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A Critical Review of the Inheritance of Mitochondrial DNA in Humans

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ABSTRACT

The dogma of mitochondrial inheritance states that mitochondrial DNA (mtDNA) is inherited solely down the maternal line. Traditionally, the elimination of paternal mtDNA happens through various mechanisms. Elimination mechanisms such as autophagy degradation or endonuclease G (EndoG) degradation have been widely studied in model organisms, while ubiquitin-mediated elimination has been identified in mammals and humans. Despite the existence of several research papers that support maternal inheritance, a controversial 2018 research paper by Luo et al. counters this longstanding dogma and proposes biparental inheritance of mtDNA. Since its publication, multiple studies have tried to justify this transmission through nuclear-mitochondrial DNA (NUMTs) or nuclear mtDNA concatemers (mega-NUMTs). It has been established that the phenomenon of biparental inheritance is not frequent since maternal inheritance entirely dominates over paternal transmission in the evolutionary timescale. This review explores the research linked with mitochondrial elimination and mitochondrial transmission, showing that further research is required to thoroughly understand this area of genetics.

Introduction

Mitochondria are double-membrane organelles found in the cytoplasm of nearly all eukaryotic cells. Mitochondria are mainly responsible for the production of energy in the form of adenosine triphosphate through oxidative phosphorylation (Taanman, 1999). Unlike other eukaryotic organelles, mitochondria contain one or more copies of their own DNA (mtDNA). This DNA is housed in the matrix of the organelle. In contrast to nuclear DNA (nDNA), mtDNA is circular and thus packaged into structures called nucleoids. This occurs with the help of various proteins, most notably prohibitins and the mitochondrial transcription factor A. Due to this, the replication pathway of mtDNA is significantly different to that of nDNA (Yan et al., 2019).

Research dating back to as early as 1974 noticed that the mitochondrial genome is transmitted only maternally in various eukaryotic organisms (Hutchison et al., 1974). In other words, offspring display only the mtDNA of the mother, indicating uniparental transmission. The first instance in which this unique inheritance pattern was demonstrated in humans occurred in 1980 (Giles et al., 1980). Mitochondrial DNA from peripheral blood platelets was screened for nucleotide sequence polymorphisms (variant forms of specific DNA sequences). Giles et al. (1980) discovered that the cleavage patterns of restriction endonucleases differed significantly between the mothers and fathers of multiple independent families, and all offspring exhibited solely the maternal cleavage. Following this significant breakthrough, the central dogma of mitochondrial inheritance was established. The dogma states that mtDNA is transmitted exclusively through the maternal germ line, suggesting that paternal mtDNA is eliminated or discarded.

Mechanisms of elimination of paternal DNA

Eliminating Paternal DNA

The question of why paternal mtDNA is eliminated in humans remains unanswered. The degradation of sperm mitochondria, also referred to as sperm mitophagy, is thought to occur since certain components of sperm mitochondria may provide defects to the fertilized oocyte. For instance, one study hypothesizes that sperm mitochondria are destroyed because they contain the prohibitin protein. Prohibitin may interfere with the egg's entry into the S-phase of the cell cycle, during which DNA is replicated (Sutovsky et al., 2000). Moreover, some evidence suggests that paternal mitochondria may accumulate mutations from oxidative stress caused by free radicals. Such mutations result from the energy expenditure involved in fertilizing eggs and may cause mitochondrial diseases in developing embryos (Kujoth et al., 2005; Shokolenko et al., 2009).

Simple Dilution Model of Sperm Mitophagy

For many years, scientists have relied on the 'simple dilution model' to describe the elimination of paternal mitochondrial DNA. The number of mitochondria containing mtDNA is significantly smaller in sperm (50-75 copies) than in the egg (over 100,000) (Taanman, 1999). Due to this, it was thought that the father's mtDNA is diluted in the offspring due to the abundance of the mother's mtDNA (Gyllensten et al., 1991). However, in a recent paper, Pyle et al. (2015) failed to detect paternal mitochondrial haplotypes (group of alleles) in human offspring. The study identified parent-offspring trios with differing mtDNA of two or more variant differences. Employing extreme-depth mitochondrial DNA sequencing of the trios revealed that the variant mtDNA were not compatible with the haplotype of the father, thus disproving the 'simple dilution model' in humans. While the study fails to propose an alternative model, recent research has revealed that paternal mitochondria are actively degraded before or after fertilization (Carelli, 2015).

