, pp. 6975–6980, 2004.

[89] S. Kobayashi, P. Volden, D. Timm, K. Mao, X. Xu, and Q. Liang, "Transcription factor GATA4 inhibits doxorubicin-induced autophagy and cardiomyocyte death," *The Journal of Biological Chemistry*, vol. 285, no. 1, pp. 793–804, 2010.

[90] S. Pikkarainen, H. Tokola, R. Kerkelä, and H. Ruskoaho, "GATA transcription factors in the developing and adult heart," *Cardiovascular Research*, vol. 63, no. 2, pp. 196–207, 2004.

[91] L. Li, G. Takemura, Y. Li et al., "Preventive effect of erythropoietin on cardiac dysfunction in doxorubicin-induced cardiomyopathy," *Circulation*, vol. 113, no. 4, pp. 535–543, 2006.

[92] R. Jeyaseelan, C. Poizat, R. K. Baker et al., "A novel cardiac-restricted target for doxorubicin. CARP, a nuclear modulator of gene expression in cardiac progenitor cells and cardiomyocytes," *The Journal of Biological Chemistry*, vol. 272, no. 36, pp. 22800–22808, 1997.

[93] T. Arimura, J. M. Bos, A. Sato et al., "Cardiac ankyrin repeat protein gene (ANKRD1) mutations in hypertrophic cardiomyopathy," *Journal of the American College of Cardiology*, vol. 54, no. 4, pp. 334–342, 2009.

[94] C. Poizat, P. L. Puri, Y. Bai, and L. Kedes, "Phosphorylation-dependent degradation of p300 by doxorubicin-activated p38 mitogen-activated protein kinase in cardiac cells," *Molecular and Cellular Biology*, vol. 25, no. 7, pp. 2673–2687, 2005.

[95] T. L'Ecuyer, S. Sanjeev, R. Thomas et al., "DNA damage is an early event in doxorubicin-induced cardiac myocyte death," *American Journal of Physiology*, vol. 291, no. 3, pp. H1273–H1280, 2006.

[96] W. Zhu, M. H. Soonpaa, H. Chen et al., "Acute doxorubicin cardiotoxicity is associated with p53-induced inhibition of the mammalian target of rapamycin pathway," *Circulation*, vol. 119, no. 1, pp. 99–106, 2009.

[97] K. Lemmens, K. Doggen, and G. W. De Keulenaer, "Role of neuregulin-1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure," *Circulation*, vol. 116, no. 8, pp. 954–960, 2007.

[98] A. Gumà, V. Martínez-Redondo, I. López-Soldado, C. Cantó, and A. Zorzano, "Emerging role of neuregulin as a modulator of muscle metabolism," *American Journal of Physiology*, vol. 298, no. 4, pp. E742–E750, 2010.

[99] X. Peng, B. Chen, C. C. Lim, and D. B. Sawyer, "The cardiotoxicology of anthracycline chemotherapeutics: translating molecular mechanism into preventative medicine," *Molecular Interventions*, vol. 5, no. 3, pp. 163–171, 2005.

[100] H. Cheng and T. Force, "Why do kinase inhibitors cause cardiotoxicity and what can be done about it?" *Progress in Cardiovascular Diseases*, vol. 53, no. 2, pp. 114–120, 2010.

[101] T. F. Chu, M. A. Rupnick, R. Kerkela et al., "Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib," *The Lancet*, vol. 370, no. 9604, pp. 2011–2019, 2007.

[102] R. Kerkela, K. C. Woulfe, J. B. Durand et al., "Sunitinib-induced cardiotoxicity is mediated by off-target inhibition of AMP-activated protein kinase," *Clinical and Translational Science*, vol. 2, no. 1, pp. 15–25, 2009.

[103] M.-B. Chen, X.-Y. Wu, J.-H. Gu, Q.-T. Guo, W.-X. Shen, and P.-H. Lu, "Activation of AMP-activated protein kinase contributes to doxorubicin-induced cell death and apoptosis in cultured myocardial H9c2 cells," *Cell Biochemistry and Biophysics*, vol. 60, no. 3, pp. 311–322, 2011.

[104] R. Kerkelä, L. Grazette, R. Yacobi et al., "Cardiotoxicity of the cancer therapeutic agent imatinib mesylate," *Nature Medicine*, vol. 12, no. 8, pp. 908–916, 2006.

[105] A. Fernández, A. Sanguino, Z. Peng et al., "An anticancer C-Kit kinase inhibitor is reengineered to make it more active and less cardiotoxic," *The Journal of Clinical Investigation*, vol. 117, no. 12, pp. 4044–4054, 2007.

[106] J. L. V. Reeve, E. Szegezdi, S. E. Logue et al., "Distinct mechanisms of cardiomyocyte apoptosis induced by doxorubicin and hypoxia converge on mitochondria and are inhibited by Bcl-xL," *Journal of Cellular and Molecular Medicine*, vol. 11, no. 3, pp. 509–520, 2007.