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Review Article

The Importance of Mitochondrial DNA in Aging and Cancer

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Mitochondrial dysfunction has been implicated in premature aging, age-related diseases, and tumor initiation and progression. Alterations of the mitochondrial genome accumulate both in aging tissue and tumors. This paper describes our contemporary view of mechanisms by which alterations of the mitochondrial genome contributes to the development of age- and tumor-related pathological conditions. The mechanisms described encompass altered production of mitochondrial ROS, altered regulation of the nuclear epigenome, affected initiation of apoptosis, and a limiting effect on the production of ribonucleotides and deoxyribonucleotides.

1. Introduction

Mitochondria are semiautonomous organelles present in almost all eukaryotic cells in quantities ranging from a single copy to several thousands per cell. Important mitochondrial functions include ATP production by oxidative phosphorylation, β-oxidation of fatty acids, and metabolism of amino acids and lipids. Furthermore, mitochondria have a prominent role in apoptosis initiation. The circular mitochondrial DNA (mtDNA) is more susceptible to DNA damages in comparison to nuclear DNA (nDNA). Importantly, mtDNA molecules are not protected by histones, they are supported with only rudimentary DNA repair and are localized in close proximity to the electron transport chain (ETC), which continuously generates oxidizing products known as reactive oxygen species (ROS). Thus, the mutation rate of mtDNA has been reported to be up to 15-fold higher than observed for nDNA in response to DNA damaging agents [1].

Mitochondrial dysfunction and especially dysfunctions caused by mutations of the mtDNA have been implicated with a wide range of age-related pathologies, including cancers, neurodegenerative diseases and, in general, processes that regulate cellular and organismal aging. The mitochondrial genome encodes peptides essential for the function of the ETC and production of ATP by oxidative phosphorylation. Electrons are primarily donated to the ETC from the Krebs cycle, but other sources also contribute. The human enzyme dihydroorotate dehydrogenase (DHODHase), an integral part of the *de novo* synthesis of pyrimidines, is coupled to the ETC [2, 3]. The activity of the enzyme is dependent on its ability to transfer electrons to the ETC. ATP is the primary product of oxidative phosphorylation, but certain molecules of ROS are also generated continuously [4, 5]. At subtoxic concentrations, ROS has been demonstrated to function as second messenger molecules proposed to report oxygen availability for oxidative phosphorylation and energetic yield, affecting epigenetic marking of nDNA and regulating nuclear transcription factors, kinases, and phosphatases as reviewed by Weinberg and Chandel [6]. However, at increased levels, ROS induces oxidative damage to lipids, proteins, RNA, and DNA.

Mutation of mtDNA has been correlated with aging and cancer. Mutations of mtDNA that results in an aberrant expression of mitochondrial encoded ETC subunits have been demonstrated to impair the activity of the ETC and be correlated with a decreased capacity for oxidative phosphorylation [7, 8]. In turn, as will be reviewed, ETC dysfunction has been demonstrated to affect the production of ATP and ROS, alter the expression of several nuclear genes, affect the regulation of proteins, and disturb the synthesis of cellular nucleotides. The focus of this paper is on the immediate effects of mtDNA alterations and their potential role in premature aging and tumor progression.

2. mtDNA Fidelity Is Correlated with Aging and Cancer

The lifespan of both mice and aging human cultured cells has been associated with decreases in the number of mitochondria and changes of mitochondrial morphology [9–11]. These alterations are accompanied by an accumulation of mutations in the mtDNA [12–16]. Accordingly, an age-related decline of mitochondrial capacity for oxidative phosphorylation has been demonstrated in both human skeletal muscle and rat hearts [7, 8, 17].

Increasing evidence suggests an important role of accumulating mtDNA mutations in the pathogenesis of many age-related neurodegenerative diseases as well as a number of age-related pathological alterations of heart, skeletal muscle, and the vascular system [18–21]. A strong correlation between the fitness of mtDNA and age-related pathologies have been demonstrated with the independent construction of two mouse lines expressing mutated versions of the mitochondrial polymerase gamma. Both mouse lines developed a mtDNA mutator phenotype, linking the increase of somatic mtDNA mutations with symptoms of premature aging and reduced lifespan [22, 23]. Furthermore, in a longevity study, human life length could be associated with the life length of the mother but not the father, suggesting an influence of the maternal inherited mitochondrial genome [24, 25].

