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Review

Effect of mtDNA point mutations on cellular bioenergetics ☆

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ABSTRACT

This overview discusses the results of research on the effects of most frequent mtDNA point mutations on cellular bioenergetics. Thirteen proteins coded by mtDNA are crucial for oxidative phosphorylation, 11 of them constitute key components of the respiratory chain complexes I, III and IV and 2 of mitochondrial ATP synthase. Moreover, pathogenic point mutations in mitochondrial tRNAs and rRNAs generate abnormal synthesis of the mtDNA coded proteins. Thus, pathogenic point mutations in mtDNA usually disturb the level of key parameter of the oxidative phosphorylation, i.e. the electric potential on the inner mitochondrial membrane ($\Delta\psi$), and in a consequence calcium signalling and mitochondrial dynamics in the cell. Mitochondrial generation of reactive oxygen species is also modified in the mutated cells. The results obtained with cultured cells and describing biochemical consequences of mtDNA point mutations are full of contradictions. Still they help elucidate the biochemical basis of pathologies and provide a valuable tool for finding remedies in the future. This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).

1. Introduction

Mitochondria play a crucial role in the maintenance of cellular homeostasis and participate in numerous cellular processes, for example: ATP production, generation of reactive oxygen species (ROS), generation and transmission of calcium signals and, last but not least, apoptosis. All these processes depend on and mutually affect one another, making the functional analysis of mitochondria very complex. Moreover, mitochondrial proteins are encoded by two genomes (mitochondrial, mtDNA and nuclear, nDNA).

Mitochondrial genome encodes 13 proteins of the respiratory chain complexes (complex **I** — ND1-6 and ND4L, **III** — cytochrome *b*, **IV** — COX I–III) and ATP synthase (ATPase6 and ATPase8), 2 rRNAs and 22 tRNAs. It is particularly vulnerable to mutations, since it is in close proximity to ROS generation sites and is not protected by histones or membranes. Up to now, more than 250 pathogenic mtDNA mutations have been described [1]. Since there are several hundred to several thousands of circular mtDNA molecules in a single cell, mtDNA mutations are usually heteroplasmic: only a certain proportion of mtDNAs within the cell contains the mutation.

Mutations in mitochondrial genes lead to complex and multisystemic disorders known as mitochondrial diseases [2–4]. The age of their onset is diverse and ranges from infancy to adulthood, and so does the severity of the symptoms which, in the first place, affect tissues with high energy demand: the central nervous system, cardiac and skeletal muscles, retina and the endocrine system. Thus, cardiomyopathy, neurodegenerative diseases, blindness, cancer or diabetes are often associated with mitochondrial dysfunction [1].

Since the same mtDNA mutation can manifest itself with different symptoms and severity, the pathophysiological analysis of mitochondrial diseases is difficult. Most studies have focused on understanding of the variable consequences of mtDNA mutations on the cellular level using cells obtained from patients, cybrid and Rho0 cells and cells with dysfunction of individual respiratory chain complex or synthase ATP. In this review, we will concentrate on the mitochondrial metabolic consequences of primary mtDNA diseases which are caused by mtDNA point mutation. Their clinical expression is wide and includes phenotypes such as: MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes), MERRF (Myoclonic Epilepsy and Ragged Red Fibres), LHON (Leber's Hereditary Optic Neuropathy) and NARP (Neuropathy, Ataxia, and Retinitis Pigmentosa).

MELAS is the most common mtDNA mutation caused by the point A3243G mutation in the tRNALeu gene (typical mutation for 80–90% of patients with MELAS), but other mutations like G1642A in tRNAVal gene, G3244A, T3258C, T3291C, A3243G and T3271C in tRNALeu gene, and various other mutations like: A12770G, A13045C, G13513A and A13514G in ND5 gene as well as in ND1 gene have also been identified. Typical for the MELAS syndrome are stroke-like episodes, seizures, dementia, diabetes, ataxia, epilepsy, optic atrophy, deafness, migraine, cortical blindness, cardiomyopathy, myopathy, exercise intolerance, lactic acidosis and vomiting.

