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REVIEW

Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies

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Traumatic brain injury (TBI) is a major health and socioeconomic problem throughout the world. It is a complicated pathological process that consists of primary insults and a secondary insult characterized by a set of biochemical cascades. The imbalance between a higher energy demand for repair of cell damage and decreased energy production led by mitochondrial dysfunction aggravates cell damage. At the cellular level, the main cause of the secondary deleterious cascades is cell damage that is centred in the mitochondria. Excitotoxicity, Ca^{2+} overload, reactive oxygen species (ROS), Bcl-2 family, caspases and apoptosis inducing factor (AIF) are the main participants in mitochondria-centred cell damage following TBI. Some preclinical and clinical results of mitochondria-targeted therapy show promise. Mitochondria- targeted multipotential therapeutic strategies offer new hope for the successful treatment of TBI and other acute brain injuries.

Abbreviations

AIF, apoptosis inducing factor; ANT, ADP/ATP carrier adenine nucleotide translocase; BH, Bcl-2 homology; CBF, cerebral blood flow; CK, creatine kinase; CL, cardiolipin; CsA, cyclosporin A; Cyp D, cyclophilin D; cyt, cytochrome; ER, endoplasmic reticulum; ETC, electron transport chain; Fis1, fission1; HSPs, heat-shock proteins; IAP, inhibitor of the apoptosis protein; IBM, inner boundary membrane; IM, inner membrane; IMS, intermembrane space; iNOS, inducible NOS; HK, hexokinase; LP, lipid peroxidation; MAC, mitochondrial apoptosis-induced channel; MAM, mitochondria-associated endoplasmic reticulum-membrane; MEK, MAPK/ERK kinase; mGlu receptor , metabotropic glutamate receptor; MMP, mitochondrial membrane permeabilization; MOMP, mitochondrial outer membrane permeabilization; MPT, membrane permeability transition; mtDNA, mitochondrial DNA; mtPARP, PARPs within mitochondria; NCXs, Na^+/Ca^{2+} exchangers; OM, outer membrane; Opa, optical atrophy protein; PARL, presenilin associated rhomboid-like protein; Pi, mitochondrial phosphate carrier; PMCA, plasma membrane Ca^{2+} ATPase pump; PTPC, permeability transition pore complex; ROS, reactive oxygen species; SAPKs, stress-activated protein kinases; TBI, traumatic brain injury; tBid, truncated Bid; TSPO, translocator protein; UCP, uncoupling protein; VDAC, voltage-dependent anion channel; VDCC, voltage-dependent Ca^{2+} channels; $\Delta\Psi m$, membrane potential

Traumatic brain injury (TBI) constitutes a major health and socioeconomic problem throughout the world. According to the report (2002–2006) of Centers for Disease Control and Prevention, at least 1.7 million people sustain a TBI in the United States each year. About 1.365 million of those individuals are treated in an emergency department, 275 000 are hospitalized and 52 000 die. Although many preclinical studies have shown positive results in treating TBI, almost

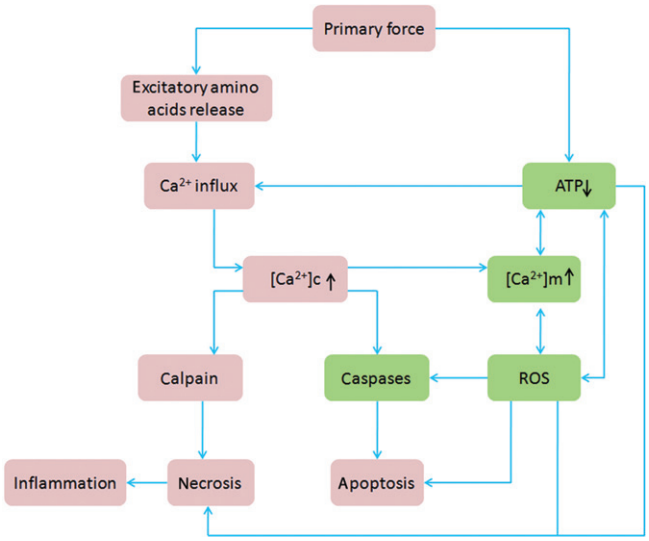

Figure 1

Pathological changes of TBI. The primary force (trauma) will cause direct tissue damage, impaired regulation of CBF and metabolism and excessive release of excitatory neurotransmitters. This results in an abnormally high level of free intracellular and mitochondrial Ca^{2+}. The consequent Ca^{2+} overload leads to self-digesting (catabolic) intracellular processes that involve overproduction of free radicals, activation of cell death signalling pathways and up-regulation of inflammatory mediators. Together, these events lead ultimately to cell death. Note: the green boxes show the main pathological changes in mitochondria. $[Ca^{2+}]c$ indicates intracellular Ca^{2+} concentration. $[Ca^{2+}]m$ indicates mitochondrial Ca^{2+} concentration.

none have proved effective in clinical settings. This might reflect the diverse nature of clinical TBI and/or an incomplete understanding of the mechanisms of secondary neuronal loss.

TBI is a heterogeneous disorder with different forms of presentation. In past decades, research of TBI made great progress in clarifying the pathophysiological mechanisms. These consist of a primary insult resulting from the direct biomechanical forces and a secondary insult that plays an important role in the brain damage and death following TBI. At the cellular level, two initiating events related to energy depletion and Ca^{2+} homeostasis are of particular importance in the response to primary injury. The first is an 'ischaemia-like' pattern that is characterized by direct tissue damage and impaired regulation of cerebral blood flow (CBF) and metabolism. The ATP stores are depleted, and failure of energy-dependent membrane ion pumps occurs. The second is characterized by nerve terminal membrane depolarization along with excessive release of excitatory neurotransmitters (i.e. glutamate, aspartate), activation of NMDA, AMPA and voltage-dependent Ca^{2+} and Na^{+} channels. This, in turn, releases additional Ca^{2+} from intracellular stores, thus producing abnormally high levels of free intracellular and mitochondrial Ca^{2+}. The consecutive Ca^{2+} overload leads to self-digesting (catabolic) intracellular processes that involve overproduction of free radicals, activation of cell death signalling pathways and up-regulation of inflammatory mediators (Figure 1). Together, these events lead to membrane degradation of vascular and cellular structures and ultimately cell death (Werner and Engelhard, 2007).

As the 'power plant of the cell', ATP production via oxidative phosphorylation is the primary function of mitochondria, and Ca^{2+} is the characteristic stimulatory signal for activation of numerous mitochondrial enzymes (Graier *et al.*, 2007). Several studies in recent years have indicated that mitochondria play a pivotal role in neuronal cell survival. Mitochondrial dysfunction is considered to be an early event in the CNS injury that can cause neuronal cell death.

Structure and function of mitochondria

The widely accepted model of mitochondria is the 'crista junction' model that was first introduced by Daems and Wisse in 1966 (Figure 2) (Daems and Wisse, 1966). In this model, there are three membrane systems – an outer membrane (OM) and inner membrane (IM) that is further divided into an inner boundary membrane (IBM) and a cristae membrane (criM) – and three distinct compartments. The latter are the intermembrane space (IMS), the matrix and the additional intracristal space (intracriS).

The OM contains large numbers of integral proteins that are called porins. Molecules of less than 5 kDa can diffuse freely through the channels that are formed by these porins, but larger proteins must be actively translocated by a translocase of the OM (Herrmann and Neupert, 2000). The voltage-dependent anion channel (VDAC) is the most common pathway. Under physiological conditions, this channel is closed and there is free exchange of metabolites of molecular mass of up to 5 kDa in size and cations, like Ca^{2+}, K^{+} and Na^{+}, through the OM (Colombini, 2004). Three isoforms (VDAC1, VDAC2 and VDAC3) have been identified in multicellular organisms (Lemasters and Holmuhamedov, 2006).

The IM contains compartmentalized proteins with five types of functions: those that perform the redox reactions of oxidative phosphorylation, ATP synthase, specific transport proteins that regulate metabolite passage into and out of the matrix, protein import machinery, mitochondria fusion and fission protein (Walter, 1994). The IBM is enriched in proteins that are involved in mitochondrial fusion and in mitochondrial protein import, whereas the protein complexes of the respiratory chain and the proteins that are involved in the iron/sulfur cluster biogenesis accumulate in the criM. Moreover, the proteins redistribute dynamically in response to changes in the physiological state of mitochondria (Vogel *et al.*, 2006). The IM is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix. Cardiolipin (CL), the only membrane component that is synthesized by the IM and it acts as a platform for multiple apoptotic signals that converge to coordinate apoptotic cell death (Cristea and Degli Esposti, 2004; Mari *et al.*, 2008; Schlame, 2008; Schug and Gottlieb, 2009; Zaltsman *et al.*, 2010). There is an electrochemical proton gradient across the IM that is ctically involved in the import of mitochondrial proteins and regulation of metabolite transport across the mitochondrial membrane (Magder, 2006).

