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Mitochondrial dysfunction in humans by cyto-nuclear incompatibility

Evolutionary genetics

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Abbreviations

mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
LHON	Leber's Hereditary Optic Neuropathy
OXPHOS	Oxidative Phosphorylation
PGC	Primordial Germ Cell
ROS	Reactive Oxygen Species
SNP	Single Nucleotide Polymorphism
RCC	Respiratory Chain Complex

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Summary

Mitochondria provide energy for the cell by using sugars and lipids and converting stored energy in the form of a proton gradient into usable ATP. Mitochondria are also responsible for calcium signalling, cell metabolism regulation, steroid synthesis and apoptosis. Mitochondrial proteins are mostly encoded by nuclear DNA (nDNA), but mitochondria also have their own DNA (mtDNA). This mtDNA is 17 kb long circular double stranded DNA, and has a high mutation rate because of its proximity to oxidative phosphorylation in mitochondria. Different mtDNA genomes exist within the human population, this is caused by mutations in mtDNA and subsequent mutations in nDNA over the course of generations that reverse the pathogenic effect. These different mtDNA genomes are called haplotypes and suggested to be linked to several diseases, for example Leber's hereditary optic neuropathy (LHON).

Pathogenic point mutations can be transferred maternally and transfer mitochondrial disease to next generations. Treating or preventing mitochondrial disease is very difficult because many mechanisms are not yet fully understood. Examples are the mtDNA 'bottleneck' and mitochondrial segregation in cell division. There are currently several therapies to prevent mitochondrial disease, all of which involve using a donor oocyte with 'healthy mtDNA'. There are however still many uncertainties using these techniques and more knowledge about mitochondrial evolution and interaction with nDNA can improve these therapies.

Introduction

Mitochondria are the only organelles with their own DNA (mtDNA) in eukaryote cells and are responsible for meeting the energy demand in the cell (McBride et al. 2006, Lanza and Nair 2010). Mitochondria were incorporated in eukaryote cells early in evolution, about 1,5 billion years ago and adapted to the cell environment (Salvatore & Davidzon, 2005). Structures that resembled mitochondria were first observed in 1840 and named mitochondria by Carl Benda in 1898 (Ernster and Schatz, 1981). Since then a lot has been discovered about mitochondria and the field of mitochondria and mitochondrial disease is expanding still. Producing and storing energy is not the only function of mitochondria, because mitochondria are also key components in calcium signalling, involved in regulation of cell metabolism, steroid synthesis and, very importantly, programmed cell death (Chinnery & Hudson 2013, McBride et al. 2006). With that in mind it has become increasingly clear that dysfunction of mitochondria is the source of many metabolic and degenerative diseases, cancer and aging (Park and Larsson, 2011).

MtDNA is a circular, 16 569 base pair long double stranded DNA molecule and each mitochondrium has 2 – 10 copies of mtDNA (Rudolf et al. 1992). The number of mitochondria varies in each tissue depending on energy demand. For example, red blood cells have no mitochondria whereas liver cells contain more than 2000 (Alberts et al. 1994). The total number of nuclear and mitochondrial encoded genes is estimated at ~1500 (Calvo and Xie, 2006). MtDNA is a circular DNA molecule and contains on both strands altogether 37 genes (Chinnery & Hudson, 2013). MtDNA does not undergo recombination and is dependent on the nucleus for replication and maintenance. Replication happens continuously and is not governed by the cell cycle (eukaryotic cell division). Keeping in mind the importance and many functions of mitochondria, dysfunction in these organelles can be fatal for the cell (Burgstaller et al. 2014, Poulton et al. 2010).

The relationship between mitochondrial DNA and the nucleus can break down when mutations arise in either mtDNA or nDNA. Thousands of mtDNA copies are present in every nucleated cell and normally humans are homoplastic, which means that all their mtDNA copies are identical. However patients diagnosed with mtDNA diseases are usually heteroplastic and most of their tissues contain both normal and mutated mtDNA. These mutations may cause epilepsy, liver failure, cardiomyopathy or more common milder disorders such as age-related deafness and loss of vision. The spectrum of mitochondrial disease is wide but cells that require a lot of energy are typically affected, such as the central nervous system or the hart (Chinnery & Hudson, 2013).