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MERRF is a progressive, neurodegenerative disease mainly caused by a point mutation A8344G in the tRNALys gene. It usually presents itself between childhood and early adulthood. The clinical manifestation of this mutation depends on the percentage of the affected mtDNA copies and includes myoclonic epilepsy, encephalomyopathy, ataxia, myopathy, dementia, deafness and others.

The **LHON** syndrome is mostly caused by three primary mutations: G11778A, G3460A and T14484C in the ND4 genes. Typical features of the syndrome are impaired visual acuity and blindness, more predominant in males.

The **NARP** syndrome is accompanied by the T8993G or T8993C mutations in the ATP6 gene and is characterized by ataxia, retinitis pigmentosa, mental retardation, encephalopathy, developmental delay, cardiomyopathy, epilepsy, dementia and gastrointestinal disorder. Higher than 90% of heteroplasmy cause the Leigh Syndrome (subacute necrosing encephalomyopathys) a neuropatologically defined multisystem disorder of infancy. Its clinical picture is dominated by seizures, weakness, hypotonia, developmental delay and lactic acidosis.

Since mtDNA codes exclusively for proteins of the oxidative system its phenotypicaly manifested mutations usually alter the activity of the oxidative phosphorylation system with typical reduction in ATP production, enhanced generation of ROS, changes in calcium homeostasis and reorganization of the mitochondrial network. The main aim of this review is to provide a comprehensive overview of disturbed bioenergetics of the cell corresponding to the most common mitochondrial diseases caused by mtDNA point mutations.

2. Mitochondrial membrane potential and ATP levels in cells with mtDNA point mutations

The driving force for mitochondrial ATP synthesis is the electrochemical proton gradient created by the respiratory chain. Thus, pathogenic mutations in mtDNA affecting the activity of mitochondrial respiratory chain and/or mitochondrial ATP synthase result in decreased efficiency of mitochondrial ATP synthesis. This was confirmed by many studies performed on cells with mtDNA mutations, both within the primary cells of patients with mitochondrial diseases as well as cybrids [5–11]. Impaired mitochondrial ATP synthesis was shown in cells with mutations in genes coding for the respiratory chain complexes [11,12], for tRNAs [6,8] as well as in ATP synthase [9,10]. Studies on homoplasmic cybrids with different mtDNA mutations demonstrated a dramatic drop of mitochondrial ATP synthesis in case of two different MERRF mutations in tRNALys gene (above 90% decrease in ATP synthesis) and also strong effect of two MELAS mutations in tRNALeu gene (83% and 63% decrease) [8]. Interestingly, significant differences were observed between the effects of two NARP mutations located in the same nucleotide of ATPase subunit 6 gene: T8993C and T8993G mutations (22% and 95% decrease in ATP synthesis, respectively). This phenomenon was confirmed also in lymphocytes from patients carrying such mutations at high heteroplasmy level (above 90%) — T8993G mutation led to about 60–70% drop in the ATP synthesis rate, while T8993C mutation had only slight effect [9]. This confirms the key importance of this locus for ATP synthase activity.

The degree of impairment depends also on the heteroplasmy level. In cells from NARP patients carrying the same mutation, strong correlation was found between the mutation load and the decrease in mitochondrial ATP synthase activity [7,13]. Interestingly, the severity of the bioenergetic impairment resulting from mtDNA mutations can also be affected by polymorphisms in other mtDNA genes. This was shown in cybrid cells with the same nuclear background and the same homoplasmic mtDNA mutation in ATP synthase subunit 6, where the level of the ATP synthesis impairment strongly varied (between 20% and 75% loss of activity) [10].

