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Review Article

Mitochondrial Therapy for Disease Treatment and Prevention: What's New?

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Abstract

Many diseases caused by mitochondrial dysfunction are caused by mitochondrial DNA abnormalities. The concept of mitochondrial therapy is based on the repair or removal of the existing anomaly. This can be done by replacing affected mitochondria by healthy ones, by in situ correction of the damaged mitochondrial DNA or by selective destruction of the affected mitochondria. In theory, all these concepts are applicable at the preconception stage, thus preventing the mother-to-child disease transmission, as well as at more advanced developmental stages (embryonic, fetal and postnatal) to alleviate disease symptoms in specific organs and tissues. Animal reasearch has paved the way to human therapies at all of these levels. So far, the only successful human application concerned the preconception stage and was based on replacement of diseased mitochondria in patients' oocytes with healthy ones originating from oocytes donated by healthy women. However, new developments in molecular biology techniques, enabling a direct reparation of mitochondrial DNA mutations or selective destruction of the affected mitocondria, will hopefully make it possible to treat mitochondrial diseases at both preconception and postconception stages, without the need for recourse to third-party (donor) biological material.

Keywords: Mitochondrial Therapy; Mitochondrial Diseases; Mitochodrial DNA; Mitochondrial Donation; MitoTALENs; Zinc Figure Nucleases; CRISPR; Disease Prevention and Treatment; Stem Cells, Regenerative Medicine

Abbrevations: CRISPR: Clustered Regularly Interspersed Short Palindromic Repeats; Mito TALENs: Mitochondrial-targeted transcription activator-like Effector Nucleases; mt DNA: Mitochondrial DNA; NOS: Nitric Oxide Synthase; SCs: Stem Cells: ZFNs: Zinc Figure Nucleases

Introduction

Mitochondria are ubiquitous cell organelles that are responsible for generating energy needed for a variety of cell functions, but they are also involved in multiple other cellular processes including calcium homeostasis, celular signaling and apoptosis [1]. Phylogenetically, mitochondria in eukaryotic cells have evolved from procaryote, bacteria-like cells, by creating a kind of symbiosis. During this evolution, a dual genetic control of mitochondrial function has been established, the optimal function resulting from a cooperation of proteins coded by mitochondrial own DNA and those coded by DNA of the hosting eukaryotic cell [1].

If the function of mitochondria is disturbed, the resulting dysfunction of cell energy generation, calcium homeostasis and intracellular signalling events, including those responsible for the removal of irreparably damaged and pathologically transformed cells, leads to the development of a number of different pathological conditions [2]. In addition to several tens of known mitochondrial diseases, related to mostly maternally inherited mutations in the human mitochondrial genome, it is now suspected that de novo mutations in mitochondrial DNA (mtDNA) are also linked to other complex traits, including neurodegenerative diseases, aging and cancer [2].

Consequences of mitochondrial dysfunction are mainly observed in those types of cells that are actively involved in energy-consuming and cell signalling-dependent processes, such as cell division, cell restructuring, cell movement or secretory functions. Thus, clinical consequences of defective mitochondrial function are mainly observed in cells and tissues with high demand on energy, such as muscle, nerve, liver or heart cells, but also, temporarily, in those cells whose demand on energy and precise cell signalling is temporarily increased by an ongoing biologically important process, such as oocytes engaged in the final phase of meiotic maturation.

In fact, mt DNA is a fossil molecule proving that endosymbiosis did occur, when – about 1.5 billion years ago – protobacteria populated primordial eukaryotic cells and took permanent residence in the new environment [1]. Unlike a fossil, however, mtDNA has lost its independence but not its life and it keeps

functioning, and sometimes malfunctioning causing a disease [2]. The human mtDNA is formed by a tiny 16.6 kb circle containing 37 genes, 13 of which encode proteins and the remaining 24 are used for translation of those 13 polypeptides [3]. It is a double-stranded supercoiled ring molecule which is not associated with histones and, unlike nuclear chromatin, is not packaged in the form of nucleosomes [4]. Consequently, mtDNA is less protected against de novo point mutation as compared with nuclear DNA, and the physiological DNA repair mechanisms, based on homologous recombination and non-homologous end joining, appear to be less effective in mitochondria as compared with somatic cell nuclei [5].

