Mitochondrial Biogenesis: Pharmacological Approaches
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Organelle biogenesis is concomitant to organelle inheritance during cell division. It is necessary that organelles double their size and divide to give rise to two identical daughter cells. Mitochondrial biogenesis occurs by growth and division of pre-existing organelles and is temporally coordinated with cell cycle events [1]. However, mitochondrial biogenesis is not only produced in association with cell division. It can be produced in response to an oxidative stimulus, to an increase in the energy requirements of the cells, to exercise training, to electrical stimulation, to hormones, during development, in certain mitochondrial diseases, etc. [2]. Mitochondrial biogenesis is therefore defined as the process via which cells increase their individual mitochondrial mass [3]. Recent discoveries have raised attention to mitochondrial biogenesis as a potential target to treat diseases which up to date do not have an efficient cure. Mitochondria, as the major ROS producer and the major antioxidant producer exert a crucial role within the cell mediating processes such as apoptosis, detoxification, Ca^{2+} buffering, etc. This pivotal role makes mitochondria a potential target to treat a great variety of diseases.

Mitochondrial biogenesis can be pharmacologically manipulated. This issue tries to cover a number of approaches to treat several diseases through triggering mitochondrial biogenesis. It contains recent discoveries in this novel field, focusing on advanced mitochondrial therapies to chronic and degenerative diseases, mitochondrial diseases, lifespan extension, mitohormesis, intracellular signaling, new pharmacological targets and natural therapies. It contributes to the field by covering and gathering the scarcely reported pharmacological approaches in the novel and promising field of mitochondrial biogenesis.

There are several diseases that have a mitochondrial origin such as chronic progressive external ophthalmoplegia (CPEO) and the Kearns-Sayre syndrome (KSS), myoclonic epilepsy with ragged-red fibers (MERRF), mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), Leber's hereditary optic neuropathy (LHON), the syndrome of neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP), and Leigh's syndrome. Likewise, other diseases in which mitochondrial dysfunction plays a very important role include neurodegenerative diseases, diabetes or cancer.

Generally, in mitochondrial diseases a mutation in the mitochondrial DNA leads to a loss of functionality of the OXPHOS system and thus to a depletion of ATP and overproduction of ROS, which can, in turn, induce further mtDNA mutations. The work by Yu-Ting Wu, Shi-Bei Wu, and Yau-Huei Wei (Department of Biochemistry and Molecular Biology, National Yang-Ming University, Taiwan) [4] focuses on the aforementioned mitochondrial diseases with special attention to the compensatory mechanisms that prompt mitochondria to produce more energy even under mitochondrial defect-conditions. These compensatory mechanisms include the overexpression of antioxidant enzymes, mitochondrial biogenesis and overexpression of respiratory complex subunits, as well as metabolic shift to glycolysis. The pathways observed to be related to mitochondrial biogenesis as a compensatory adaptation to the energetic deficits in mitochondrial diseases are described (PGC-1α, Sirtuins, AMPK). Several pharmacological strategies to trigger these signaling cascades, according to these authors, are the use of bezafibrate to activate the PPAR-PGC-1α axis, the activation of AMPK by resveratrol and the use of Sirt1 agonists such as quercetin or resveratrol. Other strategies currently used include the addition of antioxidant supplements to the diet (dietary supplementation with antioxidants) such as L-carnitine, coenzyme Q_{10}, MitoQ_{10} and other mitochondria-targeted antioxidants, N-acetylcysteine (NAC), vitamin C, vitamin E vitamin K1, vitamin B, sodium pyruvate or α-lipoic acid.

As aforementioned, other diseases do not have exclusively a mitochondrial origin but they might have an important mitochondrial component both on their onset and on their development. This is the case of type 2 diabetes or neurodegenerative diseases. Type 2 diabetes is characterized by a peripheral insulin resistance accompanied by an increased secretion of insulin as a compensatory system. Among the explanations about the origin of insulin resistance Mónica Zamora and Josep A. Villena (Department of Experimental and Health Sciences, Universitat Pompeu Fabra / Laboratory of Metabolism and Obesity, Universitat Autònoma de Barcelona, Spain) [5] consider the hypothesis that mitochondrial dysfunction, e.g. impaired (mitochondrial) oxidative capacity of the cell or tissue, is one of the main underlying causes of insulin