Many studies fail to observe differences in intracellular ATP levels between wild-type cells and cells with mutated mtDNA, despite clearly defective mitochondrial ATP synthesis machinery [6,14,15]. Lower ATP synthesis in the mitochondria does not have to be followed by decreased cytosolic ATP levels. Studies on cybrids with NARP or MELAS mutations revealed a decrease in intramitochondrial ATP levels in cells with mtDNA mutations, without affecting cytosolic ATP levels if the cells were cultured in the presence of glucose [16]. This is because under resting conditions the energetic needs of the cell can often be satisfied with glycolysis. Indeed, in cells with mutated mtDNA the upregulation of glycolysis is usually observed, resulting in high lactate production levels [16,17]. The compensatory role of glycolytic stimulation is evidenced by inverse correlation between lactate synthesis and respiratory activity reflecting ATP synthesis efficiency [8]. Nevertheless, under conditions of increased energetic demands or low glucose accessibility, glycolysis is not sufficient to satisfy the energetic needs of the cell and ATP depletion usually occurs in respiratory-impaired cells [6,16,17]. Moreover, studies performed on myoblasts (which are the cells with high energy demand) carrying MELAS mutations demonstrated not only decrease of ATP level but also general depletion of the intracellular adenine nucleotides pool [18]. The assumption that loss of efficient mitochondrial ATP synthesis is a disease-causing factor is supported by the following observation in patients with NARP mutation present at different heteroplasmy levels: the severity of the clinical symptoms correlated with the degree of loss of the ATP synthase activity [7].

Apart from being the driving force for mitochondrial ATP synthesis, mitochondrial membrane potential is crucial also for protein import into the mitochondria, respiratory substrates uptake or for ion transport across the inner mitochondrial membrane. Thus, maintenance of $\Delta\Psi$ is extremely important for proper functioning of mitochondria. $\Delta\Psi$ is built up due to proton extrusion from the mitochondrial matrix mediated by the respiratory chain and is consumed mainly by ATP synthesis. An obvious consequence of the mutations in genes coding for the respiratory chain proteins is decreased mitochondrial membrane potential, while ATP synthase dysfunction shall be accompanied by an increase in $\Delta\Psi$. Many experimental data confirm such tendency, demonstrating low $\Delta\Psi$ levels in cells with mtDNA mutations in genes for respiratory chain complexes [19], while mutations in genes for the ATP synthase subunits usually result in elevated mitochondrial membrane potential [9,13]. In addition, Sgarbi et al. have found a clear correlation between increased $\Delta\Psi$ and loss of ATP synthase activity in lymphocytes from patients with NARP mutation at various heteroplasmy levels [13]. Mutations in tRNA genes impair the activities of both: ATP synthase and respiratory chain complexes. In that case usually a clear decrease of the $\Delta\Psi$ is observed, due to strongly decreased efficiency of respiratory chain-mediated proton extrusion from the matrix [6,17]. On the other hand, some investigators fail to observe changes in $\Delta\Psi$, despite a clear genetic and enzymatic defect of the respiratory chain or ATP synthase; sometimes even the opposite effect is detected (e.g. elevated $\Delta\Psi$ in cells with respiratory dysfunction) [20,21]. This may reflect the existence of compensatory mechanisms maintaining appropriate polarization of mitochondrial inner membrane despite the respiratory chain defect [1,21].

Even under conditions of strongly dysfunctional respiratory chain a limited but significant value of the proton gradient across the inner mitochondrial membrane is maintained, which allows to support the $\Delta\Psi$-dependent processes, such as protein import, transport of ions and metabolites across the inner mitochondrial membrane [22]. In cells with severely impaired respiratory chain function, $\Delta\Psi$ is created mainly due to reversed action of ATP synthase and adenine nucleotides transporter (ANT). ATP hydrolysis and production of ADP^{3-} provides substrate for the electrogenic exchange of this nucleotide and ATP^{4-} generated glycolytically in the cytosol [5]. Therefore in cells with mtDNA mutations mitochondrial depolarization can be observed upon inhibition of ATP synthase or ANT, while in unaffected

cells such treatment usually increases ΔΨ [19]. Here, it has to be mentioned, that ANT activity reversal requires stronger decrease in ΔΨ than needed for reversal of ATP synthase activity [23]. The potential to generate ΔΨ despite dysfunctional respiratory chain was shown in cybrid cell lines derived from embryonic stem cells, differentiated into neuronal or glial cells [21]. In cells with a mutation in respiratory complex I ND5 and ND6 subunits, accompanied by strong impairment of respiratory chain efficiency (about 90% decrease in complex I activity), ΔΨ appeared to be even higher than in control cells. Moreover, the source of elevated mitochondrial membrane potential seemed to be different in stem cells than in differentiated neurons, since only in neurons ΔΨ was dissipated upon ATP synthase inhibition.

