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Forum Editorial

Mitochondria-Directed Therapeutics

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ABSTRACT

Mitochondria are key regulators of cell life and death and play an important role in a wide range of diseases, including cancer, diabetes, cardiovascular disease, and the age-related neurodegenerative diseases. The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain. This forum discusses (a) potential new mitochondrial targets for therapeutic intervention, including components of the electron transport chain, the permeability transition, and the membrane dynamics protein mitofusin-2; (b) the role of mitochondria-targeted antioxidants including MitoQ and SS peptides in modulating reactive oxygen and chlorine species induced mitochondrial permeabilization and cell death; and (c) the potential use of SS peptides in ischemia and reperfusion tissue injury. In the future, mitochondrial drug-targeting strategies will be expected to open up avenues for manipulating mitochondrial functions and allow for selective protection or eradication of cells for therapeutic gain in a variety of diseases. *Antioxid. Redox Signal.* 10, 575–578.

THE MITOCHONDRION: MORE THAN JUST A REGULATOR OF CELL LIFE

After Peter Mitchell's landmark discovery of the regulation of mitochondrial energy production by chemi-osmosis, many scientists considered the role and function of the mitochondrion solved (29). It took little more than two decades after this discovery for mitochondria to reemerge in the spotlight; first, with the observation that a number of diseases are associated with specific mutations in mitochondrial DNA (mtDNA), and second, by the seminal discovery of Wang and colleagues that mitochondria are key regulators of programmed cell death by apoptosis (24). These findings rekindled scientific interest in this organelle, and in its potential role in a variety of diseases in which abnormalities in energy production, reactive oxygen species (ROS), and deregulated cell death are now considered key features. The number of diseases linked to abnormalities in mitochondrial function continues to grow and has provided a major impetus behind the idea to design and develop drugs that specifically target mitochondria. Indeed, the major diseases responsible for morbidity and mortality in the developed world, including cancer, cardiomyopathy, diabetes, and neurodegenerative diseases, all appear to have a significant mitochondrial component (6, 7, 23). Less commonly, although the number is rapidly growing, there are a number of *mitochondrial diseases* associated with specific mutations in mtDNA, or nuclear DNA (nDNA) coding for mitochondrial proteins, that have been discovered and which also make mitochondrial targeting of therapeutics an attractive idea. Therapeutic targeting of specific mitochondrial diseases has been dealt with, in detail, elsewhere. For recent reviews on this subject, see References 13, 14, 34, 41. The main focus of this forum is to discuss emerging ideas on novel mechanisms, and potential new targets, implicated in the *mitochondria-associated diseases* discussed above and to, herein, show the potential value of recently developed mitochondria-directed therapeutic strategies as candidates for primary or adjunct therapies to treat these diseases at the mitochondrial level.

MITOCHONDRIA: A NODAL CONTROL POINT REGULATING APOPTOSIS

Following the discovery of the role of mitochondria in apoptosis, interest in this area has risen almost exponentially. For

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example, a Pub Med search using the words "apoptosis and mitochondria" reveals <10 hits for the year 1990. This number rose to ~1,000 in the year 2000, while in 2005 there were in excess of 5,000 publications in this area. Apoptosis is regulated by extrinsic (receptor-mediated), as well as intrinsic (mitochondrial) pathways; however, the importance of the mitochondrion in apoptosis is emphasized by the fact that "crosstalk" often appears to occur between these two pathways and has been suggested to be required to amplify the overall apoptotic response (33). This observation clearly substantiates the role of the mitochondrion as a nodal control point regulating apoptosis and indicates that understanding the mechanisms regulating mitochondrial apoptosis is crucial to controlling this event for therapeutic gain. Indeed, recent reports have proposed a variety of novel methods for triggering the release of cytochrome *c* from mitochondria to activate apoptosis for potential use in cancer therapy including the use of hydrocarbon-stapled Bid BH3 domains to trigger Bax activation (37) and Smac/DIABLO mimics to block the action of the inhibitors of apoptosis proteins (IAPs) (22). Other emerging ideas have suggested the use of antisense "knockdown" (1) or glutathione redox strategies to target the antiapoptotic Bcl-2 protein (3, 42).

THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN: A NOVEL TARGET FOR THERAPEUTIC INTERVENTION

Recently, other mitochondrial targeting ideas have identified the electron transport chain (ETC) as a key regulatory point for potential therapeutic intervention (6, 7). For example, it is known that a variety of chemical agents deplete mtDNA in mammalian cells, and theoretically some of these drugs could be used in cancer therapy (6, 7, 21). More specifically, new ideas are focusing on specific sites within the ETC sites for possible intervention, including respiratory complex III (the bc_1 complex). Although this ETC site is known for its role in ROS production (18), our laboratory has found that the bc_1 complex plays a key role in regulating glutathione-dependent mitochondrial membrane permeabilization and caspase-independent cell death (3), which is effectively blocked by coenzyme Q_{10} analogues such as decylubiquinone (4) and as shown in this forum the mitochondria-targeted ubiquinone derivative MitoQ (26). Also, importantly, other laboratories have shown that the bc_1 complex acts an oxygen sensor for hypoxia regulating the transcription factor HIF1α, which firmly places this respiratory site complex as a potential new target for therapeutic intervention (8, 17). In this forum issue, Bell and colleagues review the role of the bc_1 complex as an oxygen sensor and discuss its potential as a target for cancer therapy (9).

