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REVIEW ARTICLE

Milestones and recent discoveries on cell death mediated by mitochondria and their interactions with biologically active amines

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Abstract Mitochondria represent cell "powerhouses," being involved in energy transduction from the electrochemical gradient to ATP synthesis. The morphology of their cell types may change, according to various metabolic processes or osmotic pressure. A new morphology of the inner membrane and mitochondrial *cristae*, significantly different from the previous one, has been proposed for the inner membrane and mitochondrial *cristae*, based on the technique of electron tomography. Mitochondrial Ca^{2+} transport (the transporter has been isolated) generates reactive oxygen species and induces the mitochondrial permeability transition of both inner and outer mitochondrial membranes, leading to induction of necrosis and apoptosis. In the mitochondria of several cell types (liver, kidney, and heart), mitochondrial oxidative stress is an essential step in the induction of cell death, although not in brain, in which the phenomenon is caused by a different mechanism. Mitochondrial permeability transition drives both apoptosis and necrosis, whereas mitochondrial outer membrane permeability is characteristic of apoptosis. Adenine nucleotide translocase remains the most important component involved in membrane permeability, with the opening of the transition pore, although other proteins, such as ATP synthase or phosphate carriers, have been proposed. Intrinsic cell death is triggered by the release from mitochondria of proteic factors, such as cytochrome c, apoptosis inducing factor, and Smac/DIABLO, with the activation of caspases upon mitochondrial permeability transition or mitochondrial outer membrane permeability induction. Mitochondrial permeability transition induces the permeability of the inner membrane in sites in contact with the outer membrane; mitochondrial outer membrane permeability forms channels on the outer membrane by means of various stimuli involving Bcl-2 family proteins. The biologically active amines, spermine, and agmatine, have specific functions on mitochondria which distinguish them from other amines. Enzymatic oxidative deamination of spermine by amine oxidases in tumor cells may produce reactive oxygen species, leading to transition pore opening and apoptosis. This process could be exploited as a new therapeutic strategy to combat cancer.

Keywords Mitochondria · Polyamines · Reactive oxygen species · Mitochondrial permeability transition · Apoptosis · Amino oxidase

Abbreviations

ADC	Arginine decarboxylase
AdNT	Adenine nucleotide translocase
AGM	Agmatine
AIF	Apoptosis-inducing factors
Bak	Bcl-2 agonist or killer

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Bax	Bcl-2 associated X protein
Bcl-2	B cell lymphoma 2
BID	BH3 interacting domain
BSAO	Bovine serum amine oxidase
CypD	Cyclophylin D
Cyt.c	Cytochrome c
FAD	Flavin adenine dinucleotide
H_2O_2	Hydrogen peroxide
I_2	Imidazole receptor type 2
IAPs	Inhibitor of apoptosis proteins
IM	Inner membrane
MAO	Monoamine oxidase
MOMP	Mitochondrial outer membrane permeabilization
MPT	Mitochondrial permeability transition
MPTP	Mitochondrial permeability transition pore
OM	Outer membrane
PAO	Polyamine oxidase
PUT	Putrescine
RLM	Rat liver mitochondria
ROS	Reactive oxygen species
Smac/DIABLO	Second mitochondria-derived activator of caspase/direct IAP binding protein with low pI
SMO	Spermine oxidase
SPD	Spermidine
SPM	Spermine
TPP^+	Tetraphenylphosphonium
VDAC	Voltage-dependent anion channel
ΔE	Electrode potential variation
ΔpH	Chemical gradient
$\Delta\Psi$	Electrical transmembrane potential
$\Delta\mu_H^+$	Transmembrane electrochemical gradient

Mitochondria

Mitochondria, also called cell "powerhouses," due to their ability to convert redox energy into ATP, are small double-membrane organelles involved in the regulation of life and death of eukaryotic cells. They are in fact involved in phospholipid and heme synthesis, and mainly in modulating several metabolic processes culminating in the synthesis of ATP. In this regard, a very recent report states that mitochondria may function as key regulators of several types of skeleton muscle fibers, important for sustaining muscle strength in both exercise and clinical settings (He et al. 2016). In addition, besides regulating the levels of intracellular signaling molecules (e.g., Ca^{2+} and ROS), they also activate the "intrinsic" apoptotic pathway (Osellame et al. 2012; Tait and Green 2012).

Mitochondrial bioenergetic activity depends to a great extent on the integrity of the mitochondrial membranes, particularly the inner one, as its insulating properties are essential for correct electron flux in respiratory complexes and, consequently, for establishment of the electrochemical gradient ($\Delta\mu_{H+}$) characteristic of energy-transducing membranes. It is well known that $\Delta\mu_{H+}$ is given by the sum of the electrical transmembrane potential ($\Delta\Psi$) and the chemical gradient (ΔpH), and it is expressed in mV.

Mitochondrial morphology differs among the various cell types: from solitary organelles in hepatocytes to intricate networks in many epithelial cells (Scorrano 2013). In hepatocytes and fibroblasts, the organelles are typically characterized by a length of 3–4 μm and a diameter of 1 μm, thus resembling small bacteria in size and shape. The number of mitochondria per cell also varies from one cell type to another. According to the classic model, mitochondria have an outer membrane (OM) and an inner membrane (IM), the latter being characteristically invaginated and forming pleated-like structures called *cristae*. As shown in Fig. 1a, this organization was hypothesized to generate an intermembrane space (enclosed by the OM and IM) and a matrix (enclosed by the IM) (Scheffler 2001).

However, recent electron tomography studies have modified this view by revealing new information on the mitochondrial membrane structure, with new evidence of organelle compartmentalization. These observations show that the surface area of the inner membrane, topologically continuous, is divided into two distinct domains, the surfaces of which are contiguous. The first domain, forming the peripheral space of the inner membrane, i.e., the

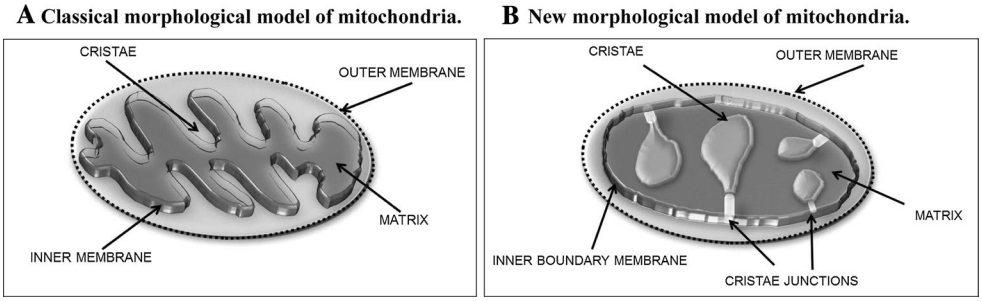

Fig. 1 Classical morphological model of mitochondria (**a**). New morphological model (**b**). The classical model shows the *cristae* formed by pleated-like structures due to invagination of IM (invagination model). In the new model, the *cristae* are separated component from IM connected with the intermembrane space by the *cristae* junctions