Warburg formulated a relationship between mitochondria and cancer with the discovery that most tumors relied on ATP production by glycolysis rather than oxidative phosphorylation [26]. Alterations of mtDNA have been correlated with tumor progression and have been reported in a variety of cancers including ovarian, thyroid, salivary, kidney, liver, lung, colon, gastric, brain, bladder, head and neck, and breast cancers [27–29]. Reported alterations include point mutations, deletions, and depletions. Alterations of mtDNA might merely be a consequence of tumor progression, however, it has been demonstrated that the invasive phenotype of human cells depleted of mtDNA can be reversed by reintroducing exogenous wild-type mitochondria [30, 31]. Furthermore, construction of a cybrid cell line of prostate cancer cells harboring specific mtDNA mutations has in nude mice been demonstrated to have a growth advantage over cybrids of prostate cells with functional mtDNA [32, 33]. As a result, cybrid cancer cells with mtDNA mutations have the potential of forming a tumor 7-fold larger than cybrid cancer cells with functional mtDNA [32]. Together these indications suggest that mtDNA alteration is directly involved in tumor progression and not merely a consequence of it.

3. Accumulation of mtDNA Mutations

The circular human mtDNA are present in several copies in each mitochondrion. The mtDNA molecule consists of 16539 base pairs that constitute 37 genes encoding 22 tRNAs, 2 rRNAs, and 13 polypeptides. The encoded polypeptides comprise few but essential subunits of the ETC and ATP synthase [34, 35]. The ETC maintains an electrochemical potential gradient between the intermembrane space and the matrix of the mitochondria. This gradient is utilized by the ATP synthase to generate ATP by oxidative phosphorylation. The ETC consists of four membrane-bound enzyme complexes (complex I–IV), two electron carriers (ubiquinon and cytochrome c), and is located in the inner membrane of the mitochondria. Mutations in mtDNA affect the function of the ETC, the electrochemical gradient, and the generation of ATP by oxidative phosphorylation. Furthermore, mutations of mtDNA can result in an elevated production of ROS.

Somatic mutations in the mtDNA have been suggested to accumulate during the life span of humans. Accordingly, it has been demonstrated that brain, muscle, heart, and skeletal muscle of aging humans harbor an increased mutational load of the mitochondrial genome when compared to corresponding tissues of young [5, 8, 12, 36]. The age-correlated accumulation of mtDNA mutations has been proposed to be the cumulative result of a mitochondrial vicious circle and a preferential accumulation of specific mtDNA mutations [37]. According to the theory of a mitochondrial vicious circle, mutations in either nuclear or mitochondrial genes encoding subunits of the ETC will impair electron transfer leading to increased ROS production [38]. Consequent oxidative damage to mtDNA induces alterations of the mtDNA-encoded polypeptides of the ETC leading to further ROS production, thus establishing a vicious circle resulting in an accumulation of mtDNA mutations.

In a variety of diseased and aging tissue, cells of the tissue are a mosaic of cells containing non-mutated mtDNA molecules and cells containing mutated mtDNA molecules [16, 36, 39, 40]. Cells containing mutated mtDNA are often homoplasmic indicating that the mutations are not the result of random oxidative damages from mitochondrial ROS. Rather, these mutations are the consequence of a preferential accumulation of a specific mutated mtDNA molecule, which has become the predominant species within a single cell. The mechanism of this selection is unknown, however it has been hypothesized that mitochondria containing mutated mtDNA causing a reduced respiratory function will have a lower production of ROS and consequently have a lower risk of oxidative damage of the mitochondrial membrane. Mitochondria with a reduced respiratory function will therefore have a lower risk of suffering lysosomal degradation allowing the affected mitochondria to populate the cell [41]. In contrast, *in Silico* models have demonstrated the potential of a single mutated mtDNA molecule to clonally expand in postmitotic cells without the requirement of any types of selection but rather through random genetic drift in a pool of replicating mtDNA molecules [42]. Age-related accumulation of mtDNA mutations is therefore likely either a result of a mitochondrial vicious circle, preferential accumulation of specific mtDNA mutations, or the cumulative outcome of both factors.