Contribution of different mechanisms to ΔΨ formation in respiratory deficient cells was quantified in rho0 cells derived from osteosarcoma 143B cell line [5]. In these cells around 13% of ATP derived from glycolysis was used for maintenance of ΔΨ, and ATP hydrolysis together with ADP^{3-}/ATP^{4-} exchange allowed to keep ΔΨ at the level of about 70 mV (compared to ca. 130 mV in wild-type cells). Later studies have shown that under physiological circumstances mitochondrial ATP synthase working in a reversed mode will consume mostly intramitochondrially generated ATP [23]. This is in agreement with moderate consumption of cytosolic ATP for ΔΨ maintenance in Rho0 cells [5]. Other investigations, on HeLa Rho0 cells revealed the additive nature of the effects of mitochondrial ATP synthase and ANT inhibitors on ΔΨ and on cell viability [24]. This demonstrates that reversal of ATP synthase and adenine nucleotide translocase activities are partially independent mechanisms of ΔΨ formation in mitoDNA-defective cells.

3. Calcium metabolism in cells with mtDNA point mutations

The potential involvement of mitochondria in cellular calcium metabolism was recognized 50 years ago [25,26]. After decades of doubts and debates, this involvement is now well documented and wildly accepted.

On the inner mitochondrial membrane there is a calcium uniporter transport system, responsible for Ca^{2+} uptake into the mitochondrial matrix. Its molecular nature was very recently described [27]. This highly efficient, although relatively low affinity (K_d 10 μM) calcium transporter, catalizes the uptake of Ca^{2+} and is driven by ΔΨ. When activated due to rising local calcium concentrations it increases calcium concentration in the mitochondrial matrix, followed by activation of key mitochondrial dehydrogenases and consequently increased oxidative phosphorylation capacity [28].

The transient activity of the uniporter also modulates specific cellular functions through modulation of the spatio-temporal dynamics of calcium signals in the cytosol. Excess Ca^{2+} uptake by the mitochondria can cause a bioenergetic catastrophe, opening of the permeability transition pore, release of cytochrome c and cellular death by apoptosis or necrosis. Therefore, abnormal Ca^{2+} homeostasis is linked to many pathogenic conditions, mitochondrial diseases included.

Even subtle deficits in the electron transfer chain, caused by genetic mutations, affect ΔΨ and thus might influence Ca^{2+} homeostasis. Abnormal calcium signals influence multiple signal cascades within the cell.

Abnormal calcium homeostasis has been reported in a variety of non-neuronal cell lines with oxidative phosphorylation defects and diminished ΔΨ. In fibroblasts derived from MELAS patients, the basal cytosolic Ca^{2+} level was increased [14]. In osteosarcoma MELAS cybrid cells intramitochondrial calcium homeostasis was also disturbed [15]. In cybrid cells with MERRF syndrome derangement of mitochondrial calcium homeostasis was found [29]. In NARP cybrids (with ΔΨ increased or the same as in control cells), cytosolic and mitochondrial Ca^{2+} homeostasis was unaffected, and these cells could accumulate Ca^{2+} as well as WT cells [29]. In SH-SY5Y neuroblastoma Rho0 cells (lacking mtDNA) with drastically reduced ΔΨ, the basal cytosolic Ca^{2+} level was reduced [30], whereas in Rho0 osteosarcoma cells it was higher than in control cells [31]. Overall, this means that even small modification of ΔΨ in cells with defective mitochondria affects mitochondrial Ca^{2+} uptake: when ΔΨ is lowered usually Ca^{2+} uptake is diminished, in the case of ΔΨ enhancement Ca^{2+} uptake can be upregulated. There are, however, cellular mechanisms compensating for abnormal respiratory chain activity.