Together with the active mitochondrial metabolism, creating reactive oxygen species susceptible to cause DNA damage, the risk of de novo mtDNA mutations during cell life is high. In fact, the mitochondrial genome has a high mutation rate, 10-17 times that of the nuclear genome [6]. The pathological consequences of this situation are mitigated by the co-existence of multiple mtDNA copies in each mitochondrion [3]. Accordingly, most de novo mutations of mtDNA lead to a situation referred to as heteroplasmy (coexistence of mitochondria with different DNA composition in the same cell [4]. When a certain level of heteroplasmy, estimated to be in the range of 60-95% mutated mtDNA depending on the severity of the mutation [7], a disease state may ensue [8,9]. New data suggest different possible approaches to both the prevention of mtDNA disease transmission and the treatment of existing pathologies in affected individuals.

About 15% of "mitochondrial diseases" are caused by pathologies caused by specific deletions or mutations of genes present in mtDNA whereas the rest is related to eukaryotic nuclear genes regulating mitochondrial function. In humans, mutations in mtDNA, are involved in a number of inherited or acquired diseases [4,7] whose list is still increasing.

The Real Prevalence and Types of mtDNA Diseases

Mitochondrial disease has long been considered one of the most common groups of genetic diseases, with a minimum prevalence of greater than 1 in 5000 in adults [10]. However, these data are based on the prevalence of known, genetically transmitted mitochondrial syndromes. If we take into account all cases of degenerative diseases involving acquired mtDNA mutations, the real prevalence would be substantially higher. In fact, acquired mtDNA mutations participate, to a greater or lesses extent, in the pathophysiology of a number of late-onset neurogenerative diseases, such as Alzheimer's, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis and hereditary spastic paraplegias [2,11], to cite only some examples.

Mitochondrial function is also altered, in numerous ways, in tumors, allowing them to survive by circumventing apoptotic cell-death pathways [12]. Other cancer-promoting pathways, though not related directly to mtDNA mutations, involve mitochondrial function indirectly. For example, nitric oxide (NO), which is synthesized by nitric oxide synthase (NOS), is closely related to carcinogenesis and progression of colon cancer [13]. The inhibition of NOS1 translocation to mitochondria was shown to reverse NO-induced apoptosis resistance of colon cancer cells [14]. According to these and other observations, delivery of anticancer drugs to mitochondria has been suggested to provide a new opportunity to overcome multidrug resistance, the main obstacle limiting the success of anticancer chemotherapy [14-16].

Based on the above data, future research into practical application of mitochondrial therapy in humans is likely to go ahead in two closely related directions: (1) prevention of the transmission of pre-existing maternally inherited mtDNA diseases to offspring, and (2) treatment of pathologies acquired during life by targeting mitochondria as part of more complex therapeutical strategies.

Prevention of mtDNA Disease Transmission to Progeny

Mitochondrial diseases due to mtDNA mutations are almost exclusively transmitted from mother to child, since paternal mitochondria have a limited life span and are actively destroyed by the egg's cytoplasmic destruction machinery short after fertilization [17]. Nonetheless, some mitochondrial functional abnormalities are caused by abnormal expression of nuclear genes controlling mitochondrial function, and these conditions can be transmitted both by the mother and the father. Moreover, in rare cases the father's mtDNA can escape the programmed destruction and contribute to the child's mitochondrial genome, too [18,19].

The transmission of mtDNA mutations from mother to child can be prevented by different means. Some of them, including the injection of healthy mitochochondria-containing donor oocyte cytoplasm into patients' oocytes [20] and the transfer of the metaphase chromosome-meiotic spindle complex from the patients' oocytes into previously enucleated donor oocytes [21], have already resulted in the birth of healthy children. Another method, using pronuclear transfer from diseased zygotes into enucleated healthy donor zygotes, is currently under investigation [22].

However, current progress in molecular biology techniques is likely to make it possible to avoid the mother-to-child transmission of mtDNA disease without the need for the recourse to a third party (oocyte donor). This possibility is particularly important for the application of germline mitochondrial treatment in those countries in which the use of donated oocytes is prohibited (mainly islamic countries) or in those, including most of the European Union countries and the United States, where a deep misunderstanding of the mechanisms governing the role of mitochondria in embryonic and adult cells [23] has led to illegalization of mitochondrial transfer therapies. These new alternatives will enable a direct treatment of diseased maternal mitochondria within the mother's oocytes.

