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Spotlight on vision

Gene therapy for mitochondrial diseases: Leber Hereditary Optic Neuropathy as the first candidate for a clinical trial

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ABSTRACT

Mitochondrial disorders cannot be ignored anymore in most medical disciplines; indeed their minimum estimated prevalence is superior to 1 in 5000 births. Despite the progress made in the last 25 years on the identification of gene mutations causing mitochondrial pathologies, only slow progress was made towards their effective treatments. Ocular involvement is a frequent feature in mitochondrial diseases and corresponds to severe and irreversible visual handicap due to retinal neuron loss and optic atrophy. Interestingly, three clinical trials for Leber Congenital Amaurosis due to *RPE65* mutations are ongoing since 2007. Overall, the feasibility and safety of ocular Adeno-Associated Virus delivery in adult and younger patients and consistent visual function improvements have been demonstrated. The success of gene-replacement therapy for *RPE65* opens the way for the development of similar approaches for a broad range of eye disorders, including those with mitochondrial etiology such as Leber Hereditary Optic Neuropathy (LHON).

Mots clés :
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NOHL

RÉSUMÉ

Les dysfonctions mitochondriales, avec une prévalence d'une naissance sur 5000, occupent une place incontournable dans de nombreux domaines de la pathologie humaine. Malgré les progrès réalisés depuis 25 ans, beaucoup reste à faire pour traiter ces affections. Des handicaps visuels sévères liés à des dysfonctions mitochondriales sont fréquents et sont dus à la perte des neurones rétiniens et à l'atrophie du nerf optique. Depuis 2007, trois essais cliniques de thérapie génique ont été développés dans le cadre de l'amaurose congénitale de Leber due à des mutations du gène *RPE65*. Ainsi, les essais qui impliquent tous des vecteurs de type adéno-associé s'avèrent, à ce jour, très prometteurs, tant sur le plan de la tolérance que sur l'efficacité chez des patients adultes et jeunes. Ces premiers succès ouvrent la voie au développement d'approches similaires pour d'autres pathologies oculaires, y compris celles présentant une étiologie mitochondriale comme la neuropathie optique héréditaire de Leber (NOHL).

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1. Introduction

Mitochondrial disorders initially considered as being uncommon, now appear as relatively frequent, although often unrecognized because symptoms are extremely variable and usually insidious at the onset. Mitochondrial impairment is observed in many widespread cardiovascular, skeletal muscle, neurological disease states [1] and age-related degenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD) [2,3]. The most severe inherited mitochondrial disorders become clinically apparent during infancy, and many of them are lethal. Additionally, mitochondrial syndromes in which symptoms do not appear until early adulthood have also been described and are characterized by striking variability in severity and symptom patterns [4,5].

Given this perplexing complexity and despite the huge advances in the understanding of molecular and biochemical bases underlying organelle dysfunction, our ability to counteract mitochondrial pathologies remains very limited [6]. Accordingly, classical interventions with vitamins or co-factors are only marginally beneficial [7], while organ transplantation is limited to the few cases in which the phenotype is dominated by an isolated organ failure [8]. For these reasons, alternative strategies, such as gene therapy, aimed at correcting disturbed energy metabolism are under development in an array of laboratories worldwide. However, a main limitation to succeed in this challenge and then proceed to its transfer to clinic is the scarcity of reliable animal models required for evaluating safety and efficacy of gene therapy [9–11]. Besides, the availability of animal models is also important considering which tissue could be triggered by gene therapy for ensuring limited body dissemination and avoiding harmful side-effects. In this respect, the eye possesses many features that make it particularly suitable as a target organ for gene therapy; its compartmentalized anatomy enables local vector delivery with low systemic dissemination and is accessible for *in vivo* assessment by optical imaging and electrophysiological techniques [12]. Up to date, 192 genes and 232 loci have been associated with inherited photoreceptor degenerations (http://www.sph.uth.tmc.edu/RetNet/). The function of their products are diverse, including: phototransduction, RNA splicing factors, intracellular trafficking molecules and cytoskeletal proteins, phagocytosis, intracellular pH regulation, and energy metabolism [13]. Accordingly, mitochondria being central to retinal cell function and survival, there is increasing evidence to support an association between mitochondrial dysfunction and a number of retinal pathologies, including age-related macular degeneration (AMD), diabetic retinopathy and glaucoma [14,15], [16,17]. In addition, inherited optic neuropathies are largely due to mitochondrial impairment, either by mutations in the organelle genome or in nuclear genes encoding mitochondrial proteins [18,19]. This review focuses on:

- mitochondrial genetics and function;
- how mitochondrial dysfunction can contribute to retinal cell death and vision loss;
- our latest developments aimed at providing a protocol safe and efficient for Leber Hereditary Optic Neuropathy (LHON), which will eventually be translated to clinical research.

2. Mitochondrial genetics and functions

Mitochondria are cytosolic organelles whose primary function is the use of oxygen to generate the energy required for cellular growth, function, and maintenance. Accordingly, tissues with high energy requirements such as brain, retina, heart, skeletal muscle, liver and endocrine systems are frequently affected in mitochondrial diseases. In mammalian cells, mitochondrial biogenesis and function require ~1500 proteins [20,21], whose encoding genes are located in either the maternally inherited mitochondrial DNA (mtDNA) or the nuclear DNA (nDNA).

2.1. Genetics

The mtDNA is a double-stranded, closed-circular molecule of 16,569 base pairs (bp) in humans [22]. In addition to the 13 polypeptide genes all of which are integral components of the respiratory chain complexes, mtDNA encodes 22 tRNAs, 12S and 16S rRNAs, that are necessary for intramitochondrial protein synthesis [23,24]. To understand mitochondrial inheritance and clinical expression of disease we should realize that mammalian cells contain approximately 10^3–10^5 mtDNA copies/cell. Mutations in an individual cell can affect all mtDNA molecules (homoplasmy), or part of them (heteroplasmy). When wild-type mtDNA copies drop below a critical level, mitochondrial function is impaired, since sustaining normal cellular functions is no longer possible. Thus, as functional mitochondria become insufficient for tissue to function, a threshold is crossed, cell death possibly initiated and disease phenotype becomes visible [25]. This "threshold effect" is tissue-specific, thus, tissues which rely heavily on adenosine triphosphate (ATP) like the central nervous system (CNS), the optic nerve, retinal pigment epithelium (RPE), and photoreceptors (PRs) may have lower thresholds for mutant mtDNA than less metabolically active tissues [26]. A higher level of complexity related to mitochondrial genetics is that mtDNA mutations accumulated throughout human history leading to the classification of human populations into a small number of mitochondrial haplogroups. A mitochondrial haplogroup is defined as a collection of groups characterized by specific Single Nucleotide Polymorphisms [7]. Several studies have shown mitochondrial haplogroups to be associated with differences in the amount of superoxide and other ROS produced by the electron transport chain [27–29]. For example, haplogroup F mtDNAs are associated with low complex I activity [30] and predilection to diabetes [31] and individuals with haplogroup J present lower oxygen consumption than other haplogroup variants [32]. Thus, specific haplogroups may constitute either a risk or a protective factor in the origin of age-related diseases such as PD, AD [28], ischemic cardiomyopathy [33,34] and cancers [35]. Obviously, this