In neuronal cells the role of mitochondria in calcium homeostasis is somewhat different. Calcium ions are involved in a number of specific cellular processes such as transmitter release and action potential conduction. It has been suggested that defective oxidative phosphorylation results in ATP deficiency, causing persistently elevated level of cytoplasmic Ca^{2+} and promoting cell death via Ca^{2+} overload [32].

In patients with mitochondrial diseases, neuronal dysfunction and neurodegeneration are the most prominent and disabling features. Ca^{2+} enters the cytosol mostly from the extracellular space through voltage-gated Ca^{2+} channels. A model of mature neurons (differentiated cybrids derived from embryonic stem cells) showed a conventional, transient Ca^{2+} response to activation by glutamate [33]. Cells with mtDNA mutation had a normal baseline response to glutamate, but after repeated stimulation showed progressive deficit in Ca^{2+} transients.

In non-neuronal cells (in electrically non-excitable cells), the most dynamic calcium pool corresponds to the endoplasmic reticulum and in Ca^{2+} homeostasis store operated Ca^{2+} channels play a key role [34]. Because store operated Ca^{2+} channels are regulated by mitochondria as well [35], in pH dependent way [36], their dysfunctions may affect neuronal and non–neuronal cells differently. Mutations in mtDNA can reduce mitochondrial Ca^{2+} influx, but do not influence the calcium content of mitochondria in NARP cybrid cells [29]. In osteosarcoma NARP and even Rho0 cells store-operated calcium influx was independent of mitochondrial energy status. Only slower Ca^{2+} influx rates were observed in these cell lines in comparison to parental cells [20].

4. ROS production and the antioxidant defence system in cells with mtDNA point mutations

Under physiological conditions mitochondria are known to produce a basal level of ROS as a result of imperfections in the respiratory chain (electron leakage) [37]. Elevated ROS generation by mitochondrial respiratory chain occurs when mitochondria are hyperpolarized and the components of the respiratory chain are highly reduced or in the case of some dysfunction of the individual respiratory chain subunits. Similarly, in some cases the defects in the mitochondrial ATP synthase may result in mitochondrial hyperpolarization and higher ROS production because the protonomotive force is not used for the ATP synthesis. To defend against ROS, cells posses defense systems which help to counteract the negative effects of ROS and protect cells from the consequences of the oxidative stress. The system contains the number of antioxidant enzymes e.g. superoxide dysmutases (SOD1 and SOD2), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) [38].

Dependences between dysfunction of the respiratory chain (caused by mutations in mtDNA), ROS production and adaptive response of the antioxidant defense system are described in numerous publications. Lack of the mitochondrial respiratory chain activity in Rho0 cell lines was accompanied by diminished ROS production and reduced antioxidant defence efficiency [38]. Moreover, detailed studies on the sites of intracellular ROS generation indicated that in osteosarcoma Rho0 cells, ROS are mainly produced extramitochondrially. It has been well demonstrated that this defect results in a decrease of glutathione reductase activity which in consequence shifts the glutathione redox to the oxidized state. Moreover, the decrease of both SOD1 and SOD2 gene expression as well as protein levels in rhabdomyosarcoma and osteosarcoma Rho0 cell lines has been observed [38].

Different effects are observed when the structure and function of a particular complex of the respiratory chain or ATP synthase is modulated due to mtDNA point mutations. This could be compared to the treatment of mitochondria by the respiratory chain inhibitors such as rotenone, antimycin or oligomycin. Such point dysfunction of the respiratory chain results in increased mitochondrial (mostly superoxide anion, $O_2^{\bullet-}$) ROS generation. Pioneering studies concerning this issue were performed on cell lines derived from patients with mitochondrial disease. It was demonstrated, that the activity of the respiratory chain complexes (I and IV) in cells harbouring the A3243G mutation (in most cases responsible for MELAS syndrome) was significantly decreased [39–41]. Subsequent studies showed that increased total superoxide dismutase and catalase activities could be considered an adaptive response to increased ROS production in cells carrying A3243G mutation in tRNALeu gene [18]. Lee at al. demonstrated that up-regulation of SOD2 in fibroblasts carrying MERRF mutations is related to the activation of protein kinase C delta (PKCδ) signalling cascades [42,43].