Active Elimination Models of Sperm Mitophagy

The mechanisms of active sperm mitochondria elimination have been widely studied in model organisms (Sato & Sato, 2017; Sato & Sato, 2013). For instance, it has been proven that in *Drosophila*, paternal mtDNA is actively degraded at the molecular level by endonuclease G (EndoG) during the process of sperm cell development. It was found that even in cases of EndoG mutations, mtDNA was removed into a waste compartment by a sequestration mechanism to prevent paternal transmission (DeLuca & O'Farrell, 2012). Meanwhile, studies on *C. elegans* showed that the clearance of the paternal mitochondria is mediated by autophagy through a lysosome-dependent regulated mechanism, yet EndoG may also play a role in the degradation (Sato & Sato, 2011; Zhou et al., 2016).

A study published in 1999 proposed that mitochondria in mammalian sperm cells may be selectively tagged by the ubiquitin protein (Sutovsky et al., 1999). This tagging, also referred to as ubiquitination, plays a crucial role in substrate degradation to ensure cell homeostasis. However, the specific mitochondrial substrates that undergo this process were not identified. It was hypothesized that ubiquitination may occur as early as the beginning of sperm cell development. Consequently, this would sentence the sperm mitochondria to death by the degradation machinery of the oocyte after fertilization. It was speculated that lysosomal and proteasomal proteolysis also contribute to this degradation.

Moreover, recent developments provided more insight into the shortcomings of the 1999 research paper. For instance, Sutovsky et al. (2000) conducted a study on bovine cells and recognized prohibitin, a protein of the inner mitochondrial membrane, as one of the possible substrates of ubiquitination. By using monospecific antibodies, Sutovsky et al. (2000) visualised the binding of ubiquitin to sperm mitochondria during the early stages of embryonic development. A different research paper strongly hypothesizes the involvement of other substrates in ubiquitination, most

notably disulfide-bond cross-linked proteins in sperm mitochondrial membranes (Thompson et al. 2003). Thompson et al. (2003) theorize that this may be due to the bonds' ability to conceal prohibitin in spermatozoa, subsequently allowing it to serve as a signal for protein breakdown after fertilization. Moreover, Song et al. (2016) identified mitophagy systems such as the ubiquitin-binding protein dislocase or the valosin-containing protein that may work alongside the ubiquitin-proteasome degradation pathway in pigs and monkeys. The researchers proved that the inhibition of different receptors in the mitophagy pathways significantly delayed the degradation of sperm mitochondria after fertilization. Thus, they concluded that sperm mitophagy relies on the combined action of varying degradation systems in the oocyte.

Despite there being no such research on humans due to ethical reasons, it can be inferred that the above-described pathways and models also play a role in the elimination of paternal mtDNA in human sperm.

