MITOCHONDRIAL STRESS IN TRANSLATIONAL MEDICINE TOWARD METABOLIC DISEASES

D. Popov*

"N. Simionescu” Institute of Cellular Biology and Pathology, Bucharest, Romania

Abstract

The recent discoveries on organelles autoregulation and their molecular mechanisms motivate a novel perspective on mitochondria involvement in cardiovascular dysfunctions related to diabetes mellitus and obesity. We present here an up-dated view on the latter topic along with a condensed perception on morphological details resultant from diabetes mellitus experimental models. This study is organized into sections covering the following topics: (I) mitochondrial stress/dysfunction, (II) the “quality controller” role of mitochondria exerted by fusion, fission, and mitophagy events, (III) the connection between mitochondria and metabolic diseases, and (IV) the perspectives of potential application of mitochondrial-targeted compounds in metabolic diseases. Critical analysis of the knowledge available so far on mitochondria-metabolic diseases relationship allows a two sides conclusion: a doubtful view, as the correlation between impaired mitochondrial function and insulin resistance is still unclear, even after 40 years since its first publication, and a hopeful view based on the novel traits of this organelle uncovered recently, such as plasticity, the “quality controller” role, the “retrograde signalling”, and the coordinate interaction with the nucleus, endoplasmic reticulum, Golgi apparatus and lysosomes. At the horizon, the essential issue of targeting mitochondria for the alleviation of diabetes/obesity complications remains to be resolved.

Key words: oxidative stress, mitochondrial dynamics, fusion, fission, mitophagy.

INTRODUCTION

Although mitochondrion is known as energy powerhouse of the cell, recent reports unveil a plethora of novel credentials of this organelle: it forms interconnected filamentous networks, moves towards the cellular sites where energy supply is locally demanded, acts as surveillance system (“quality controller”), spreads signals to the rest of the cell (known as “retrograde signalling”), scavenges excess of reactive oxygen species (ROS) produced by other cellular sources, and concludes on cells survival or death. A substantial part of this ongoing field of research is devoted to organelle’s role in pathophysiology; new terms populate this key area, such as “mitochondrial stress”, “mitochondrial dysfunction”, the “redox-optimized ROS balance”, “mitochondrial dynamics”, “fusion”, “fission”, “mitophagy”. The aim of this editorial is to draw attention on the molecular events beyond these terms, and to discuss the novel mitochondria-targeted compounds of potential relevance in metabolic diseases. Such therapies, directed to specific ROS-activated pathways in malfunctioning mitochondria, may appear as a promising alternative to the former unsuccessful systemic antioxidant approaches.

A NEW WORLD IN CELLS

PATHOPHYSIOLOGY: MITOCHONDRIAL STRESS/DYSFUNCTION

In physiological conditions, mitochondria homeostasis is a circumstance that ensures cellular life-supporting processes, as oxidative phosphorylation generates energy in the form of ATP. It is associated with production of low levels of mitochondrial ROS, important second messengers that regulate growth, development and differentiation, and has a close communication with cellular nucleus that ensures execution of “quality control mechanisms”. The main attributes of quality control mechanisms include: (I) bioenergetic capacity regulation, via “mitochondrial unfolded protein response”(UPR), (II) protection against ROS damage, via activation of antioxidant defense mechanisms, (III) adjustment of signals transduced through ROS (known as “redox signaling”), and (IV) control of organelle turnover, by engagement of autophagic degradation; all these processes are scheduled to maintain redox homeostasis and cellular

*Correspondence to: Doina Popov MD, “N. Simionescu” Institute of Cellular Biology and Pathology, Pathophysiology and Pharmacology 8, B.P. Hasdeu, Bucharest, 050568, Romania, E-mail: doina.popov@ichp.ro

Acta Endocrinologica (Buc), vol. XI, no. 3, p. 269-275, 2015
survival (1-4).