Increased ROS production was also found in fibroblasts from patients with diagnosed MERRF syndrome harbouring A8344G mutation in tRNALeu gene. Moreover, mitochondrial $O_2^{\bullet-}$ production in cells with point mutations in mtDNA encoding complex III of the respiratory chain was also elevated [44]. Upregulation of ROS production and simultaneous down-regulation of antioxidant enzymes level by the PKCbeta-dependent phosphorylation of p66Shc upon cellular response to the oxidative stress were observed in skin fibroblasts of children harbouring different mitochondrial disorders [45].

Progress in the studies of mitochondrial disorders accelerated with the use of cybrid cell lines. Using this experimental model — two cybrid cell lines with mutated mitochondrial tRNA genes (A3243G in tRNA LeuUUR and A8344G in tRNA Lys) and one carrying mutation in subunit I of cytochrome *c* oxidase (the stop-codon mutation G6930A), Vives-Bauza et al. studied dependences between antioxidant defence system and oxidative stress with mitochondrial origin [46]. They have found significant increase in ROS production in both MERRF and MELAS tRNA mutant cybrids accompanied by increased SOD1, SOD2 and catalase activities. Interestingly, no expected cellular symptoms of the oxidative stress (proteins carbonylation or lipids peroxidation) have been observed in these cells. It has been concluded that the lack of oxidative stress is a direct effect of the antioxidant enzymes upregulation. In contrast to the mt tRNA MERF and MELAS mutations, the cytochrome c oxidase mutant human osteosarcoma cybrid cells (carrying the stop-codon mutation G6930A in the COXI gene) had similar ROS production rate and levels of antioxidant enzymes to isogenic control cells. This indicates that the mutations described above, affecting complex IV do not increase ROS production [46]. More recently Wu et al. presented different results for MERRF patients with A8344G mutation in tRNALeu gene. In cells from patients with diagnosed MERRF syndrome only SOD2 (mRNA, protein, and enzyme activity levels) has been found upregulated. The other antioxidant enzymes, such as SOD1, GPx and catalase did not show significant changes [47]. Similarly, in human osteosarcoma NARP cybrid cells with 98% of T8993G mutation load, defect in the mitochondrial ATP synthase is accompanied by changes in ROS generation and antioxidant defence system. [48]. In fibroblasts derived from NARP patients direct correlation between mtDNA mutation, oxidative stress and oxidative damage were observed [49].

Studies performed with the use of three osteosarcoma cybrid cell lines carrying homoplasmic point mutations in ND4 (11778), ND1 (3460) and ND6 (14484) mtDNA genes encoding complex I subunits of the respiratory chain (associated with LHON) showed enhanced mitochondrial ROS production [50]. The increased mitochondrial superoxide anion has been observed also in another cybrid model, neuronal (NT2) cybrid cell line carrying the ND4 (11778) and ND1 (3460) LHON mutations [51]. In LHON-affected osteosarcoma cybrids 11778/ND4, 3460/ND1 and 14484/ND6 the activities of SOD1, SOD2 and glutathione reductase activity were decreased [52].

In the above described experimental models (cybrid cells, fibroblasts derived from patients), the increased ROS production seemed to be directly related to mtDNA damage. This view has been challenged by the studies on mtDNA mutator mice (homozygous knock-in mice that express a proof-reading-deficient version of PolgA, the nucleus-encoded catalytic subunit of mtDNA polymerase). Such mitochondrial DNA polymerase defect leads to strongly increased number of mtDNA mutations. However, in this model no increased ROS production (H_2O_2), protein carbonylation, lipid peroxidation and oxidative damage of nucleic acids were observed [53,54]. Unchanged expression level of antioxidant enzymes in mtDNA-mutator mice seemed to indicate that the increased level of mtDNA mutations is not associated with increased ROS production [54]. In the light of contradictory results from various experimental models, the direct relation between mtDNA mutations and elevated mitochondrial ROS production is still an opened question.