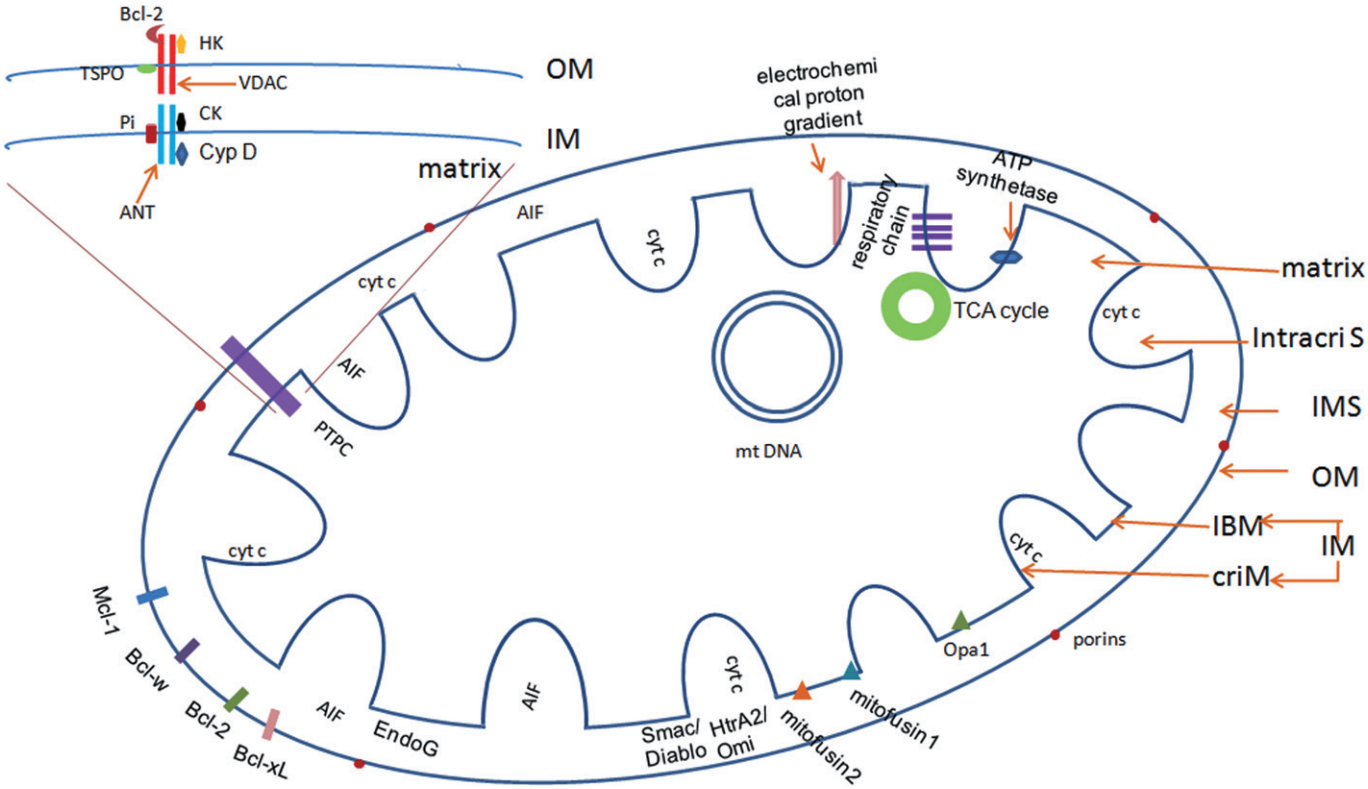

Figure 2

The structure of a mitochondrion. According to the 'crista junction' model, each mitochondrion includes three membrane systems and three compartments. The outer membrane (OM) contains large numbers of porins that allow free diffusion of small molecules. In physiological conditions, there are many anti-apoptotic Bcl-2 family members attached to the OM. The inner membrane (IM) contains compartmentalized proteins with different functions, such as those that perform the redox reactions of oxidative phosphorylation, ATP synthase, mitochondria fusion and fission protein. There is an electrochemical proton gradient across the IM. The permeability transition pore complex (PTPC) is a supramolecular channel that is assembled at the junctions between the IM and the OM, which is proposed to be composed of the adenine nucleotide translocase (ANT), cyclophilin D (Cyp D), voltage-dependent anion channels (VDAC), creatine kinase (CK), hexokinase (HK), the translocator protein TSPO and the mitochondrial phosphate carrier Pi. The IMS contains many proteins that play important roles in cell death, such as cyt c and AIF. Enzymes that carry out the citric acid cycle and mitochondrial DNA (mtDNA) are located in the matrix.

The permeability transition pore complex (PTPC) is a supramolecular channel that is assembled at the junction of the IM and the OM. Although the exact stoichiometry and molecular architecture of the PTPC remains elusive, it is proposed that it is composed of ADP/ATP carrier adenine nucleotide translocase (ANT: IM channel), cyclophilin D (Cyp D: matrix) and VDAC (OM channel) (Sullivan *et al.*, 2005; Garrido *et al.*, 2006; Tsujimoto and Shimizu, 2007). Creatine kinase (CK, periplasmic space), VDAC-associated hexokinase (HK, cytoplasm) and the 18 kDa translocator protein (TSPO) (also named as the peripheral benzodiazepine receptor), as well as Bcl-2 family proteins (Colombini, 2004; Rostovtseva *et al.*, 2004), may also have roles (Castedo *et al.*, 2002; Belizario *et al.*, 2007). The OM-located protein TSPO could modulate PTPC both directly and indirectly (Castedo *et al.*, 2002; Soustiel *et al.*, 2008). More recently, it was proposed that the mitochondrial phosphate carrier (Pi) is a PTPC constituent (Leung *et al.*, 2008) that undergoes a Ca^{2+}-induced conformational change to induce pore formation (Figure 2) (Varanyuwatana and Halestrap, 2011).

The IMS contains many proteins that play important roles in cell death, such as cytochrome (cyt) c and the apoptosis inducing factor (AIF). The matrix contains about two-thirds of the total protein in a mitochondrion, including enzymes that carry out the oxidation of pyruvate and fatty acids, and the citric acid cycle. A published human mitochondrial DNA sequence revealed 16 569 base pairs that encode a total of 37 genes: 22 tRNA, 2 rRNA and 13 peptide genes (Anderson *et al.*, 1981).

In order to provide enough ATP for the proper functioning of the cells in response to local changes, mitochondria are in constant fusion and fission (Rube and van der Bliek, 2004; Okamoto and Shaw, 2005; Chan, 2006; Cheung *et al.*, 2007; Parone *et al.*, 2008; Santel and Frank, 2008), both of which entail the participation of different proteins – Fission1 (Fis1), dynamin-related protein 1 and Endophilin B1 are three mammalian orthologues that are known to be required for mitochondrial fission in mammals (Karbowski *et al.*, 2004). Mitochondrial fusion requires both outer and inner mitochondrial components, such as mitofusin 1 and 2 and optical atrophy protein 1 (Opa1).

Close cooperation between mitochondria and other intracellular organelles is also important in maintaining normal function of the cells (Mattson and Kroemer, 2003; Green

and Kroemer, 2004; Wasilewski and Scorrano, 2009). The mitochondria-associated endoplasmic reticulum(ER) membrane (MAM) is the structure that associates the mitochondria with the ER membrane. The MAM can integrate signal transduction with metabolic pathways to regulate the communication and functional interactions between the ER and mitochondrion (Hayashi *et al.*, 2009), especially in lipid transport and Ca^{2+} exchanges (Pizzo and Pozzan, 2007). In pathological conditions, Ca^{2+} exchanges between mitochondria and ER mediated by MAM is an important apoptotic control point. The mitochondrial–lysosomal axis is also involved in the cell death cascade in TBI pathology. Lysosomal membrane permeabilization (LMP) results in the release of cathepsin proteases into the cytosol, which facilitates the release of AIF and EndoG from the IMS and results in caspase-independent DNA degradation. Such cathepsins can also trigger mitochondrial outer membrane permeabilization (MOMP), thereby stimulating the mitochondrial pathway of apoptosis (Turk *et al.*, 2001; Boya *et al.*, 2003; Luo *et al.*, 2010).

As mitochondria play a central role in the fate of the cells, the damage to mitochondria in pathological conditions, such as TBI and other acute brain injury, will have serious consequences for brain tissue. More and more evidence shows that mitochondria are among the key players in neuronal damage in the pathological processes of TBI.

Mitochondria provide a common pathway of cell death

Neuronal and glial cell death and traumatic axonal injury contribute to the overall pathology of TBI in both humans and animals. Although it is difficult to develop a generally acceptable taxonomy of cell death, apoptosis, necrosis and autophagy are three major forms of cell death that are widely recognised (Kroemer *et al.*, 2009). Today, necrosis is believed to be a programmed event, termed necroptosis, which is characterized by necrotic cell death morphology and the activation of autophagy (Li *et al.*, 2008). All three forms of cell death participate in cell damage after TBI.