4. Mitochondria-Produced ROS Can Damage Cellular Components

More than 90% of cellular oxygen uptake is utilized in the process of oxidative phosphorylation which continuously

generates ROS such as superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$), [5, 43]. ROS are generated to an extent of 1–4% of the oxygen consumed by the mitochondria [4, 5]. Complex I and especially complex III are the prime sites for electron leakage to molecular oxygen yielding $O_2^{\cdot -}$ [44, 45]. The production of ROS is inversely correlated with the rate of electron transport, increasing exponentially when complex I or III are impaired [46]. The main mediator of electron leakage is the reduced form of ubiquinone, ubiquinol, which is able to reduce molecular oxygen [47]. In order to neutralize the produced ROS, a number of antioxidant defenses are active within the mitochondria. $O_2^{\cdot -}$ is neutralized by intramitochondrial Mn superoxide dismutase (SOD2) catalyzing the formation of H_2O_2. The latter either diffuses out of the mitochondria or is inactivated by reaction with glutathione catalyzed by glutathione peroxidase [48–50]. If the amount of produced ROS exceeds the capacity of SOD2 and glutathione peroxidase, $O_2^{\cdot -}$ and H_2O_2 levels will rise. In the presence of transition metals, such as iron or copper, highly reactive $OH^{\cdot}$ can be produced by Haber-Weiss or Fenton reactions. $OH^{\cdot}$ can in turn give rise to a plethora of ROS, which can further damage proteins, lipids, and DNA [43, 51, 52].

The mitochondrial production of ROS is an essential component of the free radical theory of aging formulated by Harman [53–55]. According to the theory, mitochondria-produced ROS induces oxidative damage to lipids, proteins, and DNA in mitochondria and potentially in nucleus. Consequently, ROS production is considered one of the causes of aging and age-related pathologies [38, 56–58] and a contributing factor in the formation of cancers [59, 60]. In support of this theory it has been demonstrated that inactivation of the *Sod2* gene in mice lead to a 2-3-fold increase of oxidative damages of the nuclear DNA in heart and brain tissue when compared to mice expressing SOD2 [5]. Furthermore, it has been demonstrated that an overexpression of a human catalase targeted for the mitochondria, prolonged median and maximal lifespan of mice by approximately 20% and enhanced exercise performance when compared to wild-type littermates [61, 62]. Mice overexpressing the mitochondrial targeted catalase did not display any adverse side effects, but rather a decrease in a select group of age-related pathologies [63]. Induced mutations in genes encoding subunits for complex II have been demonstrated to result in increased production of $O_2^{\cdot -}$ and H_2O_2 in hamster fibroblasts. The increased production of ROS was cooccurring with an increase of aneuploidy that could be reversed by expression of complex II subunits without mutations [59]. This led the authors to augment that the increase of mitochondria-produced ROS resulted in genomic instability.

5. Mitochondrial ROS Functions as Second Messenger Molecules

Despite having been described mainly as a detrimental byproduct of oxidative phosphorylation for more than 50 years, it has become evident that certain types of ROS function as second messengers under subtoxic concentrations. As such, ROS has been demonstrated to regulate gene expression by controlling transcription factors and to affect protein activity by regulating kinases and phosphatases as reviewed by Weinberg and Chandel [6]. The regulative effects of ROS are exerted through their redox potential. As an example, several ROS sensitive molecules contain cysteine rich proteins, in which ROS-induced oxidation can result in formation of disulfide bonds in the same molecule or between two cysteine rich molecules. The formation of disulfide bonds can therefore lead to conformational changes of a molecule or result in the dimerizations of two or more molecules, thereby modulating activation and activity of the molecules [64].