5. Mitochondrial morphology and dynamics in cells with mtDNA point mutations

Mitochondrial morphology, dynamics and organization play an important role in living cells. It is well established that mitochondria are highly motile and remarkably plastic organelles. Two events, fusion and fission, that control mitochondrial morphology and mitochondrial network, can contribute to the repair of defective mitochondria, their proper segregation into daughter cells during cell division, the efficiency of oxidative phosphorylation, intramitochondrial calcium signal propagation, mtDNA integrity and cell death [55].

Specific proteins of the dynamin family GTPases are engaged in mitochondrial fission: dynamin-related protein 1 (DRP1) and Fis1. Drp 1 is located predominantly in the cytosol and the pool of this protein translocates to mitochondrial tubules where it interacts with Fis1 protein forming fission sites. An inhibition of Drp1 function leads to the formation of highly fused and tubular mitochondrial network [56]. Fission of mitochondria plays the main role in cell survival and cell death. For example, mitochondrial inheritance depends on mitochondrial fission during cytokinesis, and mitochondrial division is important for formation of synapses and dendritic spines in neurons. Fission machinery also participates in the process of apoptosis by inducing fragmentation of the mitochondrial network.

Fusion is controlled by mitofusin-1, mitofusin-2 and OPA 1. Mitochondrial fusion is required for maintenance of mitochondrial DNA, and its inhibition reduces the activity of the respiratory chain and mitochondrial metabolism [57].

Other important and essential aspects of mitochondrial dynamics are motility and positioning of mitochondria within the cell that involve many motor and cytoskeletal proteins. In mammalian cells, mitochondria travel along microtubules by association with dyneins and kinesins [58]. The primary functions of mitochondrial positioning at local sites are ATP provision and calcium transient buffering. Defects in mitochondrial distribution manifest themselves in highly polarized and metabolically active cells. Since the proper transport and intracellular distribution of mitochondria are crucial for normal physiology of such cells, disruptions of mitochondrial dynamics are often implicated in the pathogenesis of various neurological diseases, cellular senescence and muscle atrophy [59].

In humans, mutations in genes regulating mitochondrial morphology are linked to neurodegenerative diseases such as autosomal dominant optic atrophy — ADOA (mutation in Opa1 gene), a common form of inherited childhood blindness, and Charcot–Marie–Tooth disease type 2A (mutation in mitofusin-2 gene), a neurodegenerative disorder clinically characterized by the gradual degeneration of peripheral neurons.

It is well established that changes in mitochondrial metabolism affect mitochondrial shape and organization within the cell. Pharmacological inhibition of respiratory chain complexes alters organization of the mitochondrial network, mitochondrial membrane potential and

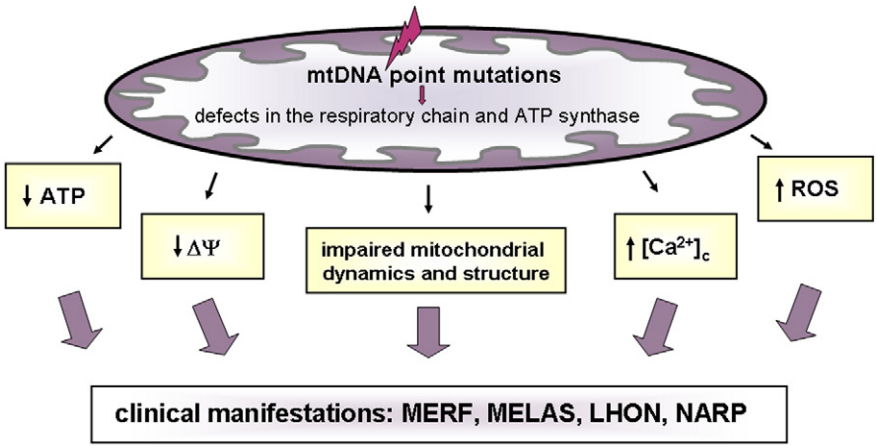

Fig. 1. Mitochondrial factors — typical consequences of mtDNA point mutations. These changes can be modified depending on a cell type (oxidative vs. glycolytic, cells with intensive metabolism vs. slow metabolism), genetic background and level of heteroplasmy.

usually increases ROS production [60,61]. Blocking cellular respiration (e.g. by cyanide) first causes shrinkage of mitochondria and then mitochondrial fragmentation and swelling. Increased ROS production also triggers dynamic changes in the morphology of the organelles by mitochondrial fragmentation [62]. An intact mitochondrial membrane potential was found to be crucial for mitochondrial fusion and shape changes as well [63]. Protonophores dissipating inner mitochondrial membrane potential completely inhibit fusion and result in rapid, but reversible fragmentation of mitochondria whereas nigericin which increases mitochondrial membrane potential, stimulates mitochondrial contacts [64].