resistance and type 2 diabetes. Although this hypothesis is not free of controversy due to the uncertainty on the sequence of events during type 2 diabetes onset, e.g. whether mitochondrial dysfunction is the cause or the consequence of insulin resistance, it has been widely observed that improving mitochondrial function also improves insulin sensitivity and prevents type2 diabetes. Thus restoring oxidative capacity by increasing mitochondrial mass appears as a suitable strategy to treat insulin resistance. The effort made by researchers trying to understand the signaling pathways mediating mitochondrial biogenesis has uncovered new potential pharmacological targets and opens the perspectives for the design of suitable treatments for insulin resistance. In addition some of the current used strategies could be used to treat insulin resistance such as lifestyle interventions (caloric restriction and endurance exercise) and pharmacological interventions (thiazolidinediones and other PPAR agonists, resveratrol and other calorie restriction mimetics, AMPK activators, ERR activators).

Mitochondrial biogenesis is of special importance in modern neurochemistry because of the broad spectrum of human diseases arising from defects in mitochondrial ion and ROS homeostasis, energy production and morphology [1]. Parkinson´s Disease (PD) is a very good example of this important mitochondrial component on neurodegenerative diseases. Anuradha Yadav, Swati Agrawal, Shashi Kant Tiwari, and Rajnish K. Chaturvedi (CSIR-Indian Institute of Toxicology Research / Academy of Scientific and Innovative Research, India) [6] remark in their review the role of mitochondrial dysfunction in PD with special focus on the role of oxidative stress and bioenergetic deficits. These alterations may have their origin on pathogenic gene mutations in important genes such as DJ-1, α-syn, parkin, PINK1 or LRRK2. These mutations, in turn, may cause defects in mitochondrial dynamics (key events like fission/fusion, biogenesis, trafficking in retrograde and anterograde directions, and mitophagy). This work reviews different strategies to enhance mitochondrial bioenergetics in order to ameliorate the neurodegenerative process, with an emphasis on clinical trials reports that indicate their potential. Among them creatine, Coenzyme Q10 and mitochondrial targeted antioxidants/peptides are reported to have the most remarkable effects in clinical trials. They highlight a dual effect of PGC-1α expression on PD prognosis. Whereas a modest expression of this transcriptional co-activator results in positive effects, a moderate to substantial overexpression may have deleterious consequences. As strategies to induce PGC-1α activation, these authors remark the possibility to activate Sirt1 with resveratrol, to use PPAR agonists such as pioglitazone, rosiglitazone, fenofibrate and bezafibrate. Other strategies include the triggering of Nrf2/antioxidant response element (ARE) pathway by triterpenoids (derivatives of oleanolic acid) or by Bacopa monniera, the enhancement of ATP production by carnitine and α-lipoic acid**.**

Mitochondrial dysfunctions are the prime source of neurodegenerative diseases and neurodevelopmental disorders. In the context of neural differentiation, Martine Uittenbogaard and Anne Chiaramello (Department of Anatomy and Regenerative Biology, George Washington University School of Medicine and Health Sciences, USA) [7] thoroughly describe the implication of mitochondrial biogenesis on neuronal differentiation, its timing, its regulation by specific signaling pathways and new potential therapeutic strategies. The maintenance of mitochondrial homeostasis is crucial for neuronal development. A mitochondrial dynamic balance is necessary between mitochondrial fusion, fission and quality control systems and mitochondrial biogenesis. Concerning the signaling pathways leading to mitochondrial biogenesis this review highlights the implication of different regulators such as AMPK, SIRT1, PGC-1α, NRF1, NRF2, Tfam, etc. on the specific case of neuronal development, providing examples of diseases in which these pathways are altered and transgenic mouse models lacking these regulators. A common hallmark of several neurodegenerative diseases (Huntington´s Disease, Alzheimer´s Disease and Parkinson´s Disease) is the impaired function or expression of PGC-1α, the master regulator of mitochondrial biogenesis. Among the promising strategies to ameliorate mitochondrial-based diseases these authors highlight the induction of PGC-1α via activation of PPAR receptors (rosiglitazone, bezafibrate) or modulating its activity by AMPK (AICAR, metformin, resveratrol) or SIRT1 (SRT1720 and several isoflavone-derived compounds). This article also presents a review of the current animal and cellular models useful to study mitochondriogenesis. Although it is known that many neurodegenerative and neurodevelopmental diseases are originated in mitochondria, the regulation of mitochondrial biogenesis has never been extensively studied. In order to find effective treatments to these up to date uncured diseases, comprehensive studies are therefore necessary on the control mechanisms of mitochondrial biogenesis, on the dynamic mitochondrial balance (fusion, fission, mitophagy and trafficking) and on the potential crosstalk among different biological processes, as expressed by the authors, along with the development of novel animal models to appropriately study this mitochondriogenesis.

