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Review

Emerging concepts in the therapy of mitochondrial disease

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ABSTRACT

Mitochondrial disorders are an important group of genetic conditions characterized by impaired oxidative phosphorylation. Mitochondrial disorders come with an impressive variability of symptoms, organ involvement, and clinical course, which considerably impact the quality of life and quite often shorten the lifespan expectancy. Although the last 20 years have witnessed an exponential increase in understanding the genetic and biochemical mechanisms leading to disease, this has not resulted in the development of effective therapeutic approaches, amenable of improving clinical course and outcome of these conditions to any significant extent. Therapeutic options for mitochondrial diseases still remain focused on supportive interventions aimed at relieving complications. However, new therapeutic strategies have recently been emerging, some of which have shown potential efficacy at the pre-clinical level. This review will present the state of the art on experimental therapy for mitochondrial disorders.

1. Introduction

1.1. Basic concepts of mitochondrial biology and medicine

Mitochondria are semi-autonomousdouble-membrane organelles, the inner membrane being folded to form mitochondrial cristae, where respiratory chain (RC) complexes reside.

The main role of mitochondria is to extract energy from nutrients through respiration, and convert it into heat, or store it as ATP, the energy currency of cells. This is ultimately carried out by the respiratory chain (RC), through a process termed oxidative phosphorylation (OXPHOS). Respiration is performed by four multiheteromeric RC complexes, CI–IV, that transfer the electrons stripped off from nutrient-derived substrates as hydrogen atoms, to molecular oxygen. Electrons are conveyed to the RC through redox shuttle moieties, NADH + H^+ for complex I, $FADH_2$ for complex II. This electron flow is coupled with the translocation of protons across the inner mitochondrial membrane from the matrix to the intermembrane space, operated by complexes I, III and IV, generating an electrochemical gradient which is then exploited by RC complex V (or ATP synthase) to carry out the condensation of ADP and Pi into ATP [1].

Mitochondria have their own DNA (mtDNA), a maternally inherited, double-stranded circular molecule of 16.5 kb in mammals, encoding 13 subunits of the RC complexes I, III, IV and V (complex II is composed of four nucleus-encoded subunit with no contribution from mtDNA). In addition, mtDNA contains 22 tRNAs, and 2 rRNA genes, which form the RNA apparatus serving the in situ translation of the 13 mtDNA-encoded respiratory chain subunits. MtDNA is present in hundreds to thousands of copies in the different cell types in an individual. In normal individuals, mtDNAs are all identical to each other, a condition termed homoplasmy. However, pathogenic mtDNA mutations are frequently co-existing in variable amount with wild-type mtDNA molecules, a condition termed heteroplasmy. The rest of the mitochondrial proteome, which is estimated to consist of approximately 1500 polypeptides, is encoded by nuclear genes, translated in the cytosol into proteins, which are eventually targeted to and imported into the organelles by an active process.

Complex I (NADH-ubiquinone oxidoreductase) contains seven mtDNA-encoded subunits (ND1–ND6 and ND4L) and at least 37 nucleus-encoded subunits of complex I; electrons are transferred from NADH, the main redox shuttle of pyruvate dehydrogenase and TCA cycle, onto a hydrophobic mobile electron carrier, ubiquinone (coenzyme Q, CoQ). Complex II (succinate-ubiquinone oxidoreductase) is composed of only four subunits, all encoded by the nuclear genome and transfers electrons from $FADH_2$, mainly derived from beta-oxidation of fatty acids, to CoQ. Complex III (ubiquinol-ferricytochrome c oxidoreductase) has a single mtDNA-encoded subunit, apocytochrome b, and 10 subunits encoded by the nuclear genome. Complex III transfers electrons from CoQ to another electron shuttle, cytochrome c, which in turn transfers them to complex IV. Complex IV (cytochrome c oxidase, COX), which is composed of three mtDNA-encoded and 11 nucleus-encodedsubunits,

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transfers electrons to molecular oxygen, with the formation of water. Complex V (oligomycin-sensitive ATP synthase), which utilizes the energy potential of the electrochemical gradient to carry out ATP synthesis, is composed of two mtDNA-encoded subunits (ATPase 6 and 8), and at least 13 nuclear DNA-encoded subunits. These subunits are arranged to form two distinct particles. The membrane-embedded F0 particle constitutes a rotor operated by protons flowing through it. The rotation of this structure is transmitted to the matrix-protruding F1 particle, which catalyzes the biosynthesis of ATP [2].

Numerous specific assembly factors and chaperons are needed to assemble the protein backbone, insert suitable prosthetic groups and metal-containing reactive centers and form each holocomplex [3].

Other components of the mitochondrial proteome are required for a huge array of biological processes, including replication, transcription, and translation of the mtDNA, formation and assembly of the respiratory chain complexes, fission–fusion of the mitochondrial network, signaling and execution pathways (e.g. ROS production and apoptosis), scavenging of toxic compounds, and many other metabolic processes, as diverse as fatty acid oxidation, biosynthesis of pyrimidines, heme, and Fe–S clusters, etc.

