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Review

Mitochondrial Diseases Part III: Therapeutic interventions in mouse models of OXPHOS deficiencies

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ABSTRACT

Mitochondrial defects are the cause of numerous disorders affecting the oxidative phosphorylation system (OXPHOS) in humans leading predominantly to neurological and muscular degeneration. The molecular origin, manifestations, and progression of mitochondrial diseases have a broad spectrum, which makes very challenging to find a globally effective therapy. The study of the molecular mechanisms underlying the mitochondrial dysfunction indicates that there is a wide range of pathways, enzymes and molecules that can be potentially targeted for therapeutic purposes. Therefore, focusing on the pathology of the disease is essential to design new treatments. In this review, we will summarize and discuss the different therapeutic interventions tested in some mouse models of mitochondrial diseases emphasizing the molecular mechanisms of action and their potential applications.

Contents

1. Introduction

Mitochondrial diseases include a wide group of human disorders affecting the oxidative phosphorylation system (OXPHOS). Common OXPHOS alterations in mitochondrial disorders are associated with

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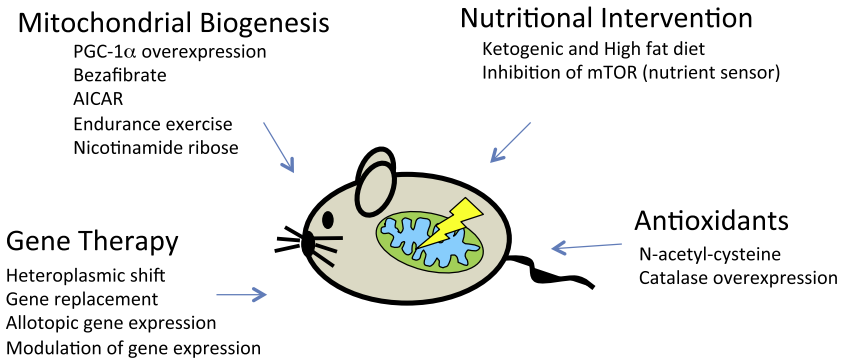

Fig. 1. General therapeutic approaches tested in mouse models of mitochondrial diseases.

mutations in multiple genes encoded by either the mitochondrial (mtDNA) or the nuclear (nDNA) genome (Schon et al., 2012; Wallace et al., 2010). Mitochondrial diseases are no longer considered "orphan diseases". Epidemiological studies predict 1 in 5000 children to be affected by them (Schaefer et al., 2004). During the last decade, many compounds have been tested to ameliorate the symptoms or delay these devastating disorders. However with a few remarkable exceptions (Hirano et al., 2012), to date no effective treatments are available to cure mitochondrial diseases. The efforts of the scientific community to find cures include different approaches, such as preventing the disease transmission from mother to child, gene therapy, exercise training, correction of metabolic alterations, special diets and antioxidant treatments [for review of current treatments in humans see (Pfeffer et al., 2013; Schon et al., 2010)]. Reliable clinical trials are hampered by the inability to collect large study groups due to the extremely heterogeneous nature of mitochondrial diseases (Pfeffer et al., 2013). For this reason, a “personalized medicine” is considered nowadays a prospect for treatment. In this chapter (Part III of review miniseries) we discuss the different therapeutic strategies that have been tested in some of the mouse models described in Parts I and II, highlighting principles, limitations and potential applications of the tested interventions. We have grouped the different treatments according to their mechanism of action in the following categories: (i) heteroplasmic shift, (ii) replacement of defective genes, (iii) activation of mitochondrial biogenesis, (iv) nutritional intervention, and (v) other alternative treatments (Fig. 1).