The major link that connects different death programs seems to be the mitochondrion and, in particular, its membranes (Jacotot *et al.*, 1999; Vander Heiden and Thompson, 1999; Scorrano and Korsmeyer, 2003; Breckenridge and Xue, 2004; Lucken-Ardjomande and Martinou, 2005; Zoratti *et al.*, 2005; Chipuk *et al.*, 2006; Kroemer *et al.*, 2007; Galluzzi *et al.*, 2010). The mitochondrial membrane permeabilization (MMP) and subsequent release of death effectors will lead to cell death (Ferri and Kroemer, 2001; Li *et al.*, 2001; Lemasters, 2005; Zoratti *et al.*, 2005; Garrido *et al.*, 2006; Galluzzi *et al.*, 2008). Essentially, two models of the formation of pores in the mitochondrial membranes have been proposed: (1) direct MOMP through pre-existing pores and *ex novo* pore formation, and (2) mitochondrial membrane permeability transition (MPT) (a process of a sudden increase in the permeability of the IM to solutes with a molecular mass of less than 1500 Da.) that occurs following the opening of the PTPC in the IM (Figure 3) (Galluzzi and Kroemer, 2008).

Structural changes in VDACs are the main reason for MOMP. *Ex novo* formatted pores include ceramide-formed, lipid channel, mitochondrial apoptosis-induced channel (MAC) (Siskind, 2005; Dejean *et al.*, 2006; Kinnally and Antonsson, 2007) and ruptured OM (Sesso *et al.*, 2004). Mitochondrial dynamics also contributes to MOMP (Cassidy-Stone *et al.*, 2008). The incomplete MOMP (iMOMP) found during apoptosis may relate to the process of mitochondrial fission. Inhibition of mitochondrial fission reduced the incidence of iMOMP, whereas the promotion of fission had the opposite effect. iMOMP may provide a critical cohort of healthy mitochondria that permits cellular recovery from MOMP, which may depend on Bax or Bak activation (Tait *et al.*, 2010).

PTPC is a highly tunable mechanism that integrates a multiplicity of signals to maintain the viability of a cell or to commit it to death (Rasola and Bernardi, 2011). In physiological conditions, the PTPC has a 'flickering' status that is characterized by reduced conductance. In the presence of Ca^{2+} overload or oxidative stress, cyclophilin D (Cyp D) can induce a conformational change in the adenine nucleotide translocator (ANT) that results in MPT (Tsujimoto and Shimizu, 2007). It is generally believed that Cyp D, a mitochondrial member of the cyclophilin family, is an indispensable component of PTPC (Galat and Metcalfe, 1995); and that the modulation of the interaction of Cyp D with ANT is a suitable target in modulating mitochondrial cell death (Temkin *et al.*, 2006). The putative role of Cyp D in regulating the MPT is derived from the observation that cyclosporin A (CsA), a specific inhibitor of the cyclophilin family, blocks the MPT (Broekemeier *et al.*, 1989). However, other evidence showed that not all apoptosis can be inhibited by CsA (Nakagawa *et al.*, 2005). Basso *et al.* (2005) proposed that there might be a CsA-insensitive MPT. Both the CsA-sensitive MPT and CsA-insensitive MPT may share a common mechanism, because both forms of MPT are inhibited by ubiquinone 0.

MOMP is always viewed as the hallmark of apoptosis, whereas MPT will lead to necrosis (Baines *et al.*, 2005; Nakagawa *et al.*, 2005). Apoptosis can be initiated by three major pathways – the extrinsic pathway, the mitochondrial pathway and the caspase-12-mediated ER apoptotic pathway. In the extrinsic apoptotic pathway, mitochondria amplify the apoptotic signal or are essential for execution of the apoptotic program (Barnhart *et al.*, 2004). The mitochondrial apoptotic pathway is classified as caspase-dependent or caspase-independent. In a caspase-dependent pathway, cyt c is the necessary participant, whereas AIF is involved in the caspase-independent pathway. During apoptosis, death signals are relayed via BH3-only proteins to Bax and Bak, which will lead to MOMP (Chipuk *et al.*, 2006). The resulting release of cyt c into the cytosol triggers apoptosome assembly and the subsequent caspase activation and apoptosis. There is some evidence that cristae remodelling is necessary for the complete release of cyt c during cell death because about 85% of cyt c is in the intracriS and only 15% is in the IMS (Bernardi and Azzone, 1981). Cristae morphology and the diameter of cristae junctions are regulated, at least in part, by the GTPase activity of Opa1 and the protease presenilin-associated rhomboid-like protein (PARL) (Gottlieb, 2006). In addition to Opa1 and PARL, the Bcl-2 family members have been shown to affect cristae remodelling. However, another study found that cristae remodelling is not required for the efficient release of cyt c, as swelling occurs only late in apoptosis after

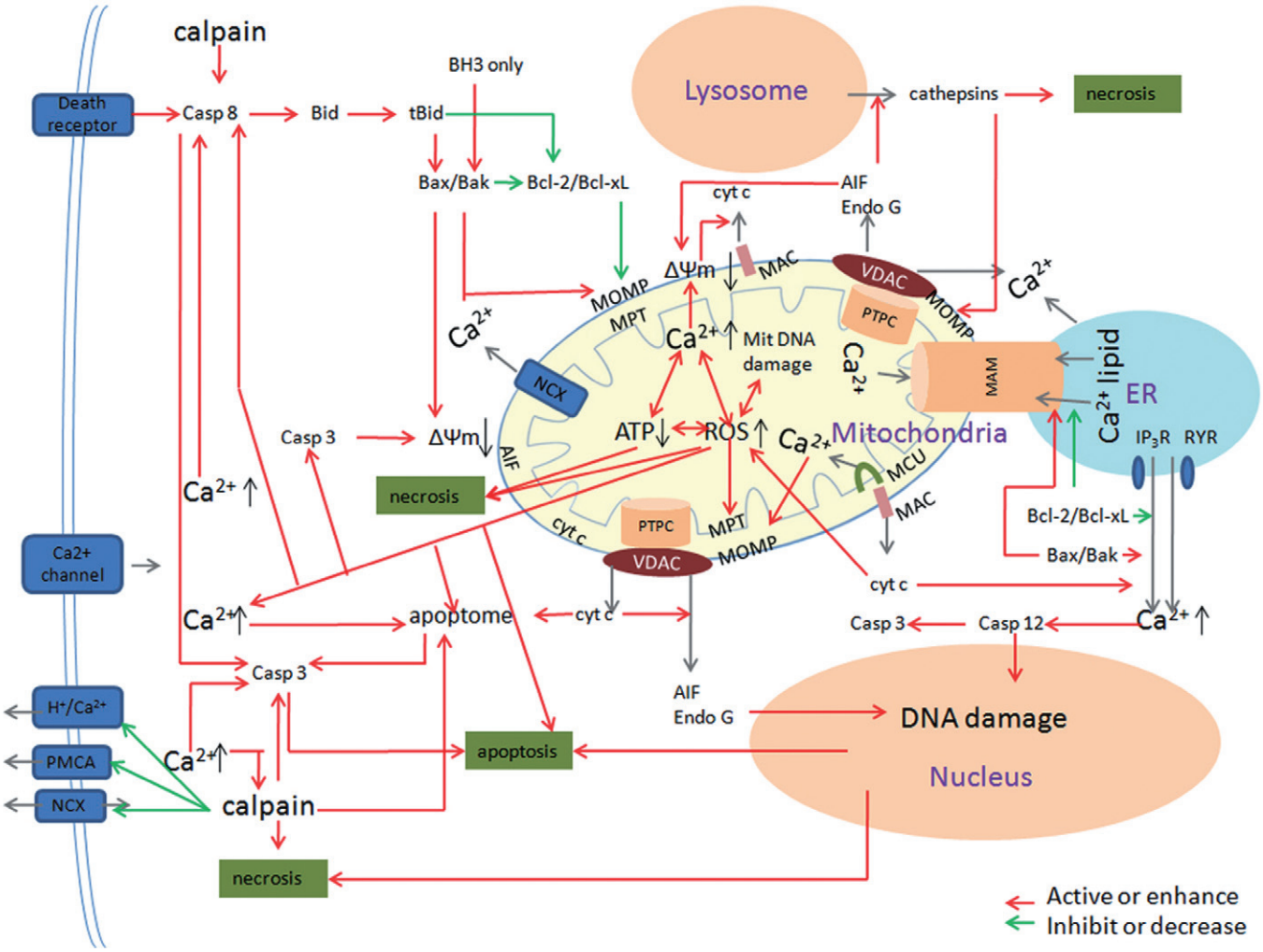

Figure 3

The role of mitochondria in cell death in TBI pathology. In TBI pathology, mitochondria provide the main platform of many intertwined factors that direct the cell to live or die. Three major pathways – the extrinsic pathway, the mitochondrial pathway and the caspase-12-mediated ER apoptotic pathway participate in cell death. In the extrinsic apoptotic pathway, mitochondria amplify the apoptotic signal or are essential for execution of the apoptotic program. The BH3-only protein Bid is one of the major links between extrinsic and mitochondrial apoptosis. The mitochondrial apoptotic pathway is classified as caspase-dependent or caspase-independent. In a caspase-dependent pathway, cyt c is the essential component whereas AIF is critically involved in the caspase-independent pathway. There are synergistic effects between mitochondrial membrane, Ca^{2+} and ROS in mediating cell damage after TBI. MOMP and MPT are two models of the formation of pores in the mitochondrial membranes. The Bcl-2 family is a critical regulator of mitochondria and cell fate. There are many amplifying loops among different pro-apoptotic effectors. In addition, there is coordination among mitochondria, ER and lysosome. In pathological conditions, Ca^{2+} exchanges between mitochondria and ER mediated by MAM is an important apoptotic control point. The release of cathepsin proteases from lysosome has a positive effect on mitochondria-mediated cell death.