MEMBRANE DYNAMICS, A NEW DIMENSION IN MITOCHONDRIAL SIGNALING AND APOPTOSIS RESEARCH

Other relatively new areas of research that are probing the mechanisms regulating mitochondrial signaling and apoptosis are focusing on the role of mitochondrial dynamics (membrane fission/fusion reactions) in this process (15, 20). Although membrane dynamics play a crucial physiological role in mitochondrial biogenesis and for energy distribution in the cell, the proteins that govern these reactions, membrane-bound dynamin-related GTPases, have recently been shown to play a key role in apoptotic cell death (15, 16). Indeed, recent evidence shows that the fusion protein Optic Atrophy 1 (OPA1) is crucial regulator of cytochrome *c* release by its effect on remodeling of the mitochondrial inner membrane cristae junction, a function that has recently been shown to be independent of its physiological role as a mitochondrial shaping protein (15). In addition to proteins such as OPA1 that remodel the mitochondrial inner membrane, there are GTPase proteins, known as the mitofusins (MFN), that reside in the mitochondrial outer membrane that also appear to possess a dual function. For example, while MFN1 appears to be primarily involved in organelle membrane dynamics, increasing evidence suggests that MFN2 is involved in multiple signaling pathways in addition to its role in the regulation of mitochondrial fusion, including the regulation of mitochondrial metabolism (40), cell cycle progression (12), and apoptosis (31). In this forum issue, Martins de Brito and Scorrano review the role of the MFN-2 in mitochondrial signaling highlighting the potential role of this protein as a novel target for therapeutic intervention (27).

MITOCHONDRIA AND NECROSIS: THE ROLE OF THE MITOCHONDRIAL PERMEABILITY TRANSITION

In addition to apoptosis, mitochondria also play a key role in mediating the necrotic cell death that occurs in ischemia and reperfusion (IR) (5–7, 10, 19). Since IR injury is associated with the cell death that occurs during myocardial infarction and stroke, it firmly links the mitochondrion to a major cause of mortality in the developed world. This mode of cell death occurs primarily through the "so-called' mitochondrial permeability transition (MPT), a nonspecific mitochondrial permeabilization regulated by the mitochondrial matrix peptidyl-prolyl isomerase cyclophilin D (Cyp-D) and induced by increased mitochondrial calcium and ROS formation by the ETC (5, 10, 19). In this forum, Burwell and Brookes propose a novel approach to modulating ETC-derived ROS utilizing the reactive nitrogen species nitric oxide (NO), which suggests that, in addition to their known function as regulators of cell death, mitochondria may also be viewed as effectors of cardioprotection during IR tissue injury (11).

MITOCHONDRIA-TARGETED ANTIOXIDANTS

The association between ROS and disease over the years has been overwhelming and has been a major impetus behind the development of a variety of strategies into blocking or modulating ROS production for therapeutic gain. In addition to the use of a variety of antioxidants including vitamins C and E, and

glutathione donors such as *N*-acetyl cysteine (NAC), recently the use of superoxide/catalase mimetics such as the catalytic antioxidant manganese 5,10,15,20-tetrakis (4-benzoic acid) porphyrin (MnTBAP) has shown promise by increasing the lifespan of mice with oxidative stress-associated neurodegenerative disease (28). Indeed, because mitochondria are a major source of intracellular ROS, it would be ideal to deliver the antioxidant therapy directly to the organelle. At the forefront of this approach are the MitoQ class of compounds, and the recent development of a novel class of mitochondria-targeted peptide antioxidants known as the Szeto–Schiller (SS) peptides (30, 35). These mitochondria-targeted antioxidants have been shown to block the myocardial cell injury associated with IR in animal models (2) and neuronal cell death in animal models of stroke, Parkinson's disease, and amyotrophic lateral sclerosis (32). In this forum, Szeto discusses the cytoprotective potential of SS peptides in IR tissue injury ischemia and compares their unique properties with other mitochondria-targeted antioxidants (36). Also in this forum, Lu *et al*. show that the antioxidant MitoQ effectively blocks mitochondrial permeabilization resulting from GSH depletion and increased ROS production in the absence of a functional ETC, which is thought to be needed for antioxidant recycling (26). This observation indicates the potential therapeutic value of MitoQ in a range of diseases associated with loss of GSH, deregulated ETC, and increased ROS production (30). In addition, mitochondria-targeted antioxidants can be used to help understand the mechanisms underlying oxidative stress-induced cell damage, as well as the sites of cellular ROS production, and therefore serve two goals. For example, MitoQ has recently been used to investigate the link between calcium and ROS formation leading to mitochondrial permeabilization during GSH-dependent cell death (25).

REACTIVE CHLORINE SPECIES, INFLAMMATION, AND LIVER DISEASE

In addition to the effects of ROS and RNS on mitochondria, reactive chlorine species (RCS), produced during a variety of inflammatory diseases, including hepatitis, also appear to target mitochondria. For example, it was recently shown that hypochlorous acid (HOCl) induced the MPT and cell death in isolated hepatic cells (38). In this forum, Whiteman and colleagues have examined the mito-protective effects of SS peptides and MitoQ on HOCl-induced mitochondrial dysfunction and cytotoxicity in human liver cells (39).

CONCLUSIONS

The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain. Recent reviews, including those in this forum, have proposed a variety of novel mitochondrial molecular targets for a diverse group of diseases, with an underlying mitochondrial etiology, in the hope that these new drug targets will promote the development of drug strategies for future disease treatment.

ABBREVIATIONS

Cyp-D, cyclophilin D; ETC, electron transport chain; HOCl, hypochlorous acid; IR, ischemia and reperfusion; HIF, hypoxia-inducible factor; MFN, mitofusins; MnTBAP, manganese 5,10,15, 20-tetrakis (4-benzoic acid) porphyrin; mtDNA, mitochondrial DNA; MPT, mitochondrial permeability transition; NAC, *N*-acetyl cysteine; NO, nitric oxide; nDNA, nuclear DNA; OPA1, optic atrophy 1; RCS, reactive chlorine species; ROS, reactive oxygen species.

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