"inner boundary membrane," is near the outer membrane and is in close contact with it at several points. The second domain originates from the first and forms the *cristae*, which extend to the inner compartment of mitochondria, forming tubular or lamellar structures. The *cristae* are connected to each other and to the intermembrane space by small tubular junctions called "*cristae* junctions." According to electron tomography results, as mentioned above, a new model has been proposed, thus replacing the "invagination" model by the "*cristae* junction" model, in which the *cristae* are not invaginations with wide openings on the intermembrane space, but are separate compartments connected among themselves and with the intermembrane space by the *cristae* junctions (Fig. 1b) (Scheffler 2001; Scorrano 2013; Frey and Mannella 2000; Frey et al. 2002). The mitochondria move freely in the cytoplasm and thicken in sites requiring maximum energy (e.g., the muscle fibers surrounding the myofibrils). Their movements have been identified as the result of intricate interactions among proteins on the outer surface and various components of the cytoskeleton, including actin, filaments, microtubules, and intermediate filaments.

Mitochondria and Ca^{2+}

Ca^{2+} is an important signaling molecule, not only in cells but also in mitochondria, which maintain a large Ca^{2+} gradient across their IM. This gradient is due to the contemporaneous activity of the electrophoretic Ca^{2+} uptake and the electroneutral Ca^{2+} efflux that impose a larger endogenous Ca^{2+} concentration than the exogenous one. The bidirectional transport of Ca^{2+} in mitochondria has been extensively studied in the past, and a large number of reviews have been reported. Among them, it is to cite an exhaustive one of Carafoli (2003). This process has been first demonstrated in isolated mitochondria, in which these organelles are clearly able to accumulate large quantities of Ca^{2+}, by a mechanism requiring the activity of oxidative phosphorylation (Vasington and Murphy 1962) and ATP, Mg^{2+} and inorganic phosphate (DeLuca and Engstrom 1961). More recently, the electrophoretic uniporter responsible for Ca^{2+} uptake has been identified as a 40-kDa protein of the IM (De Stefani et al. 2011). This transport system, most probably a channel, was identified in a computer simulation and confirmed by an extensive cellular biology study, including analysis of the location of the protein, its function in living cells, mutant construction, protein purification, and analysis in vitro. The efflux mechanism is regulated through a Na^+/Ca^{2+} exchanger, the activity of which was identified by Carafoli et al. (1974) in heart, and an H^+/Ca^{2+} antiporter, described in liver (Fiskum and Lehninger 1979) (Fig. 4). Several mitochondrial enzymes are regulated by Ca^{2+}, thus explaining the need for a bidirectional flux or, as proposed by several research groups, a Ca^{2+} cycling mechanism. The accumulation of high Ca^{2+} concentrations in the presence of phosphate, as demonstrated by Lötscher et al. (1980), may also lead to complete and irreversible depolarization of the organelle. These conditions alter the permeability of the IM, now known as the mitochondrial permeability transition (MPT). As Grijalba et al. (1999) demonstrated, Ca^{2+} alters the lipid organization of the IM by interacting with the anionic head of the phospholipid cardiolipin. These alterations may compromise the conformation and thus the functionality of membrane components (including coenzyme Q), thus affecting the function of the respiratory chain. As a result, the generation of cytotoxic reactive oxygene species (ROS) is favored (Vercesi et al. 1997).

Mitochondrial ROS production

Mitochondria clearly play a crucial role in regulating the redox state of the cell. The respiratory chain is in fact the primary putative source of ROS, mainly produced during the flow of electrons through complexes I, II, and III (Murphy 2009; Moreno-Sánchez et al. 2013). Normally, ROS is generated in sufficiently small numbers to be removed by the scavenging systems of the organelle, which include superoxide dismutase, catalase, glutathione, and thioredoxin reductase. However, when ROS production exceeds the scavenging capacity of the above safety systems (e.g., in the presence of too much Ca^{2+} and phosphate, as described previously, and/or when scavenging pathways are not activated), accumulated ROS can cause several kinds of damage in both mitochondria and other subcellular systems (Kowaltowski et al. 2001; Murphy 2009).

In mitochondria, oxidative damage can cause alterations of the redox state of both membrane and matrix components (glutathione, thiols, and electron transport chain complexes) and DNA, which, in turn, clearly hamper the mitochondria and also their metabolic functions. Mitochondrial oxidative stress has been demonstrated to be a fundamental step in the induction of apoptosis in rat liver mitochondria (RLM) (Halestrap et al. 1993). Conversely, this phenomenon has not been observed in rat brain mitochondria (Grancara et al. 2011).

Mitochondria and apoptosis

Cell death may be classified as apoptotic (programmed) or necrotic (accidental), although the boundaries between the two are not always clearly defined (Osellame et al. 2012).

Apoptosis is an active, coordinated process which requires energy: it is essential for the development of