**Mitochondrial stress description**

Mitochondrial stress is characterized by diminished energetic performance and increased release of cytotoxic ROS, such as superoxide anion radical (O$_2^-$•), hydroxyl radical (•OH) and hydrogen peroxide (H$_2$O$_2$) and reactive nitrogen species (RNS), mostly peroxynitrite (formed by reaction between superoxide anion and the vasodilator nitric oxide). The superoxide radical is generated by mitochondrial respiratory chain, while the hydroxyl radical is produced by reduction of hydrogen peroxide by endogenous iron and cooper ions. As ROS over-production is in common with dysfunctional mitochondria, several authors refer to either mitochondrial stress or dysfunction (5). Furthermore, these “defective” mitochondria display abundant DNA mutations and imbalanced expression of antioxidant enzymes.

**Mitochondrial stress inductors.** There are several inductors of mitochondrial stress such as metabolic diseases (diabetes mellitus, obesity), systemic hyperglycemia, accumulation of unfolded proteins within mitochondria, nutritional factors (high-fat high-sucrose diet, nutrient excess), the aging process, the inadequate replenishment of mitochondrial pool by mitochondrogenesis, along with genetic oxidative phosphorylation disorders (the basis of inherited mitochondrial disorders)(6-8).

**Mitochondrial stress biomarkers.** The cellular redox-sensitive pathways trigger post-translational modifications that may serve as biomarkers of mitochondrial stress /dysfunction. Examples of such biomarkers are: (I) S-(2-succinyl) cysteine (2SC) generated during succination, an irreversible chemical interaction between fumarate and the low pKa thiols in cysteine residues of adiponectin, adipocytes tubulin and actin, and endoplasmic reticulum (ER) chaperone proteins (9-11), (II) glutathionylation of the similar low pKa thiols, a process with broad cellular interventions (12), (III) lysine deacetylation catalyzed by mitochondrial sirtuins (SIRT3, SIRT4, and SIRT5), as a regulatory mechanism of energy metabolism under oxidative stress (13, 14).

**Mitochondrial stress consequences.** In pathophysiological conditions, production of excessive amounts of ROS conduct to the decline of oxidative phosphorylation and decreased ATP synthesis by mitochondria. One can delineate several features of mitochondrial stress response: (I) initiation of modifications (point mutations, deletions) in mitochondrial DNA (mtDNA) by excess superoxide radicals restrained to mitochondria (as they cannot cross the inner mitochondrial membrane, IMM), (II) altered expression of several gene clusters that portrays “mitochondrial diseases”, (III) damage of lipids and proteins within IMM, including lipid peroxidation-induced protein carbonylation, (IV) upregulation of mitochondrial chaperone and protease expression in the matrix and intermembrane space, and (V) activation of adaptive responses that encompass AMPK-PFK2, AMPK-FOXO3a, AMPK-PGC-1α, and AMPK-mTOR signaling pathways (15-18). Overall, elevated ROS production by electron transport chain overwhelm the cellular antioxidant defence capacity (19).

**Strategies aiming mitochondrial stress alleviation.** There is a general agreement that caloric restriction and exercise are benefic for mitochondrial stress/dysfunction alleviation (20). On the other hand, the use of mitochondria-targeted molecules is still a matter of debate. Some authors consider as inconclusive the results implying mitochondrial antioxidants such as MnSOD (EUK-8, EUK-134 and MitoSOD), choline esters of reduced glutathione and N-acetyl-L-cysteine, of triphenylphosphonium ligated vitamin E, lipoic acid, plastoquinone and of Szeto-Schiller (SS)- peptides (SS-02 and SS-31); but the use of coenzyme Q10 is considered worthwhile for mitochondrial stress amelioration, as the clinical trials reveal it is a safe adjunct to conventional therapies in cardiovascular disease (reviewed by 16). Other authors employed pharmacological or biochemical approaches (e.g. inhibition of succinate accumulation and oxidation, and S-nitrosylation of mitochondrial complex I, respectively) to achieve decreased ROS production and impaired oxidative damage and tissue necrosis during ischemia-reperfusion injury (IRI) (21, 22).