One possibility is based on the Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) technology, initially developed for nuclear gene editing (cutting the mutated genes and pasting their healthy counterparts in their place) but adapted to be used for mtDNA editing [24]. However, the CRISPR-based technologies have aroused some concern as to their safety with regard to the suspected potential of the CRISPR system to produce collateral side-effects, also including nuclear DNA [25]. Alternative treatments, avoiding

these collateral risk factors, have emerged recently. It was shown that mitochondrial-targeted transcription activator-like effector nucleases (mitoTALENs) can correct mtDNA mutations in cultured human cells from patients with a mtDNA disease [26,27] and correct induced mtDNA mutation in vivo in the mouse model [28]. The use of mitochondrially targeted zinc-figure nucleases (mtZNFs) [29] is another tool for specific removal of mtDNA mutations with a relatively low risk for interaction with the cell's nuclear DNA. This technique has been shown recently to remove a pathogenic mtDNA mutation in an in vivo mouse experiment [30].

It is well-known that almost all cells of adult organisms are heteroplasmic for mtDNA, due to age-related acquired mtDNA mutations, and that the degree of mutation load is decisive for the onset of clinical symptoms, a phenomenon known as the threshold effect [22]. What is new in this field is that the clinical symptomatology can be alleviated by shifting the existing equilibrium between healthy and DNA-mutated mitochondria in different ways [31]. In fact, unlike the CRISPR and mitoTALENs technologies, used to correct mtDNA mutations, thus shifting the equilibrium in the positive sense, the mtZFNs techniques are acting in the opposite direction, destroying mtDNA mutation-bearing mitochondria. This action leads to the repopulation of cells with healthy mitochondria, thus leading to the same clinical effect as the above two methods. This strategy, based on the assumption that after selective destruction of the mutated mtDNA, healthy mtDNA will prevail [32], is particularly interesting for applications in human clinical medicine because it avoids the use of gene-editing techniques which might erroneously disrupt the function of normal genes of the treated cells.

Mitochondrial Therapy to Improve Outcomes of Regenerative Medicine

Regenerative medicine is an interdisciplinary field that applies engineering and life science principles to enable regeneration of tissues and organs damaged through disease, injury or aging [33]. Regenerative medicine is increasingly using stem cells (SCs) obtained from the same patient, in order to treat different types of diseases, including degenerative disease of different organs, injury (occurring in young persons during sports activities or in older persons having suffered an accident), or age-related pathologies, such as Parkinson and Alzheimer disease or joint dieases. However, the outcomes of stem cell-based regenerative medicine is highly dependent on the age of the person from whom the cells have been obtained. The use of cells from young patients is more efficient as compared with those recovered from older patients [33].

To understand this issue, it is useful to recall what SCs really are. SCs are undifferentiated or partially differentiated cells that can, through changes in gene expression, alter their properties to adopt more specialized fates [33]. Accordingly, some SCs are pluripotent (capable of giving rise to different types of cells, but always from the same developmental line related to their origin) while others may be totipotent (able to differentiate into any cell type of the body, independent of the cell origin). In most of the current clinical applications in regenerative medicine, pluripotency of the SCs used is sufficient, whereas totipotency will be important for the development of therapies aimed at the formation of artificial oocytes and spermatozoa to be used for assisted reproduction.

Irrespectively of the clinical need for either totipotent or pluripotent SCs, there is one common denominator – the age of the patient of origin. Even though the use of donated SCs is possible, after excluding donor versus host incompatibilities, most clinics prefer the use of the patient's own cells to for somatic regenerative therapy. The main problem of this treatment strategy is the age-related decline of the quality of the SCs to be used, and this quality decline is mainly due to the accumulation of acquired mtDNA mutations during life [34,35]. In other words, the selection of naturally occurring SCs, or the induction of pluripotency or totipotency by artificial means, cannot erase the pre-existing mitochodria-related problems (associated or not with the patient's age) [36]. Consequently, SCs originating from older patients are prefential targets for future mitochondrial therapy (see above) to improve regenerative medicine outcomes.

Conclusion

Mitochondrial therapy, aimed at shifting the existing mitochondrial heteroplasmy in favor of mitochondria carrying healthy, non-mutated DNA, is currently a subject of intensive research which has led to the development of new molecular biology techniques that could either repair the pathological mtDNA mutations or selectively destroy the affected mitochondria, thus allowing the healthy ones to repopulate the cells. Further studies are needed to compare the efficiency and safety of these new techniques in different types of cells and clinical indications. In the case of SCs, especially those from older patients, mitochondrial therapy is expected to improve outcomes of different types of regenerative medicine interventions. As to oocytes and early embryos, mitochondrial therapy may be considered as an alternative to mitochondrial donation for prevention of mother-to-child transmission of hereditary mtDNA diseases, actually banned in many countries.

Conflict of Interest

There is no financial interest, nor any other conflict of interest associated with this article.

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