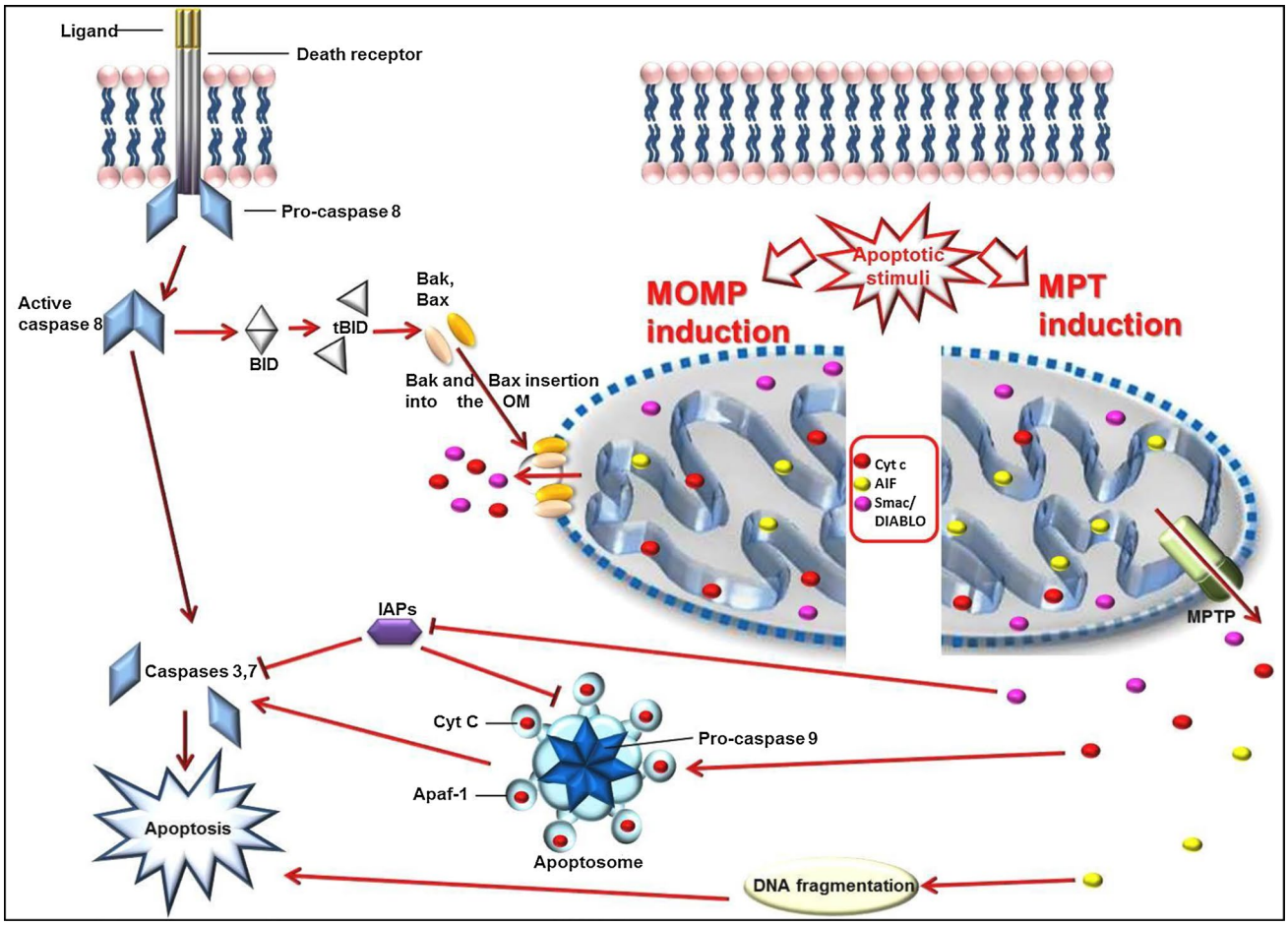

Fig. 2 *Intrinsic pathway* In response to pro-apoptotic stimuli leading to OM permeabilization (MPT or MOMP), cyt.c, Smac/DIABLO, and AIF are released in the cytosol. Cyt.c together apoptotic protease activating factor (Apaf) and dATP forms the apoptosome that, in turn, activates caspase cascade leading to intrinsic apoptosis. The inhibitory effect on apoptosis by IAPs is removed by Smac/DIABLO. AIF induces apoptosis in a caspase-independent manner by inducing DNA fragmentation. Activation of BID by caspase 8, or other activators, induces a Bax or Bak translocation to OM. Then, they undergo a change in conformation leading to their oligomerization and induction of MOMP and consequent cyt.c release. *Extrinsic pathway* Binding of appropriate ligands to plasma membrane death receptors activate pro-caspase-8 that, in turn, activates caspase-3 leading to extrinsic apoptosis. Death receptors and mitochondrial pathway converge on caspase-3 activation

organisms, removing excessive damaged or aged cells. It occurs along two signaling pathways: extrinsic, which involves cell surface receptors and culminates in the activation of caspase 8; and intrinsic, which requires mitochondrial permeabilization (Fig. 2).

Necrosis is the result of a metabolic failure which gives rise to an "energy collapse" of the cell, leading to the breakdown of ion gradients, cell swelling, and structural disorganization.

According to Osellame et al. (2012), the rupture of mitochondrial membranes is crucial for both apoptotic and necrotic processes, but the above authors state that, although MPT activation is one of the major mechanisms driving necrosis, mitochondrial outer membrane permeabilization (MOMP) is characteristic of intrinsic apoptosis. Instead, Halestrap (2005) distinguishes between the induction of transient MPT (in which ATP production is maintained) leading to apoptotic cell death, and permanent MPT induction (in which ATP content is depleted), leading to necrotic cell death. In both hypotheses, permeabilization of mitochondrial membranes clearly appears to be crucial to the mechanism of cell death.

Mitochondrial permeability transition and controversies

In normal physiological conditions, the mitochondrial IM is permeable to a few selected metabolites and ions. However, in response to noxious stimuli (e.g., Ca^{2+} overload,

oxidative stress, and cytotoxic agents), a non-specific pore, the mitochondrial permeability transition pore (MPTP), opens at the points of contact between IM and OM, allowing the passage of molecules with molecular masses of up to 1.5 kDa. It should be stressed that although both membranes seem to be involved, the MPT is essentially a process regarding the IM, since experiments on mitoplasts (i.e., mitochondria deprived of the OM) showed a typical MPT in these structures (Dalla Via et al. 2014; Siemen and Ziemer 2013), with disrupted permeability of the IM, allowing the passage of solutes across it. The free passage of protons then induces uncoupling of oxidative phosphorylation and hydrolysis of ATP. As a consequence, retention of proteins in the matrix (due to pore dimensions) gives rise to colloid-osmotic pressure, allowing water to enter the matrix, with swelling of mitochondria, unfolding of *cristae*, and expansion of the matrix, all of which contribute to disrupting the OM, with the release of mitochondrial proteins, including cytochrome c (cyt.c), Second mitochondria-derived activator of caspase/Direct IAP binding protein with low pI (Smac/DIABLO) and apoptosis inducing factor (AIF) (Halestrap 2005; Brenner and Moulin 2012) (Figs. 2, 4).

Several studies have addressed the problem of MPTP as a dynamic multiprotein complex. Its proposed composition involves various interacting proteins: the adenine nucleotide translocator (AdNT), the matrix peptidyl-prolyl isomerase cyclophilin D (CypD), the phosphate carrier, and also the OM protein voltage-dependent anion channel (VDAC) and the cytosolic and intermembrane space hexokinase and creatine kinase, respectively. Since the mid-1990s, several MPTP candidates have been "knocked out" in mice, but these genetic studies have failed to support their implication in the pore. In particular, they suggest a regulatory function for AdNT and CypD, but exclude any essential role for VDAC (Brenner and Moulin 2012). Conversely, a recent hypothesis indicates that MPTP is composed of dimers of F_0F_1-ATP synthase which, when incorporated into lipid bilayers, can form Ca^{2+}-activated channels, with characteristics in common with MPTP studied in situ (Giorgio et al. 2013).