**Novel assessments: “redox-optimized ROS balance” and “ROS-induced ROS release”**. The mitochondrial ROS generators are respiratory chain complex I (NADH:ubiquinone oxidoreductase) and complex III (ubiquinol:cytochrome c oxidoreductase; cytochrome bc1 complex); both complexes produce superoxide anions and hydrogen peroxide, but the mitochondrial compartments of delivery are different, i.e. matrix for complex I and the intermembrane space, for complex III (23). Another cause of ROS generation consists in Ca$^{2+}$ deposition into mitochondrial matrix (16). Figure 1a shows the classic image of double-membranated mitochondria, highly dynamic organelles displaced here near the lipid droplets of a diabetic, steatotic myocardium.
According to the hypothesis/concept of "redox-optimized ROS balance" the respiratory chain functions both as a source as well as a target of redox processes. Recent studies emphasize that biomarkers for this dual role can be considered mitochondrial complex I and complex III (23). Other studies show that the balance between the regulatory and damaging effects of ROS is controlled by mitochondrial and cytoplasmic aconitase isoforms (24). A special attention receives the redox environment of mitochondria that functions as intermediary between mitochondria respiratory rate and ROS emission levels (25, 26). However, ROS levels in vicinity of mitochondria may produce opposite effects: the destruction of mitochondria or even of the whole cell, as a result of opening of permeability transition pores (mPTP) and release of ROS bursts (known as "ROS-induced ROS release") or the regression of mitochondrial dysfunction, via activation of local redox-sensitive enzymes and signaling pathways that potentially restore redox homeostasis (27). Several redox-mediated modifications govern the function of mitochondrial ion channels (Ca^{2+} uniporter pore, mitochondrial permeability transition pore, mitochondrial ATP-sensitive K+ channel) and modifications of the cysteine-thiol groups within respiratory chain complexes subunits; the latter aspect allows utilization of complex I S-nitrosylation to counteract excess ROS generation, oxidative damage

Figure 1. Cardiomyocyte mitochondria in the left ventricle of streptozotocin-induced mice (Type 1 DM) (a) The classic morphology of mitochondrion; (b) fused, elongated mitochondria; (c) fragmented mitochondria (arrows) at SR contact points (*). Id: lipid droplets. Original magnifications: (a) and (c) x 54,600; (b) x26,280
and tissue necrosis during IRI (22, 28). Collectively, the above data provide an updated image of regulatory role of mitochondria under oxidative stress conditions.

**THE QUALITY CONTROLLER ROLE OF MITOCHONDRIA DYNAMICS: FUSION, FISSION, MITOPHAGY**

The recent studies emphasize the mechanisms beyond the “quality controller” role of mitochondria, such as: fusion of malfunctioning mitochondria with “healthy” ones, aiming energy production preservation and cell survival, specific elimination of dysfunctional proteins of the outer mitochondrial membrane (OMM) via cytosolic ubiquitin-proteasome system (UPS), removal of damaged mitochondrial proteins via chaperones and proteases, and mitochondria discharge via autphagic degradation (mitophagy) (29). Mitochondrial dynamics implies changes in organelle’s morphology via processes of fusion and fragmentation/fission (30). Mitochondrial fusion is promoted by activation of fusion molecules and inhibition of mitochondrial fission pathway (31). The fusion of outer mitochondrial membranes (OMM) is regulated by dynamin GTPases mitofusin 1 (MFN1) and mitofusin 2 (MFN2), while that of inner mitochondrial membranes (IMM) is regulated by optic atrophy protein 1 (OPA1) (32). The result of fusion is formation of elongated mitochondria (longer than three sarcomeres) within the diabetic myocardium (Figure 1b). When expression of mitofusins is impaired (such as following corticosterone treatment) the oxidative stress is increased, energy production in brain is reduced, and depression-like behaviours ensued (33). The dysfunctional small size mitochondria are the result of fission either of normal organelles (when encountering a decrease in membrane potential associated with degradation) or of enlarged, fused mitochondria, aiming elimination of the malfunctioning part (committed to clearing by mitophagy) and conservation of the “healthy” part (assigned for further fusions) (34, 35). Inside cardiomyocyte, mitochondrial fission is initiated at the sites where OMM closely associates with sarcoplasmic reticulum (SR) cisternae, proving the physical contact between mitochondria and SR that allows the molecular cross-talk (Fig. 1c). The specific fission-associated molecules are dynamin-related protein-1 (DRP1) and fission-1 (FIS1) (36-38). The last step in dysfunctional mitochondria journey is mitophagy, i.e. engulfment of small size dysfunctional mitochondria within autophagosomes, followed by fusion of the latter with lysosomes and hydrolysis (39). It can be viewed as a cardioprotective process, preventing accumulation of dysfunctional mitochondria and ensuing bioenergetic efficiency (40). Every cell should maintain a dynamic balance between mitochondrial shape changes and organelle’s biogenesis, to overcome the mitochondrial reduced mass in pathophysiological conditions. A recent report demonstrates that MFN1 establishes a specific mitochondrial size that allows OMM permeabilization and proapoptotic BCL-2 family function (41). Interestingly, defects in mitochondrial dynamics are related to pathogenic gene mutations, as shown in neurodegenerative diseases (42). A safe conclusion is that mitochondrial dynamic shape changes and turnover are closely related to organelle’s bioenergetic potential.