2. Therapeutic interventions tested in mitochondria deficient mouse models

2.1. Heteroplasmic shift

Different copy numbers of wild type (wt) and mutated mtDNA can coexist in the mitochondria without being detrimental. The ratio of the levels of the two molecules defines the heteroplasmy level of the mutation. Mitochondrial DNA mutations must reach a certain level to exert their biochemical, cellular and clinical phenotype (threshold effect). Hence, a reasonable therapeutic approach to prevent mitochondrial dysfunction is based on the reduction of the mtDNA mutant load. Since the pathological threshold levels of heteroplasmy tend to be very high, a small reduction in the % of heteroplasmy is expected to be beneficial (Thorburn and Dahl, 2001; Zeviani and Di Donato, 2004). One of the approaches to change the heteroplasmic levels involves the use of restriction endonucleases that will recognize specific restriction sites only present in the mutant mtDNA. The restriction endonuclease can be expressed and targeted to the mitochondria to digest the unwanted population of mtDNA. To test the feasibility of this approach, Moraes' group took advantage of an existing mouse model carrying two different non pathogenic haplotypes of murine mtDNA, NZB and BALB (Jenuth et al., 1997). They used ApaLI (Mito-ApaLI) that recognizes a site only in BALB mtDNA causing a shift towards the NZB haplotype (Bayona-Bafaluy et al., 2005). A viral transduction of Mito-ApaLI in skeletal muscle and brain of NZB/BALB mice by local injection produced a rapid heteroplasmic shift in both tissues (Bayona-Bafaluy et al., 2005). The same approach has given promising results when delivered systemically to heart and liver tissue using adeno-associated and adenovirus in the NZB/BALB mice (Bacman et al., 2010). The heteroplasmic shift towards NZB mtDNA observed in these tissues was not followed by depletion or deletion of mtDNA, most likely because the NZB replicated faster avoiding mtDNA depletion caused by the rapid digestion of the BALB genome (Bacman et al., 2007). Systemic delivery of Mito-ApaLI in newborn NZB/BALB mice decreased the NZB haplotype specifically in skeletal muscle and heart. The expression of the transgene was stable over time and no collateral effects were observed (Bacman et al., 2012). However, the success of this type of approach in patients is somehow limited because it requires the presence of a unique restriction site in the mutant mtDNA to avoid mtDNA depletion, which is a potential problem that has to be taken in consideration when using this innovative strategy.

A refinement of this approach involves the use of re-engineered transcription activator-like effector nucleases (TALENs) targeted to the mitochondria (mito-TALEN) (Bacman et al., 2013). A heteroplasmy shift towards wild type mtDNA was detected in cells carrying the common deletion and in cybrids carrying the Leber's hereditary optic neuropathy (LHON) point mutation 14459G>A in the *ND6* gene using the mito-TALENs approach. Since not all the pathogenic mutations in the mtDNA described in humans give rise to sites recognized by restriction endonucleases, the use of mito-TALENs opens the possibility for a more effective custom design intervention. However, mito-TALENs technology remains to be tested *in vivo* in animal models.

A different strategy to enrich wild type mtDNA content in heteroplasmic cells has been achieved by using ketone bodies in culture media deprived of glucose (Santra et al., 2004). The ketone bodies mimic the ability of galactose-containing media to shift heteroplasmy levels due to the inability of the deficient cells to metabolize this carbohydrate. The selective pressure forces the cells to rely on OXPHOS and not on glycolysis to produce ATP (Santra et al., 2004). Based on this principle, the effect of a ketogenic diet has been tested *in vivo* using the Deletor mouse, which carries a dominant mutation in the *Twinkle*-helicase that leads to accumulation of multiple mtDNA deletions during aging causing a late-onset progressive respiratory chain deficiency (Tyynismaa et al., 2005). Deletor mouse fed with a ketogenic diet showed an increase in the respiratory chain activity and a general improvement on the course of the disease. Unlike what happens in cell culture, no heteroplasmic shift was detected in the Deletor mouse, as the percentage of deleted mtDNA molecules remained unchanged (Ahola-Erkkilä et al., 2010). Instead, the ketogenic diet induced mitochondrial biogenesis, diminished the amount of COX (cytochrome *c* oxidase) negative fibers in muscle and restored the metabolic and lipidomic changes observed in the Deletor mice to wild type levels. The rationale of using