Further studies are clearly required to clarify the composition of MPTP. This remains an extremely important scientific question, in view of the possible involvement of the MPTP in mitochondria-associated dysfunctions which warrants the development of mitochondria-targeting drugs.

Pro-apoptotic factors

The release of pro-apoptotic proteins from mitochondria is a well-known process, crucial for activation of the cell death mechanism. This study focused primarily on cyt.c, Smac/DIABLO and AIF.

Cyt.c is a 12.5-kDa soluble metalloprotein located on the side of the IM facing the intermembrane space, which shuttles electrons from Complex III (cytochrome c reductase) to Complex IV (cytochrome c oxidase) of the respiratory chain (van Gurp et al. 2003). The presence of various pools of cyt.c has been proved: loosely bound pools are mainly linked to membrane phospholipids (especially cardiolipin) by electrostatic interactions, whereas tightly bound pools are partially embedded in the IM, due to hydrophobic interactions (Ott et al. 2001). In response to several pro-apoptotic stimuli, leading to OM permeabilization, cyt.c is released into the cytosol, where it binds dATP and adaptor molecule apoptosis-protease activating factor I, initiating the formation of the apoptosome. This complex, in turn, activates the caspase cascade, leading to chromatin condensation, DNA fragmentation, and cell death (Dalla Via et al. 2014).

Smac/DIABLO is a 23-kDa protein in the intermembrane space which, upon OM permeabilization, is released into the cytosol (Maly 2007). The physiologic function of Smac/DIABLO is still unknown, although it has been reported that it enhances apoptosis by binding to members of the inhibitor of the apoptosis protein (IAPs) family. Smac/DIABLO competes with caspases for (inhibitory) binding to IAPs, because the first factor has regions structurally similar to those that in caspases binding IAPs. Thus, the inhibitory effect of IAPs is removed by Smac/DIABLO, and the caspases are able to act in apoptosis (van Gurp et al. 2003; Dalla Via et al. 2014).

AIF is a type-I IM protein (62 kDa), with its N-terminal portion exposed to the matrix and its C-terminus protruding into the intermembrane space (Otera et al. 2005). In physiological conditions, AIF exhibits antioxidant activity and is required for the correct assembly and maintenance of Complex I of the respiratory chain (Vahsen et al. 2004). The release of AIF into the cytoplasm is caused by its cleavage to a 57-kDa protein in response to apoptotic signals. The released AIF induces nuclear apoptosis in a caspase-independent manner by translocating to the nucleus and inducing DNA fragmentation and chromatin condensation (Dalla Via et al. 2014) (Fig. 2).

Mitochondrial outer membrane permeabilization

As noted, there are two main models explaining the release of pro-apoptotic factors during mitochondria-mediated apoptosis (Green and Reed 1998). In the first model, permeabilization of mitochondrial membranes occurs through the formation of MPTP, and involves both mitochondrial membranes; the second model conceives the main involvement of MOMP. The latter process is characterized by the formation of channels in the OM (taking place

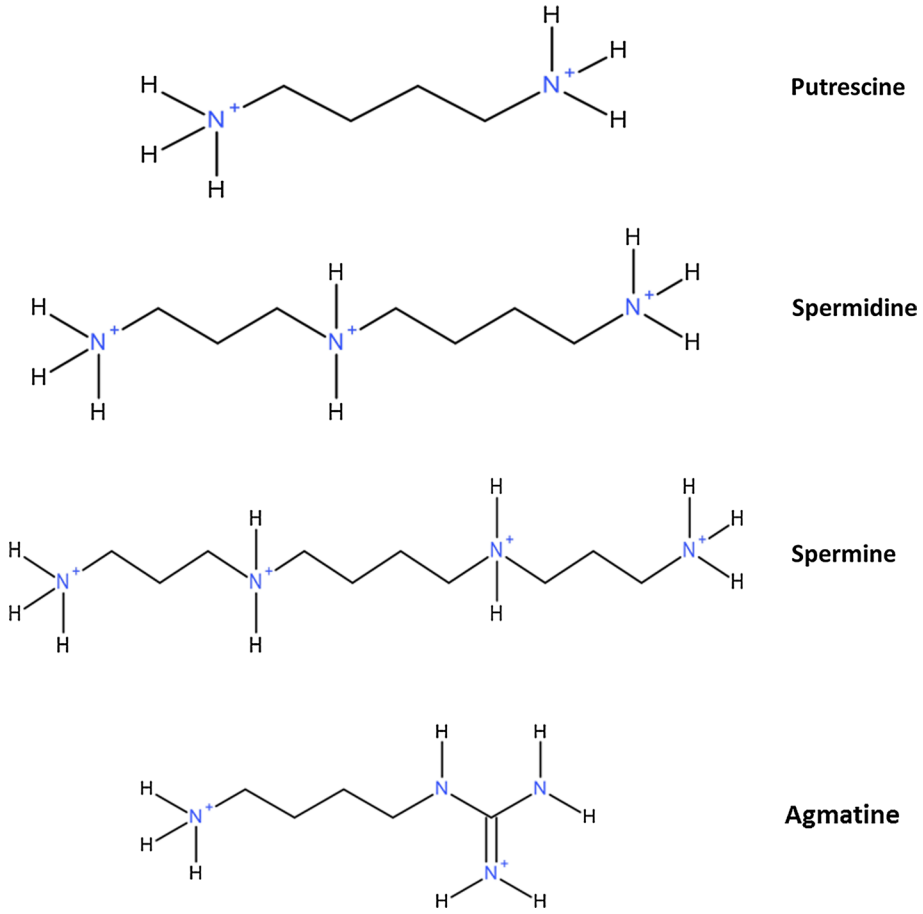

Fig. 3 Polyamines and agmatine. At physiological pH, the basic groups of all polyamines and agmatine are almost completely protonated, therefore allowing them to, respectively, behave as polycationic or dicationic compounds

independently of mitochondrial swelling), and may be induced by different stimuli, such as a state of hypotonicity, hydrogen peroxide, and/or pro-apoptotic B cell lymphoma 2 (Bcl-2) family proteins (Arnoult et al. 2002; Gogvadze et al. 2006).