Molecules that are regulated by ROS have been demonstrated to be involved in cell survival, cell cycle control, apoptosis, differentiation, and several stress responses. Abnormal signaling elicited by aberrant ROS production can therefore affect essential pathways of the cell, potentially initiating an incorrect cellular response to a given situation, increasing the risk of senescence or tumorigenesis [6, 65]. Mitochondria-produced ROS have been demonstrated to affect the transcription factor hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimer consisting of the constitutively expressed subunit HIFβ and the oxygen sensitive subunit HIF-1α. Upon expression HIF-1α is marked by ubiquitination and subsequent degraded by the proteasome [66]. However, an increased production of mitochondrial ROS has been demonstrated to stabilize HIF-1α, allowing the subunit to dimerize with HIFβ and forming the active nuclear transcription factor. Active HIF-1 is known to regulate the expression of glycolytic enzymes, the angiogenic factor VEGF and affect pathways promoting apoptosis [67–69] and aberrant activity of HIF-1 has been correlated with tumorigenesis as reviewed by Weinberg and Chandel [6]. Furthermore, loss of HIF-1α has been demonstrated to accelerate premature cellular senescence in mice [65].

Mitogen-activated protein kinases (MAPK) are a superfamily of serine/threonine protein kinases that function as signal transducers that propagate stimuli from growth factors and a wide variety of cellular and extracellular stresses [70]. The MAPKs are critical for correct regulation of gene expression, cell cycle progression, apoptosis, and other cellular activities in response to these stimuli. Mitochondria-produced ROS has been demonstrated to induce phosphorylation and thereby activation of the p38 MAPK in cardiomyocytes [71]. Furthermore, the ERK1/2 MAPK is activated by exogenously added H_2O_2 [72] and in eosinophils this activation is inhibited if the cells are treated with rotenone, an inhibitor of complex I of the ETC [73]. The authors interpreted this to the importance of mitochondrial respiration in activation of ERK1/2, however, rotenone is demonstrated to induce the production of mitochondrial ROS [74], and it is therefore possible that mitochondrial ROS rather than mitochondrial respiration is the effector of the ERK1/2 regulation. MAPKs are critical for correct cellular response to a variety of stimuli including mitogenic factors and stresses, and concurrently aberrant function and regulation of p38 MAPK and ERK1/2 has been correlated

with both senescence, misregulation of apoptosis, and tumor initiation [75–77].

HIF-1, p38 MAPK, and ERK1/2 constitute a nonexhaustive list of biological molecules regulated either directly or indirectly by mitochondria-produced ROS. The purpose of this regulation is most likely for the cell to react upon oxygen availability for oxidative phosphorylation, energetic yield, and to respond to increased oxidative load and mitochondrial stresses. As such, the targets of this regulation are therefore involved in critical cellular pathways, and it is no surprise that the majority of pathways that can be regulated by mitochondrial ROS have been correlated with cancer and senescence upon deregulation.

An abnormal production of mitochondrial ROS can therefore elicit detrimental effects on the cell, causing both oxidative damages on cellular components and most likely, an incorrectly regulated second messenger affecting critical cellular pathways.

6. Mitochondrial Regulation of the Nuclear Epigenome

Mitochondrial dysfunctions invoke mitochondria-to-nucleus retrograde responses in human cells [78]. Cells devoid of functional mitochondria (ρ^0) are useful in studies aiming to gain insight into the possible role of mitochondria in regulating or being associated with the epigenetic alterations of the nuclear genome, either gene specific or genome wide, particularly at the level of DNA methylation. It has been shown that depletion of the mitochondrial genome results in aberrant methylation of promoter CpG islands (high CG rich regions) normally unmethylated in the parental cell line. Conversely Smiraglia et al. [79] has shown that, at specific loci, the 51 UTR comprising a CpG island was partially hypomethylated in ρ^0 cells compared to the parental cell line in which this region was completely hypermethylated. It was suggested that the partial loss of genomic DNA methylation could be associated with the loss of mtDNA and mitochondrial function. Repletion of wild-type mitochondria in ρ^0 cells (mtDNA deficient) resulted in the partial re-establishment of some methylation profiles to the original parental state. Accordingly, this study provides an interesting depiction of the possible role of mitochondria in establishing or maintaining genomic (nuclear) DNA methylation. Interestingly, increasing evidence is emerging on an interdependent relationship between the mitochondria and the nuclear genome. It is well established that expression of nuclear genomic DNA is regulated by epigenetic factors, and consequently, an understanding of how mitochondria regulate nuclear epigenetics is of great importance.