A switch in bioenergetics is necessary for cancer development. Thus the control of mitochondrial bioenergetics and dynamics could be useful as potential interventions on cancer treatments. Pilar Roca, Jorge Sastre-Serra, Mercedes Nadal-Serrano, Daniel Gabriel Pons, Mª del Mar Blanquer-Rosselló and Jordi Oliver (Institut d'Investigació en Ciències de la Salut (IUNICS), Universitat de les Illes Balears, Spain) [8] describe the regulation of estrogen receptors, their implication on breast cancer, on mitochondrial biogenesis, mitochondrial function, and ROS production. It deeply reviews the group of natural compounds intimately related to estrogen receptors, flavonoids, and their application in cancer treatment and research, their action mechanisms, etc. giving an emphasis on the differences found in the response depending on the doses, timing, absorption, metabolism and hormonal status for the design of new strategies to treat breast cancer.

In the search of new targets for therapies based on targeting mitochondrial biogenesis it is of extreme importance to understand the pathways involved as well as the mediators that promote these signaling pathways. Fabian Sanchis-Gomar, José Luis García-Giménez, Mari Carmen Gómez-Cabrera and Federico V. Pallardó (Department of Physiology, University of Valencia / CIBERER / INCLIVA, Spain) [9] make a broad review on the current knowledge in this field, as well as about the diseases which course with alterations on the mitochondrial biogenesis pathways. Although the knowledge on specific treatments based on mitochondriogenesis is still poor, several drugs that are currently in the market present features potentially useful to trigger mitochondriogenesis for the treatment of specific diseases. This review compiles most of them, making an emphasis on the observed side effects of these drugs and the lack of selectivity of these strategies due to the fact that mitochondriogenesis is a ubiquitous phenomenon. Far from being a backward, this may constitute a challenge for designing more tissue-specific therapeutic approaches.

The study of mitochondrial biogenesis is especially complex, due to the endosymbiotic evolutionary origin of this organelle. Mitochondria are the most complex and unique organelles: in which eukaryotic and prokaryotic mechanisms coexist, they possess an inner and an outer membrane, own small genome and they suffer continuous fusion and fission events. Moreover, along with endosymbiosis, novel mitochondrial biogenesis pathways have evolved [1]. In order to extend our knowledge about underlying mechanisms via which mitochondriogenesis in different tissues is induced, it is crucial to use the proper techniques to measure mitochondrial mass. In living cells, the regulation of mitochondrial content or mitochondrial mass depends on the subtle balance between mitochondrial biogenesis, mitochondrial degradation (mitophagy) and mitochondrial dynamics (fusion, fission). Karl J. Tronstad, Marco Nooteboom**,** Linn I. H. Nilsson, Julie Nikolaisen, Maciek Sokolewicz, Sander Grefte, Ina K.N. Pettersen, Sissel Dyrstad, Fredrik Hoel, Peter H.G.M. Willems and Werner J.H. Koopman (Department of Biomedicine, University of Bergen, Norway / Department of Biochemistry Radboud University Medical Centre, The Netherlands) [10] describe the mechanism that maintains this equilibrium and the available techniques to quantify mitochondrial morphology and content. After reviewing the advantages and disadvantages of the most common techniques and strategies (measuring oxygen consumption, biochemical biomarkers or by electron microscopy), we can find in this work a deep analysis on fluorescence microscopy for the detection of mitochondrial content, its visualization, quantitation and interpretation of results both in 2D and in 3D imaging, along with available software and strategies developed by this group and others. This work can be of great help at the time to choose a technique to study mitochondrial biogenesis in a specific cell type. In addition, we can also find a table with several drugs known to affect mitochondriogenesis.