According to their function, Bcl-2 proteins are subdivided into anti-apoptotic and pro-apoptotic members. The latter group is further divided into two subfamilies, according to the presence of specific Bcl-2 homology domains (BH1, BH2, and BH3): Bcl-2 associated X protein (Bax) and Bcl-2 agonist or killer (Bak), sharing BH1-BH3 domains, are the effectors of MOMP. BID is another BH3-only protein involved in CD95-mediated cell death. Indeed, CD95 activation leads to BID truncation (tBID) through a caspase-8-driven process and tBID is pivotal to induce mitochondrial outer membrane permeabilization and cell death (Li et al. 1998) (Fig. 2). BH3-only proteins can interact with Bax and Bak, or with the other anti-apoptotic members (Green and Kroemer 2004). These proteins share structural similarity with bacterial pore-forming toxins, suggesting that they act similarly in the OM (Tait and Green 2010). According to Bleicken et al. (2013), after the translocation of Bax to the OM, the conformations of Bax and Bak change, leading to their oligomerization and to the formation of pores. However, the exact mechanism through which Bcl-2 proteins regulate this process is still a matter of debate.

Biologically active amines

Biologically active amines are a class of compounds physiologically synthesized in living organisms. According to a classification proposed by COST actions 917 and 922 (research programs funded by the Commission of the European Communities DG/XIIB), active amines are distinguished into polyamines: putrescine (PUT), spermidine (SPD), spermine (SPM), and biogenic amines (agmatine, serotonin, tyramine, histamine, phenylethylamine, tryptamine, and cathecolamines) (Toninello et al. 2004).

Polyamines

PUT, SPD, and SPM are decarboxylation products of ornithine and S-adenosyl-methionine metabolism in nearly all eukaryotic cells. In physiological conditions, these molecules are fully protonated (poly)cations (Agostinelli et al.

2010) (Fig. 3). The total intracellular concentration of polyamines is in the mM range, although the majority is bound to various anions in cells, such as DNA, RNA, proteins, and phospholipids (Casero and Marton 2007).

Many studies emphasize the essential activities carried out by polyamines, including activation of kinases involved in signal transduction, regulation of ion channel gating, and modulation of oxidative processes. Thus, polyamines play mandatory roles in several cell functions, such as DNA synthesis, cell proliferation, gene transcription regulation, translation, post-translational modification, membrane stability, apoptosis, membrane and cytoskeleton functioning, modulations of the cell cycle and ion channeling (Pegg and Casero, 2011).

Polyamines and diseases

Interest in polyamine research increased when polyamines and their metabolites were found to play critical roles in human diseases. Alterations in polyamine levels and metabolism have been detected in many types of diseases, including chronic renal failure, liver cirrhosis, cystic fibrosis, Alzheimer's and Parkinson's diseases, Duchenne muscular dystrophy, and cancer. Regarding cancer, increased polyamine concentrations have been detected in many solid tumors, and similar increases in urinary and serum polyamine contents have shown to be common features of malignancy. In addition, the polyamine biosynthetic pathway is more active in tumor tissues, making it an attractive target for designing chemotherapeutic drugs (Wallace and Fraser 2004).

Cellular transport of polyamines

The cellular content of polyamines which, as noted above, plays an important role in cell proliferation and differentiation, is highly regulated by their biosynthesis, degradation and transport. As regards their transport in mammalian cells, a specific transporter has not yet been identified, although polyamine accumulation has been found to be energy-dependent and selective. Polyamines have been proposed to be imported first by a plasma membrane carrier and then sequestered in pre-existing vesicles (Hoshino et al. 2005). Very recent advances in the field described by Nowotarski et al. (2013) also indicate two other mechanisms for polyamine import: the first is the binding of SPM on heparan sulfate groups of glypican 1 on the cell surface, with subsequent internalization of the amine; SPM is then freed by a nitric oxide-oxidation-mediated process. The second proposal involves caveolin-1-dependent internalization. The subsequent release of the internalized compounds has been described only for PUT through the solute carrier family 3 member 2 exporter, whereas a nitric oxide synthase 2-dependent reaction is thought to destabilize SPM-receptor complexes (Nowotarski et al. 2013).

Polyamines and mitochondria

Mitochondria apparently lack a polyamine biosynthetic pathway, although substantial quantities of SPM and SPD have been detected in heart, liver, and brain mitochondria, due to the presence of a transport system in the membranes of these organelles (Toninello 2001). The first demonstration of such a system was published by Toninello et al. (1985), showing that SPM internalization in mitochondria occurs through an energy-dependent mechanism. Further investigations (for a review, see Toninello et al. 1992, 2004) identified two binding sites for SPM and SPD, both with mono-coordination, low affinity, and high binding capacity, whereas only one binding site was evidenced for PUT. This site corresponded to one of the binding sites for SPM and SPD. Polyamines were demonstrated to be mutually competitive inhibitors, indicating a common transport system. Indeed, the above studies demonstrated that polyamines are transported by a channel having two asymmetric energy barriers and that SPM can be released out of mitochondria (Fig. 4).

The structure of these amines allows their specific interaction with mitochondrial membranes and is probably responsible for the variety of effects ascribed to these molecules (e.g., activation of the mitochondrial uptake of hexokinase and protein kinases CKI and II, stimulation of the activity of pyruvate dehydrogenase, and inhibition of ATP hydrolysis, by F_1-ATPase). The main target of polyamines seems to be the MPT, and Toninello et al. (1984) first reported the ability of SPM to prevent Ca^{2+}-induced MPT in RLM in 1984. Several later studies confirmed the inhibitory effect of SPM not only in liver, but also in other organs (e.g., heart, intestine; Anup et al. 1999). The protective effect of polyamines on MPT induction is not completely understood, although several mechanisms have been examined to elucidate their activity (for a review, see Toninello et al. 2004). Apart from the stabilization of mitochondrial membranes, due to their charged molecules (Lapidus and Sokolove 1993), one of the main actions of polyamines, particularly SPM, resides in their antioxidant properties (Sava et al. 2006). SPM exhibits scavenging by reacting with the hydroxyl radical ($OH^{\cdot}$), resulting in the production of SPM dialdehyde (1,12-bis-1,12-dioxo-4,9-diazododecane). As MPT opening is linked to the "redox catastrophe" (Susin et al. 1998), the above effect is undoubtedly fundamental in explaining the SPM inhibition of MPT (Fig. 4).