Mutations in mitochondria can be fixed due to adaptive evolution in the nuclear DNA. Because mtDNA works together with nDNA, mutations in mtDNA can be fixed with mutations in nDNA, which means that cells that adapt to the new mtDNA are positively selected for. This adaption has been observed in several organisms such as plants, flies and wasps (Chou and Leu 2014). nDNA of the cell needs to catch up quick in order to survive mtDNA mutations, and this evolutionary race between nuclear and mitochondrial genomes can lead to rapid evolution of the genes involved in the interactions. This co-evolution means that the original mutation has been fixed due to adaptive mutations in the nDNA, but the evolved cells have the same fitness as their ancestors (Chou and Leu 2014). In humans, these common inherited mtDNA mutations have created stable subgroups separated by common sequence variation known as haplogroups (Wang et al. 2013). Different haplogroups have been associated and linked to several mitochondrial diseases, for example Leber's hereditary optic neuropathy (LHON) (A-Mai et al. 2011).

Much is known about mtDNA inheritance, mtDNA replication and mutation rate, however many articles agree that the treatment for patients with mtDNA disease is inadequate and is limited to symptomatic relief (Burgstaller et al. 2014). The aim of this thesis is to explain how mitochondrial mutations arise, how these mutations are transferred to next generations and how this can be prevented and the possible problems using these treatments. This thesis will address the gap between biological knowledge about mitochondria and how this can be applied to treatment.

MtDNA experiences high mutation rates

The circular double stranded mitochondrial genome contains a total of 37 genes and is very efficient with ~93% representing a coding region (Chinnery and Hudson 2013). There are two strands that differ in base composition. The heavy strand (H-strand) is guanine rich and encodes 28 genes, the other 9 genes are located on the light strand (L-strand). At least 92 mitochondrial structural subunit genes have been identified, 79 encoded by nDNA and 13 genes are encoded by mtDNA. 22 mtDNA genes encode mitochondrial tRNA molecules, the other two genes ribosomal subunits. Although mtDNA is not part of the nDNA they are dependent on each other to form functioning mitochondria.

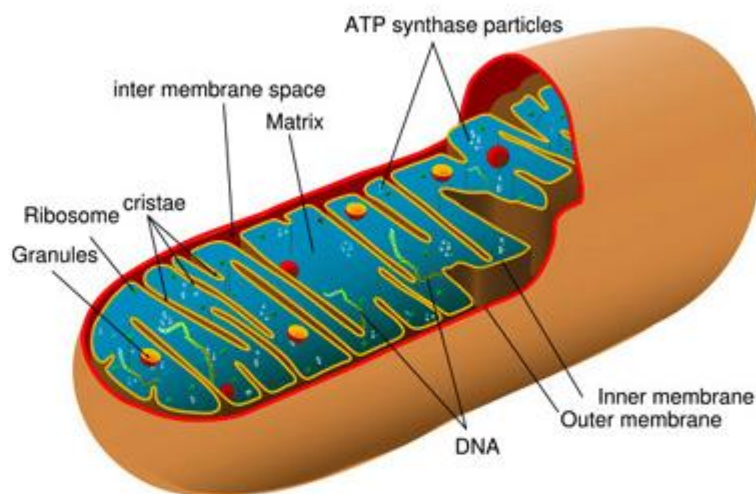

Figure 1 Structure of human mitochondrion. The inner membrane divides the matrix from the outer space and its surface is bigger than the outer membrane (TutorVista).

As briefly mentioned, in humans the adaptive changes of nuclear DNA to different mtDNA lead to stable population subgroups or haplogroups. These differences were created over 10 000 years ago due to migration of humans and isolation of the subgroups geographically (Andersson et al. 2003, Chinnery and Hudson 2013). Ten haplogroups have been defined by specific sequence variants within the European population, and given a letter (H, J, T, U, K, M, I, V, W, X). Over 95% of Europeans belong to 1 of 10 major haplogroups and are homoplasmic for this group. There are several studies which show a direct link between different background haplogroups and a functional effect on the RCC protein complex assembly (LHON). The mtDNA variants have become fixed in the population and different nuclear background can cause defects. Recent discoveries show that differences in haplogroups also contribute to age-related neurodegenerative disorders, for example Parkinson’s and Alzheimer’s disease (Ridge et al. 2013).