7. mtDNA and Apoptosis

Mitochondria play a central role in initiation of the intrinsic pathway of apoptosis by releasing mitochondrial proteins, which normally reside in the intermembrane space, into the cytosol. This triggers assembly of the apoptosome and activation of procaspase-9, leading to a cascade of events which ultimately leads to cell death. Several studies have indicated that mtDNA mutations are associated with mitochondrial induced apoptosis in aging mouse models. It has been demonstrated that a genetically engineered mouse model expressing proofreading-deficient mitochondrial DNA polymerase gamma accumulate mtDNA mutations and display a premature aging phenotype [23]. It was further demonstrated that cleaved caspase-3 levels increased with aging in various organs of the mouse model, suggesting increased apoptotic activity [23]. Furthermore, Hiona et al. [80] demonstrated an increased level of mitochondria-elicited apoptosis in muscle tissue of the same mouse line by observing increased caspase-9 activity and a significant positive correlation between caspase-9 and caspase-3 activity. The increased level of apoptosis was accompanied by a reduction in the mitochondrial membrane potential [80]. Reduction in the mitochondrial membrane potential has been demonstrated to affect the mitochondrial matrix condensation *in vitro*, and thus the release of proapoptotic cytochrome c into the cytosol [81]. It has also been demonstrated that aging human colonic cells displaying respiratory chain deficiency have a significant higher apoptotic frequency compared to normal human colonic cells [82], indicating that respiratory deficiency induces apoptosis. However, Hiona et al. [80] demonstrated that mtDNA mutations in the mouse model of their study caused no change in activity of the ETC complexes or ROS production, however, they did find a marked decrease in ETC complexes and ATP production, suggesting that accumulation of mtDNA mutations associated with ETC dysfunction and altered membrane potential may lead to activation of the intrinsic apoptotic pathway. Thus these studies indicate a link between increased apoptosis and aging.

8. Mitochondrial Dysfunction and the Cytosolic Nucleotide Metabolism

Rate-limiting steps of the metabolism of cytosolic ribonucleotides and deoxyribonucleotides take place in the mitochondria and can be affected by the fitness of the organelle as reviewed by Desler et al. [83]. In accordance, several studies have demonstrated a correlation between mitochondrial dysfunctions affecting the ETC and aberrant synthesis of cytosolic ribonucleotides and deoxyribonucleotides [78, 84, 85]. Deoxyribonucleotides are exclusively destined for DNA synthesis in the form of deoxyribonucleotide triphosphates (dNTP), but ribonucleotides have a multitude of roles in RNA synthesis in the form of ribonucleotide triphosphates (rNTP), as chemical transporters in form of ATP and in the form of basic second messenger molecules. Disruption of the intracellular levels of deoxyribonucleotides or ribonucleotides is unfavorable as imbalance of the dNTP pools can induce a variety of genetic changes such as base substitutions, frameshift mutations, delay of replication fork progression, and DNA replication as well as increase in the frequency of fragile sites [86–91]. Decreased levels of rNTP pools inhibit RNA synthesis, likely by inhibiting the initiation frequency of RNA polymerase I, and thereby inhibiting the

synthesis of rRNA [92]. Furthermore, inhibition of purine and pyrimidine synthesis induces a p53-mediated cell cycle arrest and inhibits cell proliferation, ultimately leading to increased cytotoxicity [93–96].