the release of cyt c and loss of the mitochondrial membrane potential (ΔΨm) (Sun *et al.*, 2007). Arnoult *et al.* (2003) proposed that the release of different mitochondrial apoptogenic factors is a sequential process. First, Bax/Bak-mediated MOMP leads to the release of a significant part of the cyt c, Smac/Diablo and HtrA2/Omi proteins. Then ΔΨm loss occurs, which may be required for the release of the last pool of cyt c, Smac/Diablo and HtrA2/Omi. In a third step, cyt c, Smac/Diablo and HtrA2/Omi, which were released into the cytosol, trigger caspase activation. This is necessary to alter the physical association of AIF and EndoG with the IM to enable their relocation to the cytosol. Thus, EndoG and AIF seem to define a 'caspase-dependent', mitochondria-initiated apoptotic DNA degradation pathway. Severe mitochondrial cyt c release is also a predictor of necrotic cell death (Lewen *et al.*, 2001). ER-stress leading to caspase-12 activation is the third route of apoptosis that is involved in TBI pathology. It can act either as an executioner caspase or activate caspase-3 directly or indirectly (Morishima *et al.*, 2002).

MPT will lead to profound cellular consequences in necrosis. There is a loss of Δψm that is needed to drive production of ATP from ADP. The result is rapid mitochondrial dysfunction and excessive production of ROS that ultimately lead to necroptosis. Moreover, MPT results in marked mitochondrial

swelling and potentially the outright rupture of the OM and severe release of death effectors (Kitsis and Molkentin, 2010).

In TBI pathology, mitochondria serve as signalling platforms for numerous biomolecules that are produced inside and outside the mitochondria. The coordinative role of different regulators and effectors in mitochondria determine cell death or life (Figure 3).

Excitotoxicity – one of the initiating factors in secondary insult

During TBI, building up of excitatory neurotransmitters due to synaptic release and impaired/reversed uptake mechanisms will lead to excitotoxicity. Studies have shown that excitotoxicity is location-dependent. Glutamate is one of the main excitatory neurotransmitters in CNS. AMPA receptors, NMDA receptors, voltage-dependent Ca^{2+} channels (VDCC) and metabotropic glutamate (mGlu) receptors are the main routes that mediate excitotoxicity by different mechanisms (Palmer *et al.*, 1994; Wu and Saggau, 1997; Ertel *et al.*, 2000). For example, Na^+- and Cl^--dependent influx is related to immediate cell swelling (Hossmann, 1994; Siesjo *et al.*, 1995; Seiler *et al.*, 2008; Brennan *et al.*, 2009; Forder and Tymianski, 2009). Extrasynaptic NMDA receptor activation can activate a CREB (cAMP response element binding) protein shut-off that, in turn, causes loss of $\Delta\Psi m$ and apoptosis (Hardingham *et al.*, 2002). Stress-activated protein kinases (SAPKs) are another class of signalling molecules that are implicated in NMDA receptor-dependent cell death (Legos *et al.*, 2002; Borsello *et al.*, 2003; Cao *et al.*, 2004; Soriano *et al.*, 2008).

Compared with other mechanisms, Ca^{2+}-dependent influx is the main factor that is responsible for cell death induced by excitoxicity. In physiological conditions, Ca^{2+} is an important messenger in maintaining cellular bioactivtity. In neurons, the departure of Ca^{2+} from the cell is achieved through the plasma membrane Ca^{2+} ATPase pump (PMCA), Na^+/ Ca^{2+} exchangers (NCXs) and H^+/ Ca^{2+} uniporter (Cross *et al.*, 2010). The cellular homeostasis of Ca^{2+} is maintained mainly by mitochondria via a calcium uniporter (MCU) on the IM, uncoupling proteins 2 and 3, Letm1 mitochondrial Ca^{2+}/H^+ antiporter (Pan *et al.*, 2011), NCXs or via 'calcium-induced-calcium-release' pathways (Rossier, 2006), and the interplay between the mitochondria and ER (Pizzo and Pozzan, 2007). During brain injury, intracellular Ca^{2+} influx is mediated by many deeply interconnected signalling pathways. These include the MAPK/ERK kinase (MEK)–ERK–p38 MAPK signalling axis and protein phosphatase 2A activation (Kikuchi *et al.*, 2000; Mori *et al.*, 2002; Xu *et al.*, 2006). mGluRs activation coupled to the GTP-binding protein Gq11 stimulates the release of inositol triphosphate (IP_3), which activates Ca^{2+} channels in the ER (Mattson, 2007). Mitochondria serve as very efficient Ca^{2+} buffers, taking up substantial amounts of cytosolic Ca^{2+} at the expense of $\Delta\Psi m$. As a consequence of Ca^{2+} uptake, mitochondria can suffer Ca^{2+} overload. According to the 'two-hit' hypothesis, a concurrent pathological stimulus can turn Ca^{2+} from a physiological to a pathological effector (Brookes *et al.*, 2004).

Ca^{2+} overload can cause damage to the cell by several different, cross-amplifying, cascades. First, Ca^{2+} activates (either directly or indirectly) cysteine proteases called calpains and caspases that degrade a variety of substrates, including cytoskeletal proteins, membrane receptors and metabolic enzymes (Nixon, 2003; Bano *et al.*, 2005). Second, Ca^{2+} induces oxidative stress through different mechanisms (Mattson and Sherman, 2003). Third, Ca^{2+} triggers apoptosis by induction/activation of various pro-apoptotic proteins (Ankarcrona *et al.*, 1995; Dargusch *et al.*, 2001; Culmsee and Mattson, 2005). Furthermore, excessive Ca^{2+} influx relates to a decreased ATP level and activated caspases and calpains, which contribute to impairment and inactivation of both PMCA and NCXs, thus exacerbating intracellular Ca^{2+} overload (Schwab *et al.*, 2002; Bano *et al.*, 2005; Pottorf *et al.*, 2006; Bruce, 2010).

Mitochondrial dysfunction that is caused by excessive Ca^{2+} uptake by the mitochondria through the potential-driven uniporter is a key event in severe excitotoxicity, which leads to depolarization of $\Delta\Psi m$ (Vergun *et al.*, 2003), ROS generation, depletion of ATP and the permeabilization of mitochondrial membrane that leads to apoptosis or necrosis due to mitochondrial damage. Recent studies suggest a link between mitochondrial dynamics and excitotoxic injury. NMDA-induced toxicity results in a fragmented mitochondrial phenotype and an impairment of mitochondrial fusion. Opa1 is a key regulator of mitochondrial integrity. Calpain activation by Ca^{2+} overload may impair Opa1 and result in mitochondrial morphology defects. The protective effect of inhibition of calpains can preserve mitochondrial morphology and protect neurons against excitotoxic cell death (Jahani-Asl *et al.*, 2011).

ROS-mitochondrial dysfunction-related initiating factors in secondary insult

ROS are natural byproducts of the normal metabolism of oxygen. Categorically, ROS include free radicals, such as superoxide, hydroxyl radical and singlet oxygen, as well as non-radical species, such as hydrogen peroxide. Under physiological conditions, numerous endogenous antioxidants prevent oxidative damage, including superoxide dismutase, glutathione peroxidase, catalase, low-molecular-weight antioxidants and vitagenes (Calabrese *et al.*, 2008). In the setting of TBI, each of those neuroprotective systems in the brain becomes overwhelmed and results in oxidative cell damage.

The post-traumatic sequelae establish conditions of increased metabolic demands on the reduced normal mitochondrial population resulting in abundant ROS production. The excessive production of ROS is due in part to excitotoxicity, free iron and interactions among ROS. Glutamate-mediated excitotoxicity leads to an increase in intracellular Ca^{2+} and the subsequent induction of enzymes, such as nitric oxide synthase and xanthine oxidase, that produce free radicals. Mitochondrial Ca^{2+} uptake can stimulate the net production of ROS through activation of MPT, release of cyt c, respiratory inhibition, release of pyridine nucleotides and loss of intramitochondrial glutathione necessary for detoxification of peroxides (Starkov *et al.*, 2004).