**MITOCHONDRIA-METABOLIC DISEASES CONNECTION**

Metabolic diseases are listed by The International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) according to World Health Organization classification (WHO) (http://www.who.int/classifications/icd/ICD10Volume2_en_2010.pdf). This editorial is focused on diabetes mellitus and obesity, key components of the metabolic syndrome, associated with development of severe cardiovascular complications. On these grounds, diabetes and obesity can be viewed as cardiometabolic diseases. It is generally accepted that chronic hyperglycemia is associated with mitochondrial dysfunction (8, 43). Other reports showed that methylglyoxal (a degradative product abnormally increased in hyperglycemia) increases ROS-mediated mitochondrial dysfunction and oxidative stress (44). The inefficient nutrient oxidation causes low O₂ consumption and over-production of mitochondrial ROS (45). Subsequently, the superoxide anions inhibit glyceraldehyde-3-phosphate dehydrogenase activity, and stimulate genes involved in activation of polyol, hexosamine, advanced glycation end products and protein kinase-C pathways (46). The connection between mitochondrial dysfunction and insulin resistance (IR) is still under debate, although it was first reported 40 years ago (47). Several recent reports support this connection: (I) in the pancreas, mitochondrial dysfunction has been related to impaired insulin release, and it was identified as contributor to beta cell failure (48), (II) mitochondrial malfunction was considered as main cause of IR and cardiometabolic co-morbidities (45), (III) IR and
obesity were associated to reduced mitochondrial mass and oxidative function, while the increase of these two parameters conducted to improved insulin sensitivity (49), (iv) an association has been detected between mitochondrial isoform of phosphoenolpyruvate carboxykinase and phosphoenolpyruvate generation, as a metabolic pathway regulating insulin secretion and gluconeogenesis (50). Other authors consider still controversial the relationship between mitochondrial dysfunction and development of IR, as it is not certain yet whether mitochondrial dysfunction is the cause or the consequence of IR (42, 49, 51, 52). The incomplete solution for this issue is caused by the complex etiology of IR, combining environmental and genetic factors.

In 3T3-L1 adipocyte model (commonly employed for the study of obesity) it was recently demonstrated a link between mitochondrial stress, protein succination and ER stress, unique to the condition of cells maturation in the presence of supra-physiological glucose concentration (8). The excess fuel supply (glucotoxicity) increased proteins succination and mitochondrial stress in adipose tissue in diabetes (10). Furthermore, a lower mitochondrial capacity for oxidative metabolism was reported in human obesity and type 2 diabetes (53). In this context, diet supplementation with hydroxytyrosol (an active compound in olive oil) attenuated mitochondrial abnormalities in obese mice, decreased the levels of mitochondrial carbonyl protein and improved mitochondrial complexes activity (54). The post-translational modifications of mitochondrial proteins (acetylation, phosphorylation, succinylation, nitrosylation, carbonylation and ubiquitination) emerged as a novel regulatory mechanism of the bioenergetic function of mitochondria that open a new perspective in metabolic disease pathogenesis (14, 48, 55).

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Mitochondrial dysfunction