The major cause of mitochondrial disease however is not the haplogroups. Point mutations occur with an estimated incidence of 1 in 5000 (Chinnery and Hudson 2013). That these point mutations can cause mitochondrial disease was first reported on molecular level in 1988 when patients were discovered to be heteroplasmic for a specific mutation. This mutation can cause sudden onset of blindness disease (LHON). Later studies showed that mutations in mtDNA are common and a survey of new-born cord bloods showed that one in 200 infants harbours one of ten common known pathogenic mtDNA mutations. Hence, pathogenic mtDNA mutations are very common and can arise very quickly. To understand why mtDNA has a high mutation rate one must look at the replication and location of mtDNA in the cell.

Like nDNA, mtDNA is packaged in protein-DNA complexes known as nucleoids and these nucleoids are located in the inner mitochondrial membrane, spaced evenly along the cristae (Chinnery and Hudson 2013). Cristae expand the surface area of the inner membrane and thereby make it five times larger as the outer membrane. It is however also close to the production of cellular energy through oxidative phosphorylation (OXPHOS). The proximity to the electron transport chain makes mtDNA vulnerable to oxidative damage mediated by reactive oxygen species (ROS) and may increase the mutation rate. The second reason for a high mtDNA mutation rate is the fact that DNA repair machinery in mitochondria work inefficiently and mtDNA is not protected by DNA binding proteins or histones.

Because of the high mitochondrial mutation rate, new pathogenic mutations are continuously being introduced in the human population and can affect every tissue. Hundreds of these point mutations have been discovered and the severity depends on the nature and the heteroplasmy level (Calvo et all. 2006). The ratio of mutated mtDNA and wild type mtDNA is very critical to disease aetiology. This threshold for bio-energetic breakdown is relatively high, most mtDNA mutations need to accumulate to greater than 60 – 90% of the total mtDNA in the cell before OXPHOS is compromised (Wallace and Chalkia 2013).

The point mutations in mitochondrial DNA can be inherited but primary mitochondrial rearrangements of mtDNA are observed to be not inheritable (Poulton et al. 2010). These rearrangements are usually large-scale deletions or insertions in the mitochondrial genome. In this case the ratio of mutated versus wild type mtDNA is also very imported, because the ratio can change over time. The clinical severity of mtDNA disorders is very variable because of this. There are studies that indicate that large scale mutations can accumulate quicker in non-dividing cells for example muscle tissue (Chinnery and Hudson 2013). The turnover time of these mtDNA molecules is slow and less subjected to inter cellular competition. But these studies do not explain all the observed data and phenotypes found of mitochondrial disease.

MtDNA mutation change between generations

The mitochondrial genome divides differently during cell division than nuclear DNA, and is also inherited differently. During reproduction the egg and sperm cell fuse together, so one would expect copies of both parental mtDNA and maternal mtDNA. This is however not the case, because compared to the oocyte the sperm cell contains only a few copies of mtDNA (Alberts et al. 1994). Moreover studies have shown that sperm mtDNA is removed by ubiquitination, most likely during transport through the male reproductive tract (Craven et al 2011). This means that mtDNA in the

zygote depends solely on the oocyte which contains about 100 000 mtDNA genomes. The amount of mutant mtDNA in offspring therefore depends on the development of the oocyte. This transmission is called the mitochondrial bottleneck, whereby a small number of mtDNA's become the founders for the offspring.