Although there are non-mitochondrial sources of ROS (Stout *et al.*, 1998; Vesce *et al.*, 2004; Nicholls *et al.*, 2007), the

mitochondrion is a major intracellular source of ROS (Starkov, 2008; Murphy, 2009). To date, approximately 10 potential mitochondrial ROS-generating systems have been identified (Andreyev *et al.*, 2005; Choi *et al.*, 2009). In the mitochondria, superoxide can be produced by respiratory complexes and individual enzymes on the OM, on both sides of the IM and in the matrix. The respiratory chain complexes I and III are the primary mitochondrial sources of univalent reduction of O_2 into superoxide (Mustafa *et al.*, 2010). Superoxide is very unstable and quickly reacts with nearby molecules. It naturally dismutes into hydrogen peroxide (H_2O_2) and O_2. In contrast to superoxide, H_2O_2 is membrane-permeable. H_2O_2 can, in turn, react with metal ions to form the highly reactive hydroxyl radical via the Fenton reaction (Feissner *et al.*, 2009). Each ROS has an unpaired electron in its outer electron shell and thus is highly reactive and unstable. Essentially, ROS cause cell injury by compromising the integrity of the cell membrane by lipid peroxidation, protein and DNA oxidation and inhibition of the mitochondrial electron-transport chain (Kowaltowski *et al.*, 2001; Skulachev, 2006).

Besides being the major source of ROS production, mitochondria are also targets of oxidative stress (Tavazzi *et al.*, 2005). Overproduction of ROS in the mitochondria is one of the very early events that precede the collapse of $\Delta\Psi m$, release of pro-apoptotic factors and activation of caspases. Most importantly, oxidative structural changes that are induced by ROS can impair mitochondrial energy metabolism. ROS also acts as a key contributor to necrotic cell death and also a promoter of apoptosis. Mitochondrial DNA (mtDNA) is prone to oxidative damage because it lacks introns and is close to an ROS source. mtDNA damage-induced decreased respiratory function enhances ROS generation, thus eliciting a vicious cycle of ROS-mtDNA damage that ultimately triggers apoptosis (Van Houten *et al.*, 2006). Evidence suggests possible direct roles for ROS in mediating death receptor activation and apoptotic induction through ROS-induced receptor clustering and the formation of lipid raft-derived signalling platforms. ROS–NO interaction in controlling FLICE inhibitory protein (FLIP) down-regulation was considered to be a key regulatory mechanism of Fas-induced apoptosis (Wang *et al.*, 2008). ROS are known triggers of the mitochondrial apoptotic cascade via interactions with proteins of the PTPC (Tsujimoto and Shimizu, 2007). Peroxynitrites can directly activate MPT (Vieira *et al.*, 2001; Bernardi *et al.*, 2006). Peroxidation of CL may initiate cyt c release by loosening cyt c from the IM (Kirkland *et al.*, 2002). Furthermore, oxidized CL is distributed to the outer leaflet of the mitochondrial membrane and functions as a docking platform for truncated Bid (tBid), enabling MPT and cyt c movement across the OM into the cytosol (Gonzalvez *et al.*, 2005). A significant mitochondrial loss of cyt c will lead to further ROS increase due a disrupted electron transport chain (Circu and Aw, 2010).

There are synergistic effects between mitochondrial membrane, Ca^{2+} and ROS in mediating cell damage after TBI. Ca^{2+} overload enhances ROS generation (Festjens *et al.*, 2006; Gunter and Sheu, 2009). Oxidative stress can aggravate Ca^{2+} overload and sensitize the bioactivity of Ca^{2+} (Crompton and Costi, 1990; Connern and Halestrap, 1996; Halestrap *et al.*, 1997; Kim *et al.*, 2006; Juhaszova *et al.*, 2008). Ca^{2+}-induced injury cascade can be amplified by the interaction of oxidative stress and Ca^{2+} (Camello-Almaraz *et al.*, 2006). Both Ca^{2+} and ROS can directly activate caspase or facilitate the release of caspases by increasing MPT during apoptosis (Circu and Aw, 2010). Ca^{2+} is the most important cellular permissive factor for MPT (Jambrina *et al.*, 2003). Its effect is greatly enhanced by oxidative stress (Kowaltowski *et al.*, 2001; Bernardi *et al.*, 2006). ROS or increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$) can oxidize CL and results in the detachment of cyt c (Kagan *et al.*, 2005). In this respect, strategies, such as Ca^{2+} channel blockade, reduction of oxidative stress and inhibition of the mitochondrial pore opening, appear to be promising tools for the management of acute brain-injured patients.

As the platform of death or life during the second insult in TBI pathology, Ca^{2+} and ROS are two main players, but not the only determiners of the fate of the mitochondria and the cell. The Bcl-2 family is a critical regulator in the death or life game.

Bcl-2 family- critical regulator of mitochondria and cell fate

The Bcl-2 family consists of proteins that contain Bcl-2 homology (BH) domains. The Bcl-2 family is divided into anti-apoptotic Bcl-2-like proteins according to structure and function that carry the BH1-4 domains (e.g. Bcl-2 and Bcl-xL) and pro-apoptotic Bcl-2-like proteins that contain the BH1-3 domains (e.g. Bax and Bak) or just a single BH3 domain (e.g. the so-called BH3-only proteins).

In TBI pathology, the performance of the anti-apoptotic and pro-apoptotic Bcl-2 family on the OM determines the fate of neurons (Kroemer and Reed, 2000; Green and Kroemer, 2004). The mitochondrial apoptosis-induced channel (MAC) is a pathway for cyt c release, the proposed components of which include the oligomeric Bax/Bax, Bax/Bak and/or Bak/Bak (Guihard *et al.*, 2004; Dejean *et al.*, 2006). In normal healthy cells, the anti-apoptotic proteins, such as Bcl-2, Bcl-xL, Bcl-w and Mcl-1, are found on the OM where they inhibit both MOMP (by sequestering Bax and Bak) and the MPT (through their interaction with the PTPC) (Scorrano and Korsmeyer, 2003; Scorrano *et al.*, 2003; Rostovtseva *et al.*, 2004; Rong and Distelhorst, 2008). Under pro-apoptotic conditions, Bax and Bak undergo conformational modifications and enter the OM fully, thereby creating MOMP (Kroemer *et al.*, 2007). Apoptotic signals also up-regulate the expression of BH3-only proteins (Shimizu *et al.*, 1999; Crompton *et al.*, 2002). Once they are at the mitochondria, the BH3-only proteins either directly activate the pro-apoptotic proteins Bax or Bak or inhibit the anti-apoptotic members, such as Bcl-2 or Bcl-xL. However, the exact mechanism is still elusive (Green and Kroemer, 2004; Kroemer *et al.*, 2007; Galluzzi *et al.*, 2008; Lindsay *et al.*, 2011). It is tempting to speculate that BH3-only proteins, such as Bmf, might promote apoptosis by relieving the Bcl-2/Bcl-xL-mediated inhibition of MOMP, and cause necrosis by interfering with the Bcl-2/Bcl-xL-mediated blockage of the MPT.

The BH3-only protein Bid is one of the major links between extrinsic and mitochondrial apoptosis that can be

cleaved as tBid by caspase-8 and promote MOMP (Li *et al.*, 1998; Luo *et al.*, 1998). Bcl-2 and Bcl-xL are also cleaved by caspases that enable the new fragments to promote apoptosis (Scorrano *et al.*, 2003; Lucken-Ardjomande and Martinou, 2005). A self-amplifying feed forward loop is involved in caspases, Bcl-2 and mitochondria, which may help to establish an irreversible commitment to apoptosis (Shelton *et al.*, 2009). The CL is an important platform for multiple apoptotic signals, including the Bcl-2 family. For example, the selective degradation of mitochondrial CL impairs the pro-apoptotic action of tBid plus Bax (Lucken-Ardjomande *et al.*, 2008). Furthermore, the binding of a pro-apoptotic Bcl-2 family (such as tBid, Bax and Bak) to mitochondria also plays critical roles in other apoptotic associated processes, such as cristae remodelling (Frezza *et al.*, 2006), mitochondrial fission and fragmentation interacting with proteins such as mitofusin 2 and Endophilin B1 (Youle and Karbowski, 2005; Suen *et al.*, 2008; Grohm *et al.*, 2010). A number of studies have demonstrated that members of the Bcl-2 family reside in the ER where they have opposing actions in regulating the transfer of ER Ca^{2+} to mitochondria and thus direct the cell to live or die (Breckenridge *et al.*, 2003; Forte and Bernardi, 2006).