From a genetic standpoint, primary mitochondrial diseases can be classified into two major categories, depending on which genome, mitochondrial or nuclear, carries the responsible mutations. MtDNA mutations include point mutations, either homo- or heteroplasmic, and (invariably heteroplasmic) large-scale rearrangements. Heteroplasmic point mutations have been found in all mitochondrial genes, and lead to different clinical phenotypes, including some canonical syndromes such as mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) [4], myoclonic epilepsy with ragged red fibers (MERRF) [5], neurogenic weakness, ataxia and retinitis pigmentosa (NARP) [6], and Leigh syndrome (LS). The main disease entity associated with homoplasmic mtDNA mutations is Leber's hereditary optic neuropathy (LHON) [7]. Rearrangements (single deletions or duplications) of mtDNA are responsible for sporadic progressive external ophthalmoplegia (PEO) [8], Kearns–Sayre syndrome (KSS) [8], and Pearson's syndrome [9].

Nuclear mutations have been found in a huge number of genes directly or indirectly related to the respiratory chain, encoding, for instance, (i) proteins involved in mtDNA maintenance and/or replication machinery; (ii) structural subunits of the respiratory chain complexes; (iii) assembly factors of the respiratory complexes; (iv) components of the translation apparatus; and (v) proteins of the execution pathways, such as fission/fusion and apoptosis (see [10] for an exhaustive list).

Mitochondrial diseases are hallmarked by huge clinical, biochemical and genetic heterogeneity, which hampers the collection of homogeneous cohorts of patients to establish the efficacy of a treatment. For instance, clinical outcomes in primary coenzyme Qdeficiency span from encephalomyopathy, multisystem disease, cerebellar ataxia, isolated myopathy and nephrotic syndrome [11]. For unknown reasons only 20% of the patients respond to CoQ_{10}, the only available therapy [11]. Studies in cellular models suggest that the slow pharmacokinetics of CoQ_{10} can explain the different responses observed in humans, but more studies are needed to clarify this issue. Similarly, riboflavin is effective in some cases of mitochondrial disease due to mutations in genes encoding FMN- or FAD-dependent proteins such as NDUFV1 (the FMN binding subunit of complex I), AIFM1, ACAD9 [12,13], and SDHA (the FAD binding subunit of complex II). However, not all patients respond to riboflavin supplementation [14].

1.2. Experimental therapeutic strategies

Remarkable progress has been made in recent years on understanding both the fundamental pathogenic processes underlying mitochondrial disease, and the mechanisms of mitochondrial biogenesis and signaling. Based on this knowledge, sensible therapeutic strategies have recently been proposed to combat mitochondrial disorders, for which experimental evidence is accumulating in cellular and animal models. These can be broadly divided in "generalist" strategies, which could in principle be applied to a wide spectrum of different disease conditions, and "disease-tailored" strategies, applicable to a single disease (Table 1). The first group includes: (i) regulation/activation of mitochondrial biogenesis; (ii) regulation/activation of mitochondrial autophagy; (iii) inhibition of mitochondrial apoptosis; (iv) scavenging of toxic compounds; (v) bypass of electron transfer chain defects; and (vi) nuclear transfer. The second group includes (i) scavenging of specific toxic compounds in specific diseases, (ii) supplementation of nucleotides, and (iii) gene- and cell-replacement therapies. Each of these strategies can be pursued by different approaches, such as pharmacological treatments, gene transfer to express the missing or a therapeutic protein, stem-cell/organ transplantation. This review will focus on emerging experimental (i.e. pre-clinical) therapies for mitochondrial disease. Ongoing clinical trials have recently been reviewed elsewhere [15].

2. Pharmacological and metabolic interventions

2.1. Increasing mitochondrial biogenesis

Mitochondrial diseases are hallmarked by bioenergetics defects, ultimately leading to decreased ATP synthesis. Thus, therapeutic interventions aimed at increasing the ATP levels available to cells may be beneficial. Importantly, mitochondrial disease become manifest when the residual activity of the defective gene product, either mitochondrial or nuclear encoded, falls below a critical threshold, suggesting that even partial restoration of the activity may be sufficient to rescue or at least ameliorate the phenotype. The idea that mitochondrial biogenesis is critical to determine the phenotypic outcome of disease has been boosted by the recent observation that increased mitochondrial content protects non-manifesting carriers of the LHON mutations. This can partly explain the incomplete penetrance of the disease and opens the possibility to stimulate mitochondrial biogenesis as a therapeutic strategy for LHON [16].

Increased mitochondrial biogenesis is a physiological response to stress conditions (e.g.: cold, exercise, nutritional status), which is activated to meet the energetic requirements of tissues [17].

The pathways controlling mitochondrial biogenesis (Fig. 1) have mainly been investigated in skeletal muscle and brown adipose tissue, and shown to rely, in most of the cases, on the peroxisome proliferator-activated receptor gamma (PPARγ) coactivators 1α and β (the PGC family). PGC proteins interact with and activate

Table 1
Summary of the experimental therapies for mitochondrial diseases.

	Strategy	Method
Generalist	• Activation of mitochondrial biogenesis • Modulation of autophagy • Inhibition of apoptosis • Scavenging of ROS • Endurance training • Dietary manipulation	Pharmacology
	• By-passing RC block • ZFNs or TALENs to shift heteroplasmy • Overexpressing aaRSs to stabilize mutated mt-tRNA • Somatic nuclear transfer	AAV-mediated gene therapy
Disease-tailored	• Scavenging of toxic compounds • Supplementation of nucleotides	Pharmacology
	• Replacement of the missing gene	AAV-mediated gene therapy