The *de novo* synthesis of pyrimidines is directly linked to the ETC by the flavoenzyme dihydroorotae dehydrogenase (DHODHase). DHODHase catalyzes the conversion of dihydroorotate to orotate by oxidation. Subsequent catalytic steps convert orotate into uridine monophosphates that can be further converted to UTP and CTP, and ultimately, dTTP and dCTP, respectively. DHODHase is located in the inner membrane of the mitochondria with the active site facing the inner membrane [2]. DHODHase is functionally connected to the ETC by a flavin prosthetic group that couples dihydroorotate oxidation to respiratory ubiquinone reduction [3]. From ubiquinol, the flow of electrons continues through the ETC. Leflunomide and brequinar are inhibitors of DHODHase that bind to the quinone-binding site, thereby blocking interaction between ubiquinone and the flavin prostethic group of DHODHase [97]. Treatment of human lymphocytes with leflunomide or brequinar arrests the cells in G1 phase and inhibits both RNA and DNA synthesis [98–100]. The inhibitory effects are suppressed by addition of uridine which can be salvaged to UMP, where by the *de novo* synthesis of pyrimidines is bypassed. Treating the human leukemic cell line CCRF. CEM with leflunomide or brequinar cause a significant reduction in the levels of CTP and UTP, while the levels of purine nucleotides remain unaffected by leflunomide but increased by brequinar [101]. Furthermore, our unpublished data indicate that a leflunomide mediated inhibition of DHODHase in a human cervical cancer cell line results in decreased levels of dTTP and dCTP (Data not shown). Together these results demonstrate the importance of the activity of DHODHase for cytosolic levels of pyrimidine nucleotides. It has been suggested that any dysfunction of the ETC; lack of oxygen, presence of inhibitors, or mutations of complex III and IV, would entail impairments of the *de novo* UMP synthesis, and a subsequent decrease in the *de novo* synthesis of cytosolic nucleotides [78, 85]. In support of this argument it has been demonstrated that a chemical inhibition of the ETC causing a buildup of ubiquinol has an inhibitory effect on DHODHase [85]. Inhibition of the ETC caused by dysfunctional ETC subunits encoded by mutated mtDNA is therefore likely to have an effect on the activity of DHODHase. Treatment of cells with chloramphenicol inhibits mitochondrial protein synthesis, and mimics an mtDNA-induced mitochondrial dysfunction, impairing ETC activity. In chick embryo cells, treatment with chloramphenicol was demonstrated to inhibit DHODHase activity and cell growth [102]. Growth inhibition was reversed by addition of pyrimidines to the growth media. This indicates that mitochondrial dysfunction affecting the ETC has an inhibitory effect on DHODHase activity that is comparable to inhibition with leflunomide or brequinar. This conclusion is substantiated by the fact that cultured mammalian cells devoid of mtDNA are auxotrophic for pyrimidines, and must be routinely grown in the presence of a uridine supplement [103].

In summary, the DHODHase links cytosolic nucleotide metabolism with the ETC. Inhibition of DHODHase or the ETC has been demonstrated to decrease the levels of cytosolic pyrimidines. It is possible that an inhibition of the ETC induced by damaged ETC subunits encoded by mutated mtDNA can cause an imbalance of cytosolic nucleotides, which in turn can induce detrimental genetic changes that are also apparent in tumor formation and aging.

9. Conclusion

For the better half of a century, both aging and cancer formation have been associated with divergent mitochondrial function. Since first suggested, the correlation between mitochondrial dysfunctions and aging and cancer has been debated as merely symptoms of aging and cancer. However, with the generation of mouse lines containing a mtDNA mutator phenotype [22, 23] and with the usage of cybrid mechanisms in cell cultures [30–33], it has been demonstrated that mtDNA aberrations can be correlated with at least certain aspects of aging and cancer progression. In this paper we have described different immediate effects of mtDNA aberrations, and their potential role in inducing premature aging, or promote tumor progression. These effects encompass (1) altered mitochondrial production of ROS, (2) mitochondrial regulation of the nuclear epigenome, (3) mitochondrial initiation of apoptosis, and (4) mitochondria induced inhibition of de novo synthesis of rNTP and dNTP (see Figure 1). Where 1, 3, and 4 can be directly linked to the mtDNA aberrations and their effect on the ETC, the link between mitochondria and regulation of the nuclear epigenome is unknown and likely multifaceted. All of the effects have been correlated to either cellular senescence or organismal premature aging and cancer progression. Even though, all the described mechanisms can be related to aging and cancer, the effect exerted by these mechanisms in response to mtDNA aberrations is most likely dependent on the type of tissue and cells exposed. For example, a mitochondrial induced inhibition of *de novo* synthesis of rNTP and dNTP will not have an adverse effect on cells that are primarily reliant on rNTP and dNTP produced by salvage pathways. Similarly, a burst of mitochondrial ROS will have a smaller risk of inducing cellular damage if the cell or tissue type affected has sufficient antioxidant defenses.