The BH4 domain is specific for the anti-apoptotic proteins of the Bcl-2 family and, hence, is a suitable candidate in designing agents that have anti-apoptotic effect (Shimizu *et al.*, 2000). HIV-TAT BH4 is a cell-permeable MOMP inhibitory recombinant fusion protein that is composed of the HIV-TAT plasma membrane translocation domain and the anti-apoptotic Bcl-xL-derived BH4 domain, that inhibits neuronal apoptosis in various models. The anti-apoptotic mechanisms of HIV-TAT BH4 peptide include efficient inhibition of caspase-3 activation, prevention of AIF translocation and suppression of AIF and cyt c translocation (Asoh *et al.*, 2002; Dietz *et al.*, 2002; Kilic *et al.*, 2002; Yin *et al.*, 2006). It is proposed that such BH4-like peptides can inhibit both caspase-dependent and caspase-independent apoptosis. Recently, studies found that HIV protease inhibitors can also simultaneously block caspase-dependent (activation of caspase-9, -3) and caspase-independent cell death pathways (AIF translocation) by blocking MOMP, presumably by inhibiting the ANT (Phenix *et al.*, 2001; Wan and DePetrillo, 2002; Matarrese *et al.*, 2003; Weaver *et al.*, 2005; Hisatomi *et al.*, 2008).

The fate of mitochondria is determined by the struggle between life supporters and death effectors. Once the death effectors become dominant, mitochondria will invariably direct the cell to apoptosis or necrosis mainly by caspases or AIF.

Caspases – main executors of apoptosis

Cellular caspases belong to a highly conserved family of cysteine proteases that cleave aspartate residues of caspase substrates and are the main players in the execution phase of apoptosis (Fischer *et al.*, 2003; Shi, 2004). Under physiological conditions, the activation and activity of caspases can be inhibited at different stages. First, members of the inhibitor of the apoptosis protein (IAP) family constitute an endogenous barrier against improper or excessive caspase activation (Verhagen *et al.*, 2001). Second, members like XIAP, c-IAP1, c-IAP2 and survivin bind and suppress enzyme catalytic activity (Kugler *et al.*, 2000; Perrelet *et al.*, 2000). Finally, post-translational modification of catalytic site cysteine residues is a potentially important redox mechanism in the control of caspase activity (Tzeng *et al.*, 1997; Rossig *et al.*, 1999; Kim *et al.*, 2000; 2002; Chung *et al.*, 2001; Zech *et al.*, 2003; Huang *et al.*, 2008). ROS, NO and glutathione are involved in redox regulation of caspase activity (Mannick *et al.*, 1999; 2001; Kim *et al.*, 2004; Mitchell *et al.*, 2007; Pan and Berk, 2007; Sykes *et al.*, 2007).

During apoptotic signalling, disturbance of the inhibitory mechanisms will lead to the activation of caspases and their cleavage activity. IAPs are antagonized by mitochondria-derived Smac/Diablo and Omi/HtrA2 proteins, allowing caspase-mediated execution (Du *et al.*, 2000; Yang *et al.*, 2003; Suzuki *et al.*, 2004b). Calpains may also play an important role in the triggering of apoptotic cascades by virtue of their ability to activate caspases (Stefanis, 2005). Additionally, activated caspase-3 promotes caspases-2 and -6 activation in an amplification loop that enhances caspase-9 processing (Nicholson, 1999; Slee *et al.*, 1999; Degterev *et al.*, 2003).

Depending on their roles, the caspases are divided into initiator and executioner caspases. Under the pro-apoptotic stimuli, initiator caspases are recruited at the death receptor by the death effector domain (caspases-8 and -10) or in the cytosol by the recruiting domain (caspases-2 and -9). Then executioner caspases, such as caspases-3, and -7, are cleaved by the activated initiator caspases to execute apoptosis by the cleavage of protein substrates (Knoblach *et al.*, 2002). More than 300 proteins have been characterized as caspase substrates (Marzo *et al.*, 1998). In a large-scale analysis using CaSPredictor software, Garay-Malpartida *et al.* (2005) identified 1600 predicted caspase substrates with a score >0.57, with 60% sensitivity and 97% specificity. For example, brain injury is accompanied by cell cycle progression of neurons that, it has been suggested, leads to apoptosis in a caspase-dependent manner (Di Giovanni *et al.*, 2005).

In TBI pathology, mitochondria are key targets of caspase cleavage and locations for the regulation of caspases. The cleavage of plasma membrane PMCA and NCX results in the increase of intracellular Ca^{2+} pools that may precede the opening of mitochondrial death decision pores (Schwab *et al.*, 2002; Bano *et al.*, 2005). Furthermore, activated caspases are localized in, or translocate to, mitochondria to trigger the extrinsic or mitochondrial pathways of apoptosis (Lemasters, 2005; Chipuk *et al.*, 2006). For example, procaspase-8 was shown to be predominantly localized within the cytosol but has also been shown to be loosely associated with the OM. The translocation of caspase-8 to the mitochondria was necessary for efficient caspase-8 processing and activation (Stegh *et al.*, 2000; 2002; Henshall *et al.*, 2001). *In vitro* studies have shown an important role for caspase-2 in regulation of cyt c release (Lassus *et al.*, 2002; Robertson *et al.*, 2004). The inhibition of caspase-2 by the pan-caspase inhibitor boc-aspartyl (OMe)-fluoromethylketone reduces acute cell death after TBI (Clark *et al.*, 2007). Caspases-3 and -7 are two highly related effector caspases. Active caspase-3 is required for the sustained loss of the $\Delta\Psi m$ (Waterhouse *et al.*, 2006). Mice that lack both enzymes have exhibited a resistance to

drugs that induce the mitochondrial and extrinsic pathways to apoptosis (Lakhani *et al.*, 2006). The close cooperation between mitochondria and caspases makes mitochondria the central player in both the extrinsic and mitochondrial apoptosis pathways in acute brain injury.

AIF – main participant of necrosis

In contrast to capases, AIF, a ubiquitously expressed flavoprotein, plays a critical role in caspase-independent apoptosis and necrosis in areas with less energy supply. Under normal physiological conditions, the NADH oxidase activity of AIF is required for the functioning of the respiratory chain. AIF deficiency results in reduced expression of complexes I and II and inefficient oxidative phosphorylation (Vahsen *et al.*, 2004). Under pro-apoptotic stimulation, AIF and EndoG are released from IMS and translocate to the nucleus to promote caspase-independent DNA degradation (Yamashita *et al.*, 2004; Slemmer *et al.*, 2008).

Moubarak *et al.* (2007) have reported that the sequential activation of PARP-1, calpain and Bax is essential in AIF-mediated programmed necrosis. PARP-1 is a nuclear enzyme that acts as a DNA damage sensor. If it is overactivated, it mediates cell death (Yu *et al.*, 2002; Cande *et al.*, 2004). There are also active PARPs within mitochondria (mtPARP) that induce intramitochondrial poly (ADP) ribosylation (Du *et al.*, 2003). Various poly (ADP)-ribosylated mitochondrial proteins have been identified. mtPARP over-activation may influence cellular energy stores both by depleting NAD^+ and decreasing ATP production, which can lead to cell dysfunction and death (Lai *et al.*, 2008). Recently, a novel AIF release and cell death by superoxide production and prolonged ERK1/2 phosphorylation were found (Kondo *et al.*, 2010). The cell death was not associated with Bax, cyt c, caspase-3 or PARP-1. The significance of PARP-1-independent AIF release in post-traumatic neuronal damage needs to be established.

Yu *et al.* found that about 30% of AIF loosely associates with the OM on the cytosolic side. This outer mitochondrial pool of AIF is sufficient to cause cell death (Yu *et al.*, 2006b; Wang *et al.*, 2009). Additionally, cytosolic AIF acts on the mitochondria to collapse the $\Delta\Psi m$ and initiates the release of cyt c, thus initiating caspase-dependent apoptosis (Susin *et al.*, 1999). AIF, itself, does not have any DNase activity and it induces DNA degradation only when it binds and translocates to the nucleus together with CsA (Zhu *et al.*, 2007). Therefore, pharmacological agents that prevent the interaction of AIF and CsA might act as neuroprotectors. The DNase activity of EndoG can be suppressed by the heat-shock protein (HSP) 70 in an ATP-dependent manner (Kalinowska *et al.*, 2005).

It is clear that mitochondria are the main platform and one of the main participants in determining the death or life of cells in TBI pathology. Thus, mitochondria-centred therapy is of vital importance in reducing tissue damage following TBI. Although mitochondria displayed bioenergetic deficits after one hour following injury, the damage was not exacerbated after 3 h, with peak mitochondrial dysfunction occurring at approximately 12–24 h, which provides an opportunity for effective intervention of the mitochondrial dysfunction in a clinical setting (Gilmer *et al.*, 2009). As ATP depletion led by mitochondrial dysfunction is the fundamental cause of cell damage, a combination of compounds that target different metabolic pathways of mitochondrial could diminish cellular disturbance and thereby improve or stabilize clinical features. Second, considering the complexity of the process and central role of mitochondria in the process of secondary brain damage, multipotential therapeutic strategies are necessary in mitochondria-targeted protection (Royo *et al.*, 2003; Pitkänen *et al.*, 2005; Schouten, 2007; Beauchamp *et al.*, 2008).