In the early stages of oocyte development a large increase of mitochondrial DNA is observed (Poulton et al. 2010). Primordial germ cells (PGC's) contain about 100 mtDNA copies in early stages of oocyte development, which expand to 100 000 in a mature oocyte. Moreover, mitochondrial DNA barely replicates during early stages of oocyte division after fertilisation and different mitochondria genomes from the oocyte can segregate to different cells of the blastocyst. Segregation is an important part of cell division and in this process the two sister chromatids are pulled to either side of the cell and separated from each other (Alberts et al. 1994). Segregation happens both in mitosis and meiosis and in this process the mtDNA genomes are also divided between cells. The term bottleneck revers to two cell division mechanisms, firstly the small amount of initial mtDNA in early oocytes which is replicated massively. Second the potential redistribution of mutated mtDNA during the first divisions of the blastocyst. Because a small amount of mutated mtDNA in these cells has potentially fatal effects when distributed heterogeneously it is important to understand the underlying mechanism (Craven et al. 2011). Both clonal proliferation of mitochondrial DNA in PGC's developing to mature oocytes and mtDNA division during early development contribute to the bottleneck.

The mitochondrial bottleneck can explain how distribution of mtDNA can differ between offspring but there is also another mechanism that prevents the spread of mutated mtDNA to further generations. Several studies show bias against mutated mtDNA during cell division in mouse models (Goios et al. 2007, Stewart et al. 2008). The first example is a study carried out with random mutations in mice mtDNA, and crossing these mtDNA mutation carrying mice with wild type mice. Subsequent investigations into wether or not these mice could survive these potential fatal mutations showed how the heteroplasmic mtDNA population changed in further generations. The randomly integrated mutations were introduced by making a mutation in the proof-reading domain of the mtDNA polymerase named polgA. The mutated mtDNA copies were then transferred homozygous in female mice. The results show that backcrossing the offspring that were heterozygous for the polgA mutation eliminated the mutant polgA allele. The amount of mutated mtDNA found in next generations became less and further mutations did not occur. This indicates not only clonal selection against deleterious mutations but that selection also happens in a short time frame of about four generations. Subsequent experiments showed a stronger selection against mutations in mitochondrial genes encoding mRNAs than tRNAs. Stricter selection of mRNA is also supported by the fact that in humans tRNA mutations are found more frequently than mRNA (Chinnery and Hudson 2013). A related study also suggested selection bias when two pathogenic mtDNA point mutations were introduced in a well-characterised mice cell line (Fan et al. 2008). One mutation was a deleterious frameshift by insertion and the second mutation a milder missense mutation. After introducing these homoplasmically in mouse embryonic stem cells, the mutations caused a severe respiratory chain defect. However over time one of the embryonic stem cell clones became heteroplasmic. Further experiments showed a deletion next to the inserted base thus restoring the frameshift. When this line was introduced into a germline, the following mice developed a sub-clinical myopathy and cardiomyopathy but were however able to reproduce. The offspring of these mice showed each generation less mtDNA with the frameshift mutation. These

observations are consistent with other studies in mice and humans. The mechanism behind mutated mtDNA selection is not yet understood but it is clear that it plays an important role in preventing mitochondrial disease.

Eliminating mutated mtDNA is contradictory with the bottleneck theory which states that distribution of mutant mtDNA happens randomly. Some experiments show a difference in mutated mtDNA level between that of the mother and the oocytes and offspring (Fan et al. 2008). There are a few mechanisms proposed that can explain the selection of wild type mitochondria. Some investigators suggest that specific mutations may be the cause of the differences in mutant DNA distribution. There are a few hypothesised mechanisms for the selection of mtDNA in cells seen in mice and potentially humans. One of these mechanisms is selection of the oogonia, because only 30% develop into matured oocytes. The oogonia that are not used enter apoptosis (Park and Larsson 2011). This selection may be based on the higher reactive oxygen species (ROS) level in oogonia containing mutated mtDNA. Difference in ROS levels also affects the number of mtDNA copies and this may be the signal against selection. A second theory on organelle level is simpler and suggests that mitochondria with a mutation in mtDNA are less efficient, for example in importing enzymes for replication (Poulton et al. 2010), or fusing with other mitochondria. Wild type mitochondria have an advantage over mutated mitochondria and are positively selected for. When repair is slow and the mitochondria become damaged, compromised mitochondria might be degraded by autophagy. This is the case in somatic cells but autophagy is also present in germ cells and autophagy can be involved in removal of mutant mtDNA for the next generation.