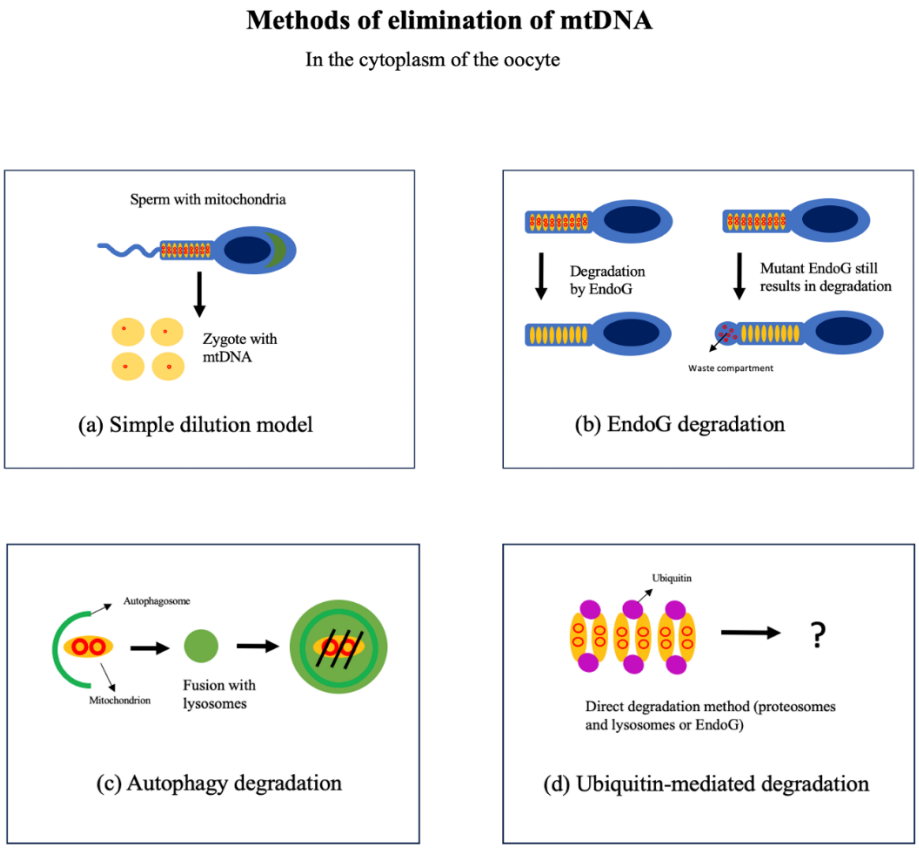

Figure 1. Mechanisms of paternal mitochondrial DNA degradation. (a) Although it is not supported by current experimental evidence, in the simple dilution model paternal mtDNA remains and is diluted in the zygote after fertilization. (b) In *Drosophila*, paternal mtDNA is degraded by EndoG during sperm cell development. In EndoG mutants, mtDNA is sequestered in waste compartments. (c) In *C. elegans*, paternal mtDNA is engulfed by autophagosomes and subsequently degraded by lysosomes. (d) In mammals, paternal mtDNA is selectively tagged with ubiquitin during sperm cell development. It is directly degraded after fertilization, presumably by proteasomes and lysosomes or EndoG.

Challenging the Dogma: Paternal Inheritance of mtDNA

As proven by prior research, mitochondrial DNA is thought to be inherited exclusively down the maternal line. However, a paper in the 2018 issue of the Proceedings of the National Academy of Sciences challenged this dogma by showing evidence of biparental transmission of mtDNA (Luo et al., 2018). Typically, patients carry only one type of mtDNA, which originates from their mother. These mtDNA genotypes are usually genetically similar (homoplasmy), yet in some cases of mitochondrial disease there can be a coexistence of normal and mutated maternal alleles (heteroplasmy). What initially started as a study of suspected mitochondrial disease turned into sequencing the whole PCR-amplified mitochondrial genome of 17 members of three multigenerational families. Luo et al.'s analysis unexpectedly showed high mtDNA heteroplasmy (24% to 76%) in 13 out of 17 studied individuals. The researchers did not observe mitochondrial disease, yet they noticed that in one patient from Family A, 30 mtDNA variants appeared to match with those of the mother while 19 mtDNA variants matched with those of the father. In other words, the patient displayed one haplotype of the father and another of the mother. A similar phenomenon was demonstrated in patients from Families B and C, thus suggesting biparental mtDNA transmission. More importantly, within the three families and haplogroups (groups of haplotypes that share a common ancestor) there were multiple variations in the transmitted mtDNA. While this transmission is unexplainable to known genetics, Luo et al. hypothesize that the inheritance pattern is the result of a mutation on a paternal mtDNA elimination nuclear gene. The authors speculate an autosomal dominant mode of transmission, meaning that one abnormal gene is enough to affect the mtDNA variants in offspring.

Moreover, an earlier study published in the New England Journal of Medicine theorises a similar mode of transmission (Schwarts & Vissing, 2002). Schwarts and Vissing present the case of a man with mitochondrial myopathy due to a novel deletion of a base pair in one mitochondrial gene. Sequencing the PCR-amplified mtDNA of the patient revealed that the haplotype of his muscle tissue matched with his father's. This suggests paternal mtDNA transmission, yet the analysis of the mtDNA in the patient's blood, fibroblasts, and hair cells identified only maternal haplotypes. The authors suggest that the instance of paternal transmission could be due to the survival of some sperm mitochondria, thus fundamentally aligning with the hypothesis presented by Luo et al.

Upholding the Dogma: Counterevidence to Paternal Inheritance

Since the publication of the aforementioned papers, several studies have aimed to trivialize the findings of the research. A comparative study by Filosto et al. (2003) monitored 10 random patients with mitochondrial myopathy mutations, half of which were like the single deletion mutation identified by Schwarts and Vissing (2002). Haplotype analysis of the mtDNA eliminated evidence of paternal transmission in two patients, while identical genes of the blood and muscle samples indicated exclusive maternal inheritance in the remainder of the patients. Filosto et al. (2003) thus question the validity of Schwarts' and Vissing's paper and establish that paternal inheritance of muscle mtDNA in mitochondrial myopathies is not a common phenomenon.

Likewise, a brief report published in 2019 elucidates the infrequency of paternal mtDNA transmission (Rius et al., 2019). The authors studied sequenced DNA from blood samples of patients with suspected mitochondrial diseases. Out of the 41 patients studied, none displayed high levels of heteroplasmy or distinct patterns of biparental inheritance. Only 5 patients displayed minimal heteroplasmy, which was also identified in the patients' mothers' blood. Additionally, 4 patients had de novo heteroplasmic variations, yet the patients shared an mtDNA haplotype with their mothers. It was thus established that biparental inheritance as observed by Lou et al. (2018) is not frequent and does not appear in all cases of suspected mitochondrial diseases.