Free radicals have been widely considered as harmful for the cellular structures and promoters of senescence. However, they also act as second messengers by triggering signals which induce gene expression. Indeed, endogenous free radicals can trigger mitochondriogenesis. Hagir B. Suliman, and Claude A. Piantadosi (Departments of Anesthesiology, Duke Cancer Institute, Medicine and Pathology, Duke University Medical Center, USA) [11] broadly review the effect of these free radicals on mitochondriogenesis during inflammation. In periods of active inflammation due to an acute tissue damage, mitochondria are frequently damaged by oxidative and nitrosative stress. The elevated levels of endogenous free radicals trigger mitochondriogenesis and mitophagy in a compensatory manner. This is the case of the NO/cGMP/PGC-1α axis, the CO/HO-1 system and the HS_2/Akt/NRF-1/-2 axis. Several well known drugs can interact with those and other signaling pathways to induce mitochondriogenesis like NO donors, CO releasing molecules, triterpenoids, erythropoietin, thiazolidinedione drugs, metformin, AICAR and several natural compounds (including nutrients and scavengers). Thus inducing mitochondrial biogenesis and quality control represents a potential valuable approach for the development of new therapies for those diseases which course with

mitochondrial damage and/or inflammation.

Much attention has been attracted by recent discoveries pointing out mitochondrial biogenesis as a key process on lifespan extension, e.g. similar molecules and pathways as well as similar interventions have been found to be common in both processes. Enzo Nisoli and Alessandra Valerio (Center for Study and Research on Obesity / Department of Medical Biotechnology and Translational Medicine, University of Milan / Department of Molecular and Translational Medicine, University of Brescia, Italy) [12] review the contribution of mitochondria and other organelles on aging and anti aging-strategies, pointing out the interplay between organelles as a potential target for the design of new therapeutic interventions against age-related diseases and to increase life- and healthspan. Some interventions include the non-pharmacological control of mitochondrial biogenesis and dynamics by caloric restriction, endurance exercise and dietary supplementation with a mixture of essential amino acids enriched in branched-chain amino acids (BCAAs). However new pharmacological strategies seem to be very promising such as the new small SIRT1 activators (SRT1720, SRT2183, SRT1460), other sirtuin activators such as oxazolo[4,4-b]pyridine and imidazol[1,2-b]thiazole derivatives, the small GSK-3 inhibitors SB216763, and ZLN005 (with unknown action mechanism) or eNOS activators such as the AVE compounds. It is worth highlighting the latest evidence that points out low concentrations of free radicals as promoters of mitochondrial biogenesis and lifespan extension.

These discoveries are closely associated with the new concept of mitochondrial hormesis or mitohormesis. Hormesis is the term that defines a positive action in response to a mild stress that would be detrimental for the cell or the organism if it would be administered at higher intensities or concentrations [13]. There are many examples of evolutionary conserved processes in which the exposure of a cell or an organism to a low dose of one stressor triggers an adaptive response that protects the cell or the organism from a moderate or severe level of stress. Indeed, free radicals, classically considered as deleterious, have been shown to act as second messengers at low concentrations to trigger different signaling pathways. Several terms have been used by the scientific community such as autoprotection, heteroprotection, preconditioning, adaptive responses, compensatory mechanisms, hormesis, xenohormesis, etc. In the same way, a wide range of terms have been used to describe the shape of the dose-response curve obtained at low concentrations such as diphasic, biphasic, bitonic, bell-shaped, U-shaped, inverted-U-shaped, etc. [14]. Although the information about this issue is diluted by the different terminology, these kinds of phenomena have been widely observed. Considering specific mitochondria, it has been observed that a modest production of free radicals by this organelle can act as second messengers to trigger mitochondriogenesis [15]. Mitohormesis is therefore the beneficial effect produced in the cell due to the moderate production of free radicals by the mitochondria and is closely related to the phenomena of mitochondrial biogenesis and lifespan extension.

This special issue tries to cover most of the current knowledge about the pharmacological approaches to trigger mitochondriogenesis, the signaling pathways involved, their regulation and the implication of mitochondriogenesis on several diseases. However, this field is still in its infancy. More research needs to be done on mitochondrial biogenesis not only with the goal of healing certain pathologies but also to find, if not the legendary Fountain of Youth, maybe an approach to reduce suffering and morbidity at advanced ages.

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