Mitochondrial disease

Mitochondrial disease occurs when one or more functions of mitochondria is compromised. The most important example is chronic loss of cellular energy, which happens when mitochondria in the cell can no longer produce enough ATP for normal cell functions (Chinnery and Hudson 2013). Because mitochondria have many more functions besides producing energy, dysfunction of mitochondria may affect different tissues differently. MtDNA works together with nuclear DNA and this relationship is fragile, any mutation in either mtDNA or mitochondrial genes encoded by nuclear DNA can cause mitochondrial disease. As described earlier, mtDNA heteroplasmy is very important in studying mitochondrial disease. Two categories of mtDNA variants can be defined, the first category is single base pair variants and are common in the population. The second category is mtDNA rearrangements, which are less common but some deletions have been found frequently (Grammage et al. 2014).

Defects in mtDNA can be inherited, usually heteroplasmic, as point mutations, most commonly in tRNA genes. This is surprising because tRNA coding regions only makes up 5% of the mtDNA genome (Chinnery and Hudson 2013). Mutation examples are m.3460 G>A, m.11778 G>A and m.14484 T>C. These three common mtDNA mutations affect a subunit of the respiratory chain complex (RCC) and can cause LHON disease (A-Mei et al. 2011). The mechanism behind this disease is not yet fully understood but these substitutions cause an acute loss of ganglion cells (RGCs) and their axons which impair optic nerves necessary for vision. Only 10 – 15 % of female mutation carriers lose their RGCs but more than half of male carriers. The reason why some mutations affect males more is because mitochondrial disease is inherited maternally and natural selection happens only in females. Males do not transmit mitochondria and thus male specific mutations have no fitness consequences for

mitochondria (Frank and Hurst 1996). LHON disease can occur at any age and most of the times in both eyes.

Nerve tissue is usually one of the most affected tissues, along with skeletal, muscle or heart tissue, because these cells require more energy to function (Chinnery and Hudson 2013). There are well defined clinical syndromes but more as half of the patients are not easily defined to one of these groups. For example in many cases are the β-cells of the pancreas involved leading to diabetes or the renal tubules leading to kidney failure. The incidence of mtDNA disease is difficult to predict but some epidemiological studies are looking for a possible connection between the presence of specific mutations and the incidence of mtDNA disease within a specific population.

Treatment and prevention of mitochondrial disease

The sources of mitochondrial disease are mutations in either mtDNA or mitochondrial genes encoded by nuclear DNA. One in 200 people carry a pathogenic mutation in mtDNA, but in most cases it does not present itself as a disease either because the amount of mutant load is not high enough or external factors are needed to cause symptoms (Burgstaller et al. 2014, Dimauro and Davidzon 2005, Craven 2011). When a patient is diagnosed with mitochondrial disease the treatment options are very limited. Only the symptoms caused by the defects in mtDNA can be treated because the source which is the mutation in a large quantity of mitochondria is almost impossible to cure. Genetic counselling is very important in families with a history of mitochondrial disease. It is however not yet possible to predict the amount of mutant mtDNA inherited because of the bottleneck effect (Wai et al. 2010). Using clinical data and comparing this to natural history of specific mitochondrial disorders has improved treatment, however there are no specific disease modifying treatments at present.

Some drugs are being tested and may hold promise in treating mitochondrial disease, but development of gene therapies may also contribute to finding a cure (Burgstaller et al. 2014, Poulton et al. 2010). Gene therapy can take three different directions, the first is replacing the gene in the nucleus causing the defect and so rescuing the pathogenic effect (Kanabus et al. 2014). The second strategy is to transfect wild-type mtDNA in the cell and so replacing the mutated mtDNA which is then integrated in mitochondria and replicated. The third option is changing the ratio of mutated mtDNA to wild type mtDNA in the cell, changing this balance can be accomplished by internal or external factors.