The activity and efficiency of the ETC and thereby the capacity for oxidative phosphorylation is directly correlated with the fidelity of the mitochondrial genome [7, 8]. The fidelity of the mitochondrial genome is determined by a variety of factors including the specific genome region, the type of mutation, and the ratio between unaffected and affected mtDNA molecules in a mitochondrion and in the cell. Correspondingly, ROS production is low in mitochondria with a functional ETC and increased when especially the mtDNA encoded subunits of complex I and III are mutated [44, 45]. However, the fidelity of the mitochondrial genome can be compromised to a threshold where the mtDNA-encoded peptides become overloaded with mutations such that the ETC is unable to support an

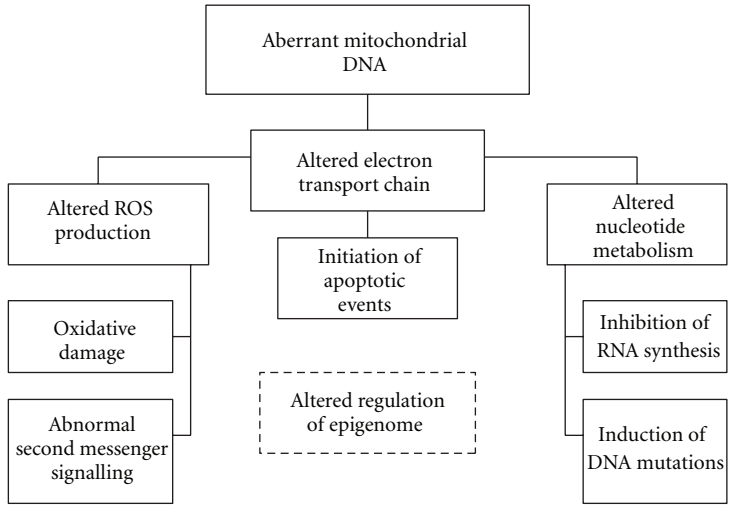

Figure 1: Overview of effects of mtDNA aberrations correlated aging and cancer progression. Aberrant mitochondrial DNA causes a reduced fidelity of the electron transport chain, which in turn can alter the production of reactive oxygen species (ROS), prematurely initiate apoptotic events and alter the nucleotide metabolism. An increased production of ROS can cause oxidative damage to cellular components including nuclear DNA, while both a decrease and increase of ROS can compromise its role as a second messenger molecule, affecting several cellular mechanisms. An altered nucleotide metabolism can result in a altered production of ribonucleotides and deoxyribonucleotides increasing the risk of inhibition of RNA synthesis and DNA mutations, respectively. Aberrant mitochondrial DNA can also alter the regulation of the nuclear epigenome even though the mechanism responsible has not been elucidated.

electron transport. In this situation, the ETC can neither perform oxidative phosphorylation nor produce ROS. As the capacity for oxidative phosphorylation and the production of mitochondrial ROS are correlated with the fidelity of the mitochondrial genome, the immediate effects of mtDNA aberrations most likely elicits their maximal destructive effects at different points in the course of the degradation of the mitochondrial genome. The ability of mitochondria-produced ROS to induce oxidative damage to cellular components are maximal in situations where the fidelity of the mitochondrial genome allows transport of electrons through the ETC, but when electron leak is so abundant that the cellular antioxidative defenses are saturated and an overload of harmful ROS molecules are allowed to form. In contrast, the role of ROS as signal molecules is affected in situations where the production is both increased and decreased. In cases where ROS as a signal molecule mediates a stress response, an increased production of ROS will potentially trigger an unnecessary range of responses, including stalling of cell cycle or initiation of apoptosis. In contrast decreased production of ROS mediate improper stress responses with affected cells more vulnerable to a given situation. Accordingly both an increased and decreased level of ROS can theoretically increase the risk of senescence or contribute to tumor initiation and progression, in light of the second messenger role of ROS. The activity of the DHODHase is inversely correlated with the activity of the ETC. Therefore, the risk of a mitochondrial induced imbalance of the cellular rNTP and dNTP pool is most pronounced when the ETC is unable to transport electrons.

It is an important realization that aberrations of mtDNA can induce the risk of cellular senescence, organismal premature aging, and cancer progression through a variety of different mechanisms, dependent on the type of mtDNA alteration and the cell or tissue type affected. As a consequence, the mechanism linking mitochondrial dysfunction to cancer and aging can no longer be perceived as a simple mechanism where oxidative damage to cellular components induced by mitochondria-produced ROS is the main mediator. Rather this pathway is complex and mediated by several critical cellular events affected by mitochondrial function.

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