Energy supply – the first level in mitochondrial-targeted therapy

The brain is highly dependent on a continuous supply of oxygen and glucose to maintain cellular integrity. In TBI pathology, energy depletion is the result of mitochondria impairment and an important cause of mitochondria damage. Inadequate O_2 supply to the traumatized brain results in the conversion of aerobic metabolism to anaerobic metabolism that results in acidosis and depletion of cellular energy. Excitotoxicity, Ca^{2+} overload and ROS overproduction that follow the primary insult and energy failure will bring severe damage to mitochondria. The impairment of mitochondrial respiratory chain-linked oxidative phosphorylation will lead to further functional failure of aerobic metabolism. On the other hand, the repair of damaged tissue needs more energy than its normal physiological condition. This results in what has been termed a 'flow/metabolism mismatch', which is the most important unfavourable factor in TBI (Rockswold *et al.*, 2007). Studies investigating the metabolic fate of glucose after TBI have shown that, after the initial hours following injury, glucose utilization changes from energy production to cellular repair mechanisms by the pentose phosphate pathway. This results in a higher temperature in the lesion core. In an energy-deprived environment, before an irreversible event,such as MOMP occurs, cells respond by maintaining a minimal survival level while awaiting a rescue in the event of a growth factor or nutrient re-addition (Chipuk *et al.*, 2006). Therefore, impaired energy metabolism is a potential target of TBI therapy.

It is generally agreed that with a reduction in temperature, the injured brain decreases its consumption of energy-producing substrates. Clinically induced hypothermia is the only therapy that demonstrates improved neurological outcomes following cardiac arrest (Bernard *et al.*, 2002). Hypothermia has been widely accepted as the gold standard method by which the body can protect the brain (Choi *et al.*, 2012). During the pathological process following TBI, induced hypothermia can protect mitochondria in at least three ways: (1) reducing the energy demand of mitochondria by reducing the cerebral metabolic rate of oxygen ($CMRO_2$); (2) direct protection of mitochondria via maintenance of $\Delta\Psi m$ and decreasing production of ROS (Shao *et al.*, 2010); (3) indirect protective effect such as the reduction of excitotoxic neurotransmitters, facilitation of anti-inflammatory responses and anti-apoptotic pathways, reduction of intracranial pressure (ICP) and stabilization of the blood–brain barrier (BBB).

Increasing the metabolic support is another important aspect in mitochondrial protection. Supporting the aerobic processes of the threatened cells could possibly preserve viable, but non-functioning, tissue. It has been proved that hyperoxia can increase the partial pressure of oxygen (pO_2) within the blood and the subsequent, improved mitochondrial metabolism/tissue oxygenation. In a rat model of fluid percussion injury (FPI), 1 h 1.5 ATA with 3 h 100% normobaric oxygen treatments significantly improved the recovery of synaptosomal mitochondrial metabolic activity (Azbill *et al.*, 1997). Palzur *et al.* (2008) hypothesized that hyperoxia-induced increases in Bcl-2 expression, and that the resultant increase in intracellular oxygen bioavailability may contribute both to preserve mitochondrial integrity and to reduce the activation of the mitochondrial mediated apoptotic pathway following TBI. Work by several investigators suggests that hyperbaric oxygen allows the injured brain to use baseline amounts of O_2 more efficiently following treatment and has a persistent effect on the injured brain tissue (Contreras *et al.*, 1988; Rockswold *et al.*, 2001). The main controversy about hyperoxia therapy concerns the increased production of ROS. In fact, brief periods of normobaric hyperoxia do not produce oxidative stress and/or change antioxidant reserves in CSF (Puccio *et al.*, 2009).

Mitochondria dysfunction in TBI makes it impossible to make use of glucose effectively. However, lactate can be used directly through the tricarboxylic acid cycle. Over the past two decades, mounting clinical and experimental evidence suggests that lactate may appropriately fuel aerobic brain energy metabolism (Schurr *et al.*, 1999; Ide *et al.*, 2000; Smith *et al.*, 2003; Gallagher *et al.*, 2009) and provide definite therapeutic effects (Izumi *et al.*, 1994; Maran *et al.*, 1994). Recently, Arun *et al.* (2010) found that glyceryl triacetate (GTA), an FDA-approved food and drug additive, is more effective than lactate in treating TBI. In several TBI models, lactate *per se* is not enough to improve the altered cerebral energy state because of the disturbance of other co-substrates, such as NAD^+ (Prieto *et al.*, 2011). However, this does not exclude the definite benefit provided by lactate.

Besides oxygen and glucose, damage to mitochondria could occur at any number of sites in the molecular machinery that might be responsible for a decline in respiration and the production of ATP. In the brain, the acetate can be converted to acetyl CoA, which can be utilized for energy production, lipid synthesis and other metabolic processes. In addition, acetate metabolism bypasses both glucose and N-acetylaspartate metabolism, which helps to compensate for the substantial reductions in N-acetylaspartate and ATP levels observed after TBI (Arun *et al.*, 2010).

In fact, the preservation of energy supply and mitochondria protection are two closely connected processes. Enough energy supply is the prerequisite for mitochondria to cope with various vicious agents and to maintain the integrity and normal function. For example, creatine not only can maintain ATP levels, but can ameliorate neuronal cell death and reduces mitochondrial ROS production following TBI (Sullivan *et al.*, 2000). Exogenous ketone bodies can improve the mitochondrial function and increase cortical levels of ATP after injury (Davis *et al.*, 2008a). Fasting and a ketogenic diet can also decrease ROS production by uncoupling protein (UCP)-mediated uncoupling (Sullivan *et al.*, 2004a; Davis *et al.*, 2008b).

Mitochondrial – targeted multipotential therapeutic strategies

The mitochondrial membrane is the last barrier against cell death. From this point on, mitochondria-centred, especially mitochondrial membrane-targeted combined therapy will hold 'the last line' and help to prevent subsequent waves of secondary cascade events. A secondary insult after TBI is a complicated pathological process that involves various stimuli, and thus one is naive to expect that patients can benefit from a single treatment that is active on a specific mechanism and administered at a single stage of the unfolding process (Saatman *et al.*, 2008). The preservation of energy supply alone is not enough for mitochondria protection. In recent years, based on new knowledge about mitochondria, some innovative strategies have shown good prospects in mitochondrial-targeted multipotential therapy (Figure 4).

A 2009 workshop that was sponsored by the National Institute of Neurological Disorders and Stroke, recommended a combined approach for the treatment of TBI, focusing on therapeutics with complementary targets and effects (Margulies and Hicks, 2009). First, the early removal of the cause of injury is known to limit the area of neuronal damage by reducing the duration of the insult. In our previous study, we found that gross-total haematoma removal was an effective way to decrease intracranial haemorrhage-induced injury to brain tissue. This effect was related to decreased perihaematomal oedema formation and secondary injury by an inflammatory cascade activated by coagulation end products (Zuo *et al.*, 2009).

The development of cerebral oedema with brain swelling is the most significant predictor of outcome. Mitochondrial dysfunction, triggered by various death stimuli, is the main cause of propagation of cytotoxic brain oedema (Marmarou *et al.*, 2000; Unterberg *et al.*, 2004) and is a leading event in the cascade of cell death in TBI (Fiskum *et al.*, 2000; Sullivan *et al.*, 2005; Wang *et al.*, 2010). Although vasogenic oedema is transient in comparison to cytotoxic oedema, vasogenic oedema is permissive for cytotoxic oedema formation (Beaumont *et al.*, 2000). Neurogenic inflammation, mediated mainly by calcitonin gene-related peptide and substance P, is the main cause of vasogenic oedema (Geppetti *et al.*, 1995; Donkin *et al.*, 2009). Neuropeptide depletion or post-traumatic administration of the neurokinin-1 receptor antagonist N-acetyl-L-tryptophan inhibits BBB permeability and any subsequent oedema formation. Early inhibition of neurogenic inflammation may present a novel approach to the treatment of post-traumatic oedema formation (Donkin and Vink, 2010).