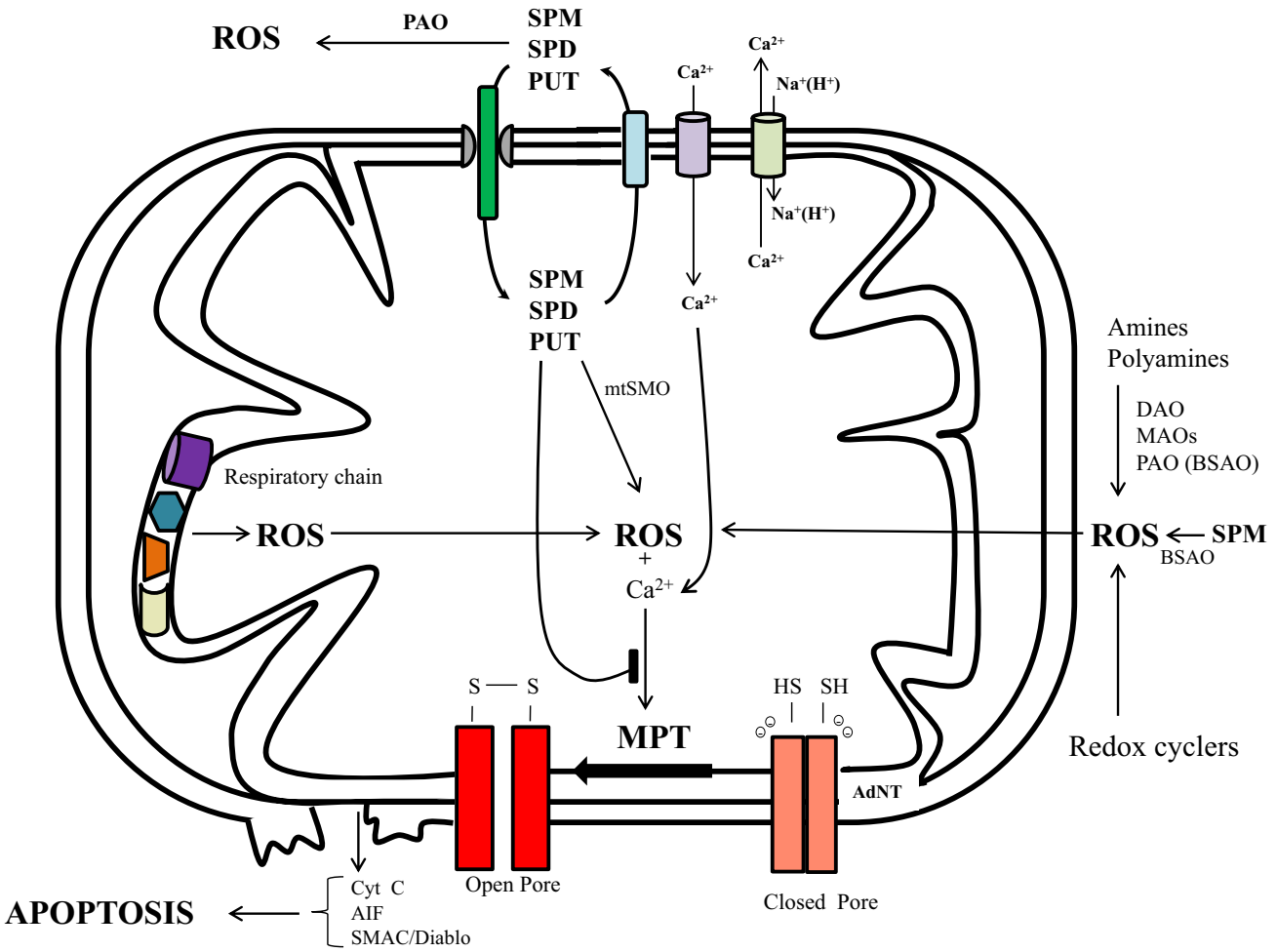

Fig. 4 Polyamine transport in mitochondria. Induction or prevention of MPT and apoptosis. Polyamines, in particular SPM, when transported in mitochondria at low concentrations can be oxidized by mitochondrial SMO with ROS production. In the presence of Ca^{2+}, MPT is induced with the opening of the transition pore and consequent release of cyt.c, AIF, and Smac/DIABLO, thus leading to apoptosis. At high concentrations, SPM behaves as a scavenger of ROS (see text) by preventing the MPT. ROS is also formed by the oxidation of exogenous amines and polyamines by cytosolic amine oxidases, as well as by the action of redox cyclers and respiratory chain activity

Despite this effect, and thus the anti-apoptotic potential of polyamines (Sava et al. 2006), it has been found that SPM causes caspase activation in leukemia cells (Stefanelli et al. 1998). Always in these cells, Stefanelli et al. (1999) demonstrated that 100 μM SPM was enough to release cyt.c in a cell-free model of apoptosis from leukemia cells. Again, Stefanelli et al. (2000) also reported the CsA-insensitive release of cyt.c induced by these molecules in rat heart mitochondria. The effect was demonstrated to be independent of mitochondrial damage, and the pool of cyt.c released was probably not part of the electron transport chain—a possibility supported by the evidence of several cyt.c pools in mitochondria (Ott et al. 2001). Furthermore, it has also been reported that the release of cyt.c is parallel with SPM uptake (Hoshino et al. 2005).

Polyamines in autophagy and mitophagy

Autophagy is a process that allows the orderly degradation and recycling of cellular components. During this process, double-membrane vesicles, known as autophagosomes, are formed. The autophagosome contains targeted cytoplasm constituents that are isolated from the rest of the cell. Thus, the autophagosome fuses with a lysosome and its contents are degraded and recycled.

Autophagy serves as an essential protective pathway to mitochondrial dysfunction and oxidative stress. In particular, in Alzheimer's and Parkinson's diseases, and stroke, mitochondrial dysfunction due to aging, genetic abnormalities, environmental damage or neuroinflammation results in decreased mitochondria oxidative phosphorylation and

accumulation of mitochondrial DNA damage (Redmann et al. 2016).

Mitophagy is a conserved, mitochondrial specific autophagic process, associated with the selective degradation of these organelles. It often occurs to defecting mitochondria following damage or stress, and it has been first mentioned by Lemasters (2005). It has been recently discovered an intricate regulatory network that balances mitophagy with mitochondrial biogenesis. Proper coordination of these opposing effects is important for stress resistance and longevity (Palikaras et al. 2015). Polyamine has shown to induce autophagy to protect cells from stress. In particular, it has been reported that induction of autophagy by SPD promotes longevity (Eisenberg et al. 2009) and that this polyamine can be consider an elixir of life (Madeo et al. 2010). Indeed, it has also been observed that SPD-triggered autophagy ameliorates memory during aging in Drosophila (Sigrist et al. 2014). The effects of synthetic polyamines activating autophagy have been investigated in cancer cells (Minarini et al. 2013), while the relationships among autophagy, mitochondria, and oxidative stress have been reported by Lee et al. (2012). The mechanism by which SPD induces autophagy is due to an inhibition of the acetyltransferase EP300 (Pietrocola et al. 2015). These investigations highlighting the role of polyamines in autophagy and, consequently, in mitophagy, open a new intriguing survey in their modulation of mitochondrial bioenergetics and cell survival.