Prevention of mitochondrial disease is also possible and at the moment more realistic. Different methods have been used or proposed in studies. Oocyte donation is the simplest way of preventing mutated mtDNA to be transferred from mother to child (Burgstaller et all 2014). This approach requires a third party oocyte donor rather than the mother oocyte, and in this way guarantees that no mutated mtDNA will be passed on. The child will also lose genetic inheritance from the mother because the oocyte used contains nDNA from the donor. Alternative options are selecting oocytes from the mother which are considered to have a low mutant load. This strategy can only be applied in heteroplasmic disease and in mtDNA disease inheritance disorders of which the phenotype is known (Wallace and Chalkia 2014). When this is unclear it is impossible to select correctly against oocytes that have a high mutant load, one of the poor predicable phenotypes is LHON. Selecting oocytes that have a low heteroplasmic ratio does not guarantee an even distribution of mtDNA in early development. A therapeutic strategy to prevent this is isolating a small sample (1-2 cells) of a

fertilized oocyte after subsequent development (Burgstaller et al 2015). Selection of these early embryos is low risk and the heteroplasmy variation is minimal. After obtaining such data the risk of possible mtDNA disorder development can be assessed. But there are still cases where the mutant load in the child was considerable higher than in the observed trophoblast cells. This difference can be attributed to distribution of mutated mtDNA during development or other selection factors causing the ratio to change. Oocyte donation of a different female has the disadvantage that the genetic information of the mother is not transferred to the child (Burgstaller et al 2015). It is however also possible to use both the DNA of the mother and the father and the mtDNA of a donor, in which case the genetic information of both parents is passed on.

Several therapies propose nuclear transfer to a healthy recipient oocyte with no nucleus and healthy mtDNA (Poulton and Oakeshott 2012). This approach involves transferring the nuclei from the mother and the father from a fertilized oocyte to an enucleated recipient donor oocyte. This way the oocyte has nDNA from the mother and father and most importantly mtDNA from a donor. There will always be some mtDNA from the mother in the recipient oocyte due to carryover, but this is a small amount compared to donor mtDNA. A closely related technique is chromosomal spindle transfer, which involves transferring the chromosomal spindle from the mother to a donor oocyte. The difference between chromosomal spindle and pronuclear transfer is that the fertilisation in chromosomal spindle happens after the spindle chromosomal complex is transferred to the donor oocyte. The donor nucleus is in both cases removed before it is ready as a recipient. First studies of these techniques prove that the transfer is possible, however practical reasons make it impossible to prevent mtDNA carryover. Ideally no mtDNA from the mother is found in the fertilized oocyte but this is unavoidable using current techniques. The carryover is very low but still detectable (St John and Campbell 2010), pronuclear transfer and spindle transfer reduce the amount of mother mtDNA to less than 2%. The average carryover of mother mtDNA in pronuclear transfer is 1,7% and 0,6% in human spindle transfer (Tachibana et al. 2013). The mtDNA bottleneck can amplify this number in the next generation but studies show that if the carryover stays under 5% of the total mitochondrial amount this effect is minimal and the child and next generations are unlikely to develop mitochondrial disease.

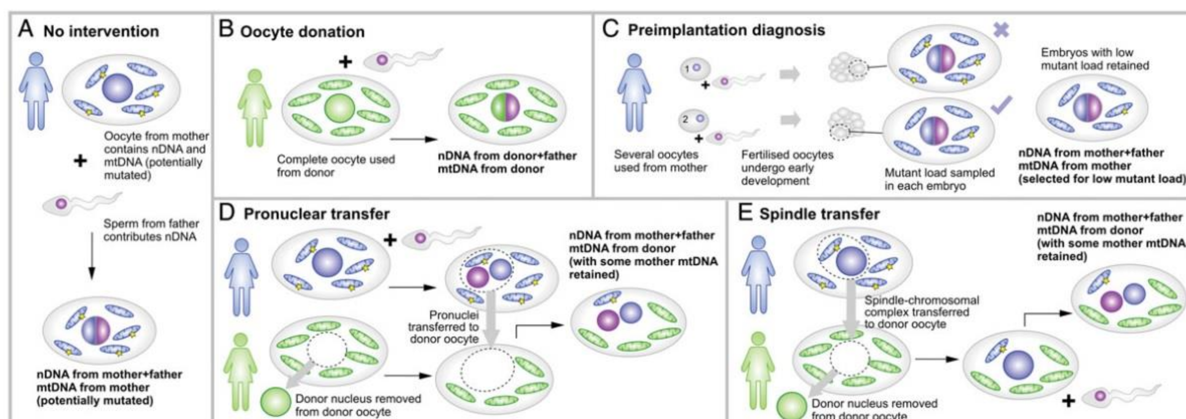

Figure 2 Schematic overview of prevention of mitochondrial disease. (Burgstaller et al. 2014)