'Paternal Inheritance' Explained Through NUMTs

In light of the counterevidence of Luo et al.'s (2018) paper, the most prominent interpretation of the paternal transmission is linked with nuclear-encoded mitochondrial sequences (NUMT). NUMTs are generated through the transposition of mitochondrial DNA into the nuclear genome. In a letter addressed to Luo et al., Lutz-Bonengel and Parson (2019) speculate that the inheritance mode of mtDNA could be derived from NUMT elements which were sequenced along with the real mtDNA. The authors note that Luo et al. (2018) did not completely dismiss this possibility, yet they do not provide a clear alternative hypothesis. A subsequent reply from Luo et al. (2019) argues against this speculation, identifying that the NUMTs would need to have very specific structures for PCR amplification. Luo et al. (2019) deemed it highly unlikely that this would happen independently in three families. Nonetheless, further research conducted after the abovementioned exchange supports the co-amplification and sequencing of NUMT elements along with real mtDNA (Cihlar et al., 2020). The study illustrated that such NUMTs may resemble mitochondrial DNA and thus may be misinterpreted as mitochondrial heteroplasmy.

In addition, there are further hypotheses that explain the trends of paternal mtDNA inheritance. Most notably, an opinion article published in the Frontiers Journal suggests that the existence of a multicopy mtDNA concatemer (mega-NUMT) in the nuclear genome may resemble paternally derived mtDNA heteroplasmy (Balciuniene & Balciunas, 2019). Balciuniene and Balciunas (2019) argue that this hypothesis is plausible since Luo et al. (2018) observed the same mtDNA haplotype segregating and contributing to mtDNA heteroplasmy. Thus, the authors infer that the mega-NUMTs segregate in an autosomal dominant manner, similar to the supposed mode of transmission of paternal mitochondria through sperm. Moreover, a more recent Nature Communications study underlines the possibility of mega-NUMTs mimicking parental inheritance (Wei et al., 2020). Out of the 11,035 trios of mother, father, and offspring studied, it has been found that 7 harbour 'biparental inheritance' as identified by Luo et al. (2018). However, after analysing the whole mitochondrial genome of the trios, the researchers detected rare mega-NUMTs in all 7 cases. Not only does this imply autosomal transmission of the haplotype, but also aligns with the hypothesis presented by Balciuniene and Balciunas (2019).

Future Outlook

Today, the exact mechanism behind mitochondrial inheritance remains unclear. Understanding the precise molecular mechanism behind this rare transmission will greatly enhance our understanding of mitochondrial inheritance. It is evident that extensive research will be necessary to comprehend and utilize the ramifications of potential biparental inheritance in the field of genetics and medicine.

For instance, current knowledge lacks the details of ubiquitin-mediated active sperm degradation in human embryos. Further research will be required to identify the specific substrates of ubiquitination in the process of paternal mtDNA degradation. This knowledge could offer a different perspective on the transfer of disease-causing mtDNA. For example, if the specific processes of mtDNA degradation were identified, mitochondria carrying mutations could be selectively modified or eliminated in the embryo. This might lead to ethical challenges, yet the potential for future genetic studies is significant.

Additionally, more research needs to be conducted on the rare instances of biparental inheritance. Repeating Luo et al.'s experiment on a larger patient dataset could give insight into the predisposing factors of biparental inheritance. Whereas Luo et al. hypothesize that a mutation on a paternal mtDNA elimination gene causes paternal transmission, the idea has never been studied. For example, mitochondrial heteroplasmy patterns could be analysed to understand inheritance patterns over generations of patients. Identifying patients more likely to experience biparental transmission could also help find an alternate explanation for paternal mtDNA leakage.

Conclusion

This review looks at the inheritance of mitochondrial DNA in humans. The inheritance of mtDNA has long been considered to occur exclusively through maternal transmission, yet recent studies have challenged this established dogma. In recent years, several controversial research papers have presented evidence for paternal or biparental mtDNA transmission. Counterevidence involving NUMTs and mega-NUMTs has been proposed to explain paternal mitochondrial DNA inheritance. In addition, this review examines the various mechanisms underlying the elimination of paternal mtDNA. Processes such as autophagy, endonuclease G degradation, and ubiquitin-mediated degradation are currently supported by studies in model organisms. Mitochondrial DNA inheritance remains a subject of ongoing research and debate, yet the studies conducted thus far highlight the need for continued exploration to shed light on this fundamental aspect of genetics.

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