Spermine and BSAO in cancer

SPM is also a substrate of several FAD-dependent enzymes, such as monoamine oxidase (MAO), polyamine oxidase (PAO), SPM oxidase (SMO), and Cu-amine oxidase like bovine serum amine oxidase (BSAO). SMO has very recently been discovered to be constitutionally present in mitochondria (Bonaiuto et al. 2015). The products of SPM oxidation, H_2O_2, aldehyde(s) and other ROS are toxic to cells (Gangas and Dewey 1981; Agostinelli et al. 1994; Calcabrini et al. 2002; Chen et al. 2001).

It has been demonstrated that SPM oxidation catalyzed by BSAO causes necrotic cell death of L1210 mouse leukemia cells (Bonneau and Poulain 2000). Both necrotic and apoptotic cell death due to oxidation of SPM products have also been observed in vitro both in cultured cells, human melanoma (M14) and colon adenocarcinoma (LoVo), as well as in vivo in melanoma B16 (Agostinelli et al. 2006a, b; Averill-Bates et al. 2005). In particular, electron microscopy observations and cytofluorometric studies have shown that these toxic products cause mitochondrial damage, leading to condensed matrix, altered and dilated *cristae*, and high membrane depolarization, resulting in MPT (Calcabrini et al. 2002). Then, MPT induces bioenergetic collapse, redox catastrophe and apoptotic cell death (Calcabrini et al. 2002; Arancia et al. 2004; Toninello et al. 2004). As SPM is largely transported in energized mitochondria (Toninello et al. 1988, 1992), the presence of SMO in the mitochondrial matrix supports the possibility that SPM is oxidized in this compartment, producing ROS and triggering the pro-apoptotic pathway by releasing pro-apoptotic factors cyt.c, AIF and Smac/DIABLO.

A similar possible event would explain the induction of apoptosis observed in tumor cells treated with exogenous BSAO and SPM (Agostinelli et al. 2010). These observations indicate the possibility of exploring a new strategy for tumor therapy. Evaluating the toxic effect of SPM when metabolized by SMO or other amine oxidases, a controversy may arise considering that SPM is a potent inhibitor of MPT. This contrasting effect, also found for agmatine (AGM) (see below), may be explained by the concentration of SPM interacting with mitochondria. At low concentrations, polyamines can be completely oxidized by amine oxidases leading to MPT induction and, eventually, to apoptosis in the cells. At high concentrations, SPM is in part equally oxidized, but contemporaneously, the aliquot not yet oxidized behaves as a scavenger against ROS produced by SPM itself, thus preventing MPT and apoptosis (Ha et al. 1998; Sava et al. 2006) (Fig. 4).

Agmatine

Discovered in 1910 by the Nobel Prize winner Albrecht Kossel (Kossel 1910), agmatine (1-(4-aminobutyl) guanidine) is a ubiquitous biogenic amine with two positive charges at physiologic pH (Fig. 3). In mammals, it is synthesized from arginine by the enzyme arginine decarboxylase (ADC) (mainly in brain), although much may be absorbed from the diet (Salvi et al. 2006). Ongoing research shows that AGM is involved in multiple biological functions: it is a ligand of α-adrenergic and imidazoline receptors type 2 (I_2), a neuromodulator and cotransmitter, modulator of some arginine metabolic pathways, inductor of nitric oxide synthesis, and modulator of polyamine metabolism (for a review, see Piletz et al. 2013).

Agmatine and diseases

After the discovery of the first neuroprotective effects of AGM in 1995, research expanded rapidly in several areas, such as neuro-, nephron-, and cardio-protection (Piletz et al. 2013). Further results showed that, from the first human clinical trials, oral AGM is safe and effective in alleviating neuropathic pain (Keynan et al. 2010). Intriguingly,

according to the cell type and its stage of proliferation, AGM has also been shown to have antiproliferative effects in rat hepatoma cells (Gardini et al. 2003) and leukemia cells (Haenisch et al. 2011), but it can also induce intrinsic apoptosis in non-proliferative hepatic cells (Gardini et al. 2001) and counteracts tumor necrosis factor-α in retinal ganglion cells (Hong et al. 2009). In conclusion, the complexity of AGM actions is summarized by the following statement by Piletz et al. (2013): "AGM is now considered to be capable of exerting modulatory actions simultaneously at multiple target sites, thus fitting the therapeutic profile of a 'magic shotgun' for complex disorders."

Cellular transport of agmatine

As previously mentioned, AGM may also come from the diet. After being absorbed by cells in the stomach, it is taken up by several other organs, particularly liver. Being amine (di-)protonated, to cross the membranes, AGM needs a transporter which, in hepatocytes (Cabella et al. 2001), endothelia (Babal et al. 2000) and kidney cells (Del Valle et al. 2001), is probably represented by the same mechanism exploited by polyamines (see above). However, in glyomal cells (Molderings et al. 2001) and in a cell line derived from human embryonic kidney, AGM may use a different route. In particular, in the latter case, the extraneuronal monoamine transporter and organic cation transporter 2 may also be involved (Grundemann et al. 2003).

Agmatine and mitochondria

AGM is transported into mitochondria, where its metabolic enzymes ADC and agmatinase are also present, as well as I_2, a 60-kDa protein located on the OM, with all of these constituting the monoamine oxidase (MAO) domain (Raddatz et al. 2000).

Previous data on AGM activity in isolated RLM have demonstrated that AGM may have different effects on mitochondrial bioenergetics, according to concentration: at low concentrations between 10 and 100 μM, in the presence of phosphate, it may act as a typical amplifier of the Ca^{2+} induction of MPT, whereas at high concentrations of about 1 mM, AGM inhibits this phenomenon (Battaglia et al. 2007). It should be noted that the different effect between low and high concentrations is not arbitrary, but is based on previously published data demonstrating that AGM concentration in hepatocyte cytosol is about 0.5 mM (Gardini et al. 2001). It should also be noted that AGM permeates both mitochondrial membranes (presumably at OM/IM contact sites) by means of a specific electrophoretic transporter, a channel, or a single-binding center-gated pore, identified in RLM (Salvi et al. 2006). The analyses carried out according to a thermodynamic approach developed by Di Noto et al. (1996, 2002) demonstrated the presence of two binding sites (S_1 and S_2) for AGM on mitochondrial membranes, both with mono-coordination (Martinis et al. 2012). S_1 is probably located on I_2 which, as previously mentioned, is itself a MAO domain. Since AGM can inhibit MAO activity, its binding to S_2 further stresses the importance of the molecule. Last, the last report mentioned above hypothesizes the existence of AGM binding sites: S_1, sensing AGM concentration, located outside the mitochondria, eventually modifies the properties of S_2, the transport site.