MtDNA incompatibility using modern treatment

The use of a donor may seem safe to use in the clinic, but several uncertainties still exist concerning nuclear transfer and chromosomal spindle. As mentioned earlier, mitochondrial DNA and nuclear DNA work together to form mitochondria and between human populations there are different haplotypes mtDNA which have many single nucleotide polymorphism (SNP) differences. Random paring show on average 130 of these SNP's and together account for 20 different amino acid changes in mitochondrial proteins (Blanco et al 2011). This differs between haplogroups but also between subgroups, and these changes may cause mtDNA – nDNA incompatibility (Wang et al. 2013). When pro-nuclear transfer is performed, the resulting oocyte contains a rather complex mixture of nuclear DNA from the mother and the father, the majority of mtDNA from the enucleated recipient oocyte with healthy mtDNA and a small amount of carryover DNA. This carryover DNA can be homoplasmic or heteroplasmic, resulting in a maximum of three different mtDNA copies present in the oocyte. The 'new' mtDNA is now dependent on both maternal nDNA as well as paternal nDNA which can cause problems when mtDNA is not compatible with nDNA. Usually the offspring's mtDNA is inherited with a haploid maternal genome, which means that only the paternal nDNA is alien to mtDNA present in the oocyte. However when mtDNA is confronted with completely unknown nuclear DNA, complications may arise (Brugstaller et al. 2014). Males are particularly at risk because mtDNA is maternally inherited and natural selection for adaptive mutations only works directly on females (Poulton et al. 2010).

The second uncertainty is the fact that there are different haplotypes present in the same cell, and in some cases three types because the carryover tDNA may be heteroplasmic. Different haplotypes show differences in OXPHOS, which can directly be linked to the fact that humans in geographically different places adapted to the various climates. Different climates mean different energy demand for humans and these mitochondria are slightly different in mtDNA sequence, copy number and replication rate. It is still not know what happens when these mtDNA – mtDNA interactions occur in humans. When different haplotypes of the same subspecies are mixed and introduced in mice, physiological changes occurred. For example the body mass of the mice changed, behaviour was altered and hypertension occurred, while homoplastic mice stayed healthy (Acton et al. 2007). The used ratio is of course not directly comparable to nuclear transfer, were the ratio is less than 2%, but it is very relevant to investigate this aspect. Because even if the amount of carryover mtDNA is very small, it may have a proliferative advantage over the recipient mitochondria. This means that even a small initial heteroplasmic ratio can eventually dominate the cell.

As mentioned earlier the human cell is able to select for wild type mtDNA over mutated mtDNA and so eliminate pathogenic mutations in following generations. How this works concerning haplotype is not yet known (Ridge et al 2013). Two scenarios are possible, one the overtime change is simply caused by replication rate, or the cells do select for haplotype and either increase the amount of carryover mtDNA or eliminate it over time. A study which addresses this in mice used a mixture of two naturally occurring but genetically different haplotypes (Goios et al 2007). A common laboratory mouse strain (CIS) mtDNA and NZB mtDNA, which is different in haplotype, were mixed and introduced in mice. This CIS/NZB mice model showed that the ratio is dependent on the type of tissue, because the proportion of NZB mtDNA increased with time in liver and kidney tissue. As described above this causes physiological changes in these mice. The next generations however showed a reduction in NZB mtDNA compared to their mothers. The mechanism behind this is still not

known, but it is safe to say that it works differently than selection for pathogenic mutations in mtDNA.