A systemic inflammatory response syndrome is a persistent pathological process that worsens patient outcome (Kemp *et al.*, 2008). Activation of the sympathetic nervous system is critical in CNS-induced immunodepression and organ inflammation. Phagocytic catecholamine production also contributes to a release of the pro-inflammatory cytokines (Flierl *et al.*, 2009). Therefore, therapeutic strategies

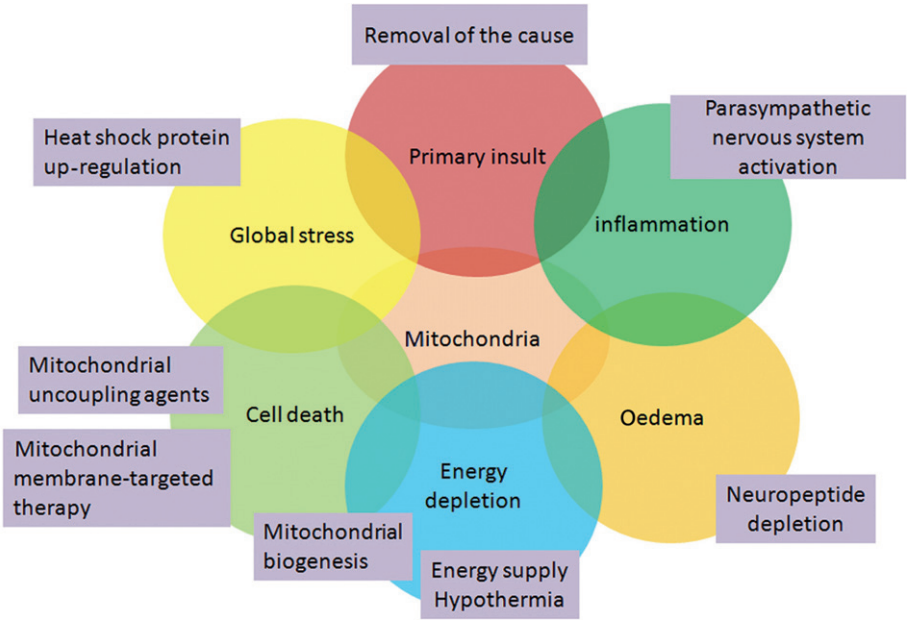

Figure 4

Mitochondrial-targeted multipotential therapeutic strategies. TBI is a pathological process with many intertwined participants that are involved in mitochondria. Combined strategies that target different pathological process will obtain the best outcome in mitochondrial-targeted multipotential therapy.

that are aimed at activating the parasympathetic nervous system could counterbalance the effects of the activated sympathetic system (Catania *et al.*, 2009). The release of chemokines by the liver is a significant aspect of the acute-phase response that is associated with CNS injury (Campbell *et al.*, 2003), and the suppression of Kupffer cell activity prevents secondary damage after acute brain injury (Campbell *et al.*, 2008).

Following acute injury, neurons and glia activate a global stress response that involves the transcriptional and translational upregulation of several several heat shock proteins (HSPs) (Wagstaff *et al.*, 1996), which contribute generally to survival by virtue of their chaperone function (Yenari *et al.*, 2005). Several HSPs (e.g.HSP27, HSP70 and HSP90) and derived molecular chaperones act as specific anti-apoptotic factors through multiple mechanisms, including the following: inhibiting both caspase-dependent and caspase-independent pathways (Saleh *et al.*, 2000; Gurbuxani *et al.*, 2003; Kalinowska *et al.*, 2005; Ruchalski *et al.*, 2006), apoptosome inhibition by direct apoptotic peptidase activating factor 1 binding (Saleh *et al.*, 2000), blockage of AIF mitochondrial release (Ruchalski *et al.*, 2006), cytosolic AIF sequestration and inhibition of EndoG DNase activity (Ravagnan *et al.*, 2001; Kalinowska *et al.*, 2005) and possible antioxidant property (Choi *et al.*, 2005).

At the cellular level, five principles of metabolic manipulation are proposed that target oxidative damage, lipid peroxidation, altered $\Delta\Psi m$, Ca^{2+} imbalance and transcription regulation (Koene and Smeitink, 2011). In this respect, pharmacological agents that offer 'mitochondrial uncoupling' ability provide new hope because they can increase proton leakage, thereby reducing oxygen stress by lowering $\Delta\Psi m$ (Cannon and Nedergaard, 2004; Jarmuszkiewicz *et al.*, 2004; Sullivan *et al.*, 2004a,bSluse *et al.*, 2006; Pandya *et al.*, 2007; 2009). Furthermore, because $\Delta\Psi m$ is the driving force for cytoplasmic Ca^{2+} entry to mitochondria, a mild 'mitochondrial uncoupling' can reduce $\Delta\Psi m$ and attenuate mitochondrial Ca^{2+} cycling (Gunter *et al.*, 1994; Mattiasson *et al.*, 2003). Stable nitroxide radicals have the ability to combine radical scavenging action with recycling capacities, which provide a new strategy to reduce ROS overproduction (Borisenko *et al.*, 2004; Mustafa *et al.*, 2010). Some stable nitroxide radical agents have shown positive results in preclinical studies (Kwon *et al.*, 2003; Macias *et al.*, 2007). ROS is produced only briefly, but the chain reaction of ROS-related lipid peroxidation (LP) persists for several days. Indirectly-acting antioxidant mechanisms that stop the 'chain reaction' propagation of LP once it has begun are a suitable choice. The combination of different antioxidant mechanistic strategies may improve the extent of neuroprotective efficacy, lessen the variability of the effect and possibly provide a longer therapeutic window of opportunity (Hall *et al.*, 2010).

As an important mediator of neuronal apoptosis and necrosis, PTPC represents a crucial target for neuroprotection (Nieminen *et al.*, 1996). An abundance of experiments have proved the efficiency of CsA in ameliorating mitochondrial functions in TBI. In an impact acceleration TBI model (Friberg *et al.*, 1998; Buki *et al.*, 1999; Crompton, 1999; Scheff and Sullivan, 1999; Sullivan *et al.*, 1999; Albensi *et al.*, 2000; Alessandri *et al.*, 2002; Friberg and Wieloch, 2002; Hansson *et al.*, 2003; Signoretti *et al.*, 2004; Mazzeo *et al.*, 2008), Okonkwo and Povlishock (1999) found that CsA protects both mitochondria and the related axonal shaft. phase II clinical trials found that CsA can improve cerebral perfusion

pressure and cerebral metabolism. Considering the proven safe and CSF pharmacokinetics of CsA, a phase III clinical trial is underway (Empey *et al*., 2006; Mazzeo *et al*., 2006; Merenda and Bullock, 2006). Because immunosupression is one of the possible side effects of CsA, other CsA derivatives, such as NIM-811 and 2-aminoethoxydiphenylborate, have been designed and show promising results (Waldmeier *et al*., 2002; Chinopoulos *et al*., 2003; Merenda and Bullock, 2006; Mbye *et al*., 2008; Readnower *et al*., 2011). The progesterone metabolite allopregnanolone is also a suitable choice to inhibit MPT and cyt c release (Sayeed *et al*., 2009). The slow-onset kinetics of necroptosis suggests that this pathway might provide a new target for neuroprotecitve intervention with an extended therapeutic window. Thus, the combination of anti-apoptotic and anti-necroptotic treatment might be suitable. Necrostatin-1 can inhibit all published examples of necrotic cell death induced by activation of death domain receptors in the presence of caspase inhibitors (Degterev *et al*., 2005). Under stress, mitochondrial biogenesis becomes an essential endogenous neuroprotective response that creates new functional mitochondria. Therefore, the stimulation or enhancement of mitochondrial biogenesis is a novel neuroprotective strategy.

TBI is a multi-factorial pathological change that affects the whole body. Thus, any potential benefits gained by targeting a single molecule or pathway may be masked by the plethora of simultaneously activated cascades. Therapies that serve to modulate multiple pathophysiological pathways and target multiple cell types may prove to be more effective. In preclinical studies, improved levels of neuroprotection have been obtained using therapeutic agents with multifunctional activities (Faden and Stoica, 2007; Vink and Nimmo, 2009), such as small cyclized dipeptides (Stoica *et al*., 2009), progesterone (De Nicola *et al*., 2009), PPAR activators (Semple and Noble-Haeusslein, 2011) and erythropoietin, among others (Stoica and Faden, 2010).

At the present, there are still many unanswered questions about the structure and function of the mitochondrion that need to be resolved. For example, the lipid composition and structural organization of the OM are among the central players in the regulation of Bcl-2 family member activity. However, the precise 3D structure that many Bcl-2 proteins adopt in this membrane is still unknown. Without this essential information, it is impossible to understand precisely how MOMP occurs (Lindsay *et al*., 2011). Yet it has been made clear that, by providing a sufficient energy supply as soon as possible, a mitochondrial-targeted multipotential therapeutic strategy will provide the best protection for TBI patients.

Conclusion

Mitochondria play a pivotal role in the secondary insult in TBI pathology. The dysfunction of mitochondria is the main cause of energy failure of damaged tissue and the platform of death. The coordinative role of different regulators and effectors in mitochondria, including excitotoxicity, ROS, caspases, the Bcl-2 family and AIF, determine cell death or life. Some preclinical and clinical results of mitochondria-targeted therapy show promise. With the clarification of the function and structure of mitochondria, mitochondrial-targeted multipotential therapeutic strategies will provide new hope for the treatment of TBI and other acute brain injury states.

Statement of conflict of interest

No conflicts of interest exist.

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