AGM induction of the MPT, at low concentrations, is triggered by the oxidation of AGM, which is probably catalyzed by a copper-dependent amine oxidase in RLM (Cardillo et al. 2009). This enzyme may be responsible for the large amounts of H_2O_2 detected in the presence of AGM, both in the presence or the absence of Ca^{2+}. The resulting ROS causes bioenergetic collapse of the organelle and activation of the intrinsic pro-apoptotic pathway. A different scenario may be observed at higher concentrations: AGM can inhibit the MPT induced by Ca^{2+} in the presence of phosphate, probably because of its antioxidant properties (Battaglia et al. 2010). In fact, in these conditions, the amine can generate oxidative stress, but the number of still unreacted molecules may act as scavengers, thus exhibiting self-protection, as also observed with spermine. This scavenging mechanism on the part of AGM in the presence of spermine is applicable when the targeted ROS is $OH^{\cdot}$: unprotonated polyamine reacts with OH to form dihydroxyaminobutyl-guanidine and, subsequently, guanidobutyric aldehyde, by spontaneous dehydration and hydrolysis, in a reaction previously proposed for SPM (Sava et al. 2006), as mentioned above.

Conclusions and perspectives

This minireview, besides evidencing milestones regarding mitochondrial structure and the main physiological role played by these organelles, such as Ca^{2+} transport, ROS production, MPT induction, and intrinsic apoptosis triggering, together with interactions between polyamines and mitochondria, also reports novel discoveries which open up new scenarios. In this regard, the latest interpretations of the structure of mitochondria suggest particular functional implications: it has been hypothesized that "*cristae* junctions" represent a barrier to free diffusion of ions from the inter-*cristae* space to intermembrane space. These junctions may in effect constitute a barrier to protein diffusion, which is probably differently distributed between the domains of the inner membrane, thus allowing compartmentalization of its functions. Another intriguing implication is that the

cristae junctions connecting the intermembrane space with the inter-*cristae* space may be involved in the phenomenon of MPT and the consequent opening of the transition pore (Scheffler 2001; Perkins et al. 1997; Frey et al. 2002; Scorrano 2013).

The discovery of the Ca^{2+} uniporter channel opens up an intriguing phase of study in which we will be able, both in vivo and in vitro, to investigate the precise function of this channel, verify its role in human diseases, and develop new drugs specifically directed toward this new molecular target.

In addition, new observations on proteins involved in assembling the transition pore and the controversies they have created, lead to further considerations about the molecular basis of MPT. In particular, one intriguing hypothesis is that AdNT, the phosphate carrier and ATP synthase constitute a single protein which can change its configuration and function according to particular transduction pathways.

Another observation, which should be taken into account and which shows the significant differences among varying cell types of mitochondria, is the induction of MPT in rat brain, not related to oxidative stress. Brain mitochondria very probably have a special MPT pathway which differs from that in other mitochondria. This observation may in turn mean that these mitochondria can undergo MPT by the mechanism proposed many years ago by Halestrap and Davidson (1990): the action of phosphate and a Ca^{2+}-dependent pyrophosphatase, transforms AdNT first into a K^{+}-channel and then into an aspecific pore. This theory, first proposed more than 25 years ago, could now be reappraised. It should also be recalled that, in the case of brain mitochondria, SPM protects against MPT induction by a mechanism which differs from that of an ROS scavenger but which is most probably due to direct interaction with the anionic charges on the proteins forming the pore structure (Grancara et al. 2011).

A noteworthy discovery is also the demonstration that both SPM and AGM, in appropriate conditions and suitable concentrations, can exhibit two opposing effects: induction or prevention of MPT or MOMP.

These effects mean that biologically active amines may be viewed as potential inducers or inhibitors of apoptotic cell death if their effects are evaluated at cellular level. All these premises may have a great influence on new strategies for therapeutic interventions against cancer.

Therefore, from a therapeutic point of view, enhancing the efficacy of the in situ formation of enzymatic cytotoxic polyamine metabolites undergoing pathological proliferation may reasonably be used to treat neoplastic diseases. Treatment of human colon adenocarcinoma and melanoma cells with BSAO and spermine has been flanked by characteristic morphological changes. Such alterations in cancer cells mirror the results of cell survival experiments (Calcabrini et al. 2002; Agostinelli et al. 2006a, b). It was assumed that phenotypic changes (transformation of elongated cells and rounded shapes) are the result of impairment of the cytoskeleton due to the reaction with H_2O_2 and aldehyde(s). Severe changes in mitochondrial structure induce various functional alterations (collapse of the mitochondrial inner membrane potential, uncoupling of the respiratory chain, overproduction of ROS, release of ions and proteins), leading to apoptosis or necrosis. These ultrastructural irregularities of the mitochondria appear to be correlated with the cytotoxic effect of the treatment. Matching this finding is the fact that the mitochondriotoxic effects of exposure to spermine metabolites are among early events.

According to the data reported here, the idea of using amine oxidase in cancer therapy should be examined. In the present experimental model, the products of enzymatic oxidation of polyamines were generated outside the cells, which mean that the main challenge will be to deliver purified BSAO into cancer cells to induce their selective killing by cytotoxic factors produced intracellularly from endogenous polyamines. Therefore, strategies could be developed to discover how the enzyme could be delivered in vivo, for possible clinical application. BSAO has previously been conjugated to poly(ethylene glycol) and immobilized in a hydrogel-type matrix (Demers et al. 2001). In mouse melanomas, using immobilized BSAO instead of free BSAO favored cell apoptosis with respect to necrosis (Averill-Bates et al. 2005).

With the aim of improving system efficacy by exploiting a nanotechnology approach, BSAO was covalently immobilized into injectable nanohydrogels (NHs) based on cholesterol–graft-hyaluronic acid, a biocompatible conjugate. The resulting NH system was more bioactive than free BSAO, indicating that this approach may represent a promising tool inducing an apoptotic effect in human cancer cell treatments (Montanari et al. 2013).

Core–shell gold nanoparticles stabilized with a hydrophilic polymer, poly(3-dimethylammonium-1-propyne hydrochloride), have recently been used to immobilize BSAO. The general availability of this enzyme may have multiple applications in the biotechnological and pharmaceutical fields as a biosensor and in medicine as an anticancer therapy (Venditti et al. 2015).

In this regard, there is a growing interest in the utilization of a coupled system of exogenous and endogenous polyamines/BSAO for inducing MPT in isolated mitochondria and apoptotic cell death in cancer cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This research did not involve human participants or animals.

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