These unknown areas do not mean a fatal flaw in the treatment, but it are uncertainties which have to be explored. Until these possible side effects are studied more thoroughly, modern treatment should only be performed when absolutely necessary, for example in families with severe phenotypes and a mtDNA mutant of known pathogenicity. Selecting the right families is important for initial studies but is difficult in this case because they are very rare.

Conclusion

Mitochondria are adapted to the cell environment and because they are in some ways independent of the nucleus they show different evolutionary trajectories. This can be observed when looking at different human haplotypes where mtDNA has adapted into a stable nuclear background within human populations. These differences in mtDNA are usually inherited but can also be caused naturally because mitochondria are very susceptible for mutations. Two types of mutations can be distinguished, long term evolutionary changes and point mutations which can be transferred to the next generation. MtDNA is stored in nucleoids in the inner membrane of the mitochondria and contain 2 – 10 mtDNA molecules per mitochondria. The reason why mtDNA has a high mutation rate is because it is very close to the OXPHOS and is exposed to reactive oxygen species which can cause substitutions or deletions during replication. MtDNA is also dependent on the nucleus for replication and DNA repair, which is less effective in mitochondria. Large scale deletions and insertions can occur and give rise to a heteroplasmic mitochondrial population and when mutated mtDNA accumulates in the cell can this impair cellular function.

Large deletions or insertions in mtDNA cannot be inherited but point mutations however can. MtDNA can only be inherited maternally which makes males more susceptible for mutations that can benefit female population, this is for example observed in LHON disease. For example a germline mutation with severe effect on males but only mild effects on females can increase in frequency because natural selection occurs only in females. When hetroplasmic mtDNA is transferred to offspring two factors are important that can cause differences in the mutant / wild type ratio which is refered to as the mitochondrial bottleneck. The first factor is the development of oocytes, because in this process the amount of mtDNA goes from 100 mtDNA copies to 100 000 which means that only a few initial mutated mtDNA molecules will amplify in this stage of development. The second bottleneck stage is after fertilization of the oocyte, when the cell starts to divide and for the first divisions the number of mitochondria stays the same. When distribution of heteroplasmic mtDNA is not uniform some tissues will acquire more mutated mtDNA than others, and will be more susceptible for mitochondrial disease.

There is however also experimental data that suggest cells can prevent the spread of pathogenic mutations to next generations. The mechanism involved in this process is not yet fully understood but wild type mitochondria may be positively selected for replication because defective mitochondria are functionally impaired. For example, if enzyme uptake is slower in mutated mitochondria than replication is also slower. Moreover damaged mitochondria will be degraded by autophagy and eliminated in the mitochondrial population. This is contradictory to the mitochondrial bottleneck and more experiments are needed in order to predict these processes in clinical cases.

To prevent the spread of pathogenic mtDNA mutations in families with a history of mitochondrial diseases it is possible to use a donor recipient oocyte to eliminate possible mitochondrial defects in the child. The nDNA of the mother and father is transferred to an recipient oocyte in which the nDNA is removed. There are other options such as selecting female oocytes with a low mutant load, but this method can only be used in some cases and outcome is still unpredictable. Another option is also using the nDNA of the donor oocyte, thus only using the donor oocyte and paternal nDNA. This means that none of the genetic information of the mother is used. Using the whole donor oocyte is the safest option, but the child will share no genetic information with the mother. Using nuclear transfer and a donor oocyte will result in a fertilized oocyte with nDNA from the mother and father, mtDNA from the donor but also some carryover mtDNA from the mother. This amount of carryover DNA is very small and likely not to cause problems, but the possible phenotypic effects are yet unknown. MtDNA copy ratios can change over time in specific tissues, especially during the first stages of development. This has been confirmed in mice models, where one type of mtDNA was favoured in specific tissues.

Knowledge about mtDNA evolution over generations may answer the questions that are still remaining. Many processes such as the mitochondrial bottleneck, mtDNA segregation and haplotypes are not yet fully understood. This knowledge can in future help formulate strategies for gene therapy to treat mitochondrial disease or prevent the spread of pathogenic mtDNA in families with a history of mitochondrial disease.

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