Studies of mitochondrial organization in cells with mtDNA mutations show some abnormalities of mitochondrial structure and shape. These observations were made with respect to skin fibroblast cells with complex I dysfunction caused by mutations in nuclear genes encoding complex I subunits and cells with complex I dysfunction induced by pharmacological agents. Koopman et al. [65,66] showed that mitochondria were fragmented and/or less branched. Studies of skin fibroblasts from patients with mitochondrial complex I deficiency and healthy fibroblasts during rotenone or antimycin A treatment showed an increased number of swollen, filamentous mitochondria and nodal filament [67].

Investigation of other cells with complex I dysfunction caused by mtDNA point mutation, and LHON muscle samples (obtained *post mortem*) revealed abnormalities and disruption of mitochondrial network in some muscle fibres. Many mitochondria had lost their inner cristae, formed a “ghost profile” or obtained the cristae shape defined as “onion ring” as well as exhibited an increased volume [68]. Ultrastructure of brain tissue obtained from patients with Leigh disease (fragments of spongy degenerated white matter) also showed abnormal enlarged mitochondria with aberrant configurations of cristae, sometimes with few of them situated in the centre [69]. In MELAS cerebral tissue biopsy electron microscopy revealed abnormal, irregular and concentric cristae structure in the mitochondria [70]. Moreover, in a liver sample obtained from patients diagnosed with Pearson syndrome, some mitochondria had few or revealed no cristae at all [71].

The mitochondrial morphology study was also performed on cybrid MELAS and NARP cells that harbored mtDNA mutations, and revealed mitochondrial fragmentation [20,72]. In MERRF cybrids, it was found that proteolytic OPA1 processing was accelerated [72]. An increased fragmentation and density of mitochondria was also shown in proximal nerves and muscles of *Drosophila* with mtDNA depletion [73]. Yeast cells lacking the mitochondrial gene encoding the ATP synthase subunit 6 also exhibited deformed mitochondria with condensed mitochondrial compartment [74].

Apart from diseases caused by mutations in mitochondrial genome, many studies suggested a possible role of mitochondrial mtDNA mutations in sporadic neurodegenerative diseases such as stroke, Parkinson's, Huntington's, and Alzheimer's diseases [75].

6. Summary

The genetics and pathophysiology of mitochondrial diseases are slowly unravelled. Some of these diseases are initiated by point mutations in mtDNA. Most of the relevant biochemical studies were carried out on cell lines derived from patients, usually on fibroblasts or lymphocytes. On the cellular level, consequences of mtDNA mutations (Fig. 1) depend on cell type (cells predominantly oxidative vs. glycolytic; cells with intensive metabolism vs. slow metabolism), genetic background, level of the penetration of a given mutation (heteroplasmy) and physiological state of cell (resting vs. activated). Thus, results describing the consequences of mtDNA mutations on cultured cells are contradictory. However, a clearer picture starts to emerge. Pathogenic point mutations in mtDNA disturb the activity of mtDNA-encoded components of the respiratory chain or/and mitochondrial ATP synthase. Thus, modifications of mitochondrial membrane potential ($\Delta\Psi$) and the redox state of components of the respiratory chain occur. The change in these two mitochondrial parameters may lead to lower ATP level in the cell, abnormalities in cellular calcium metabolism and changes in mitochondrial ROS production. Moreover, $\Delta\Psi$ influences mitochondrial dynamics.

In a future, more studies are needed to correlate changes of mitochondrial factors in cells with mtDNA point mutations with a phenotype of a given cell line.

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