Published in final edited form as:
Mech Ageing Dev. 2008 ; 129(7-8): 383–390. doi:10.1016/j.mad.2008.03.002.

Mitochondrial DNA repair in aging and disease

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Introduction

Mitochondria are membrane-enclosed organelles that are found in eukaryotic cells. Often they are described as “cellular power plants”, because they generate most of the cell’s supply of ATP which is used as a source of chemical energy. The synthesis of ATP occurs through the respiratory or electron transport chain (ETC) which is located at the inner mitochondrial membrane and consists of five protein complexes (complex I to V). Mitochondrial respiration accounts for approximately 90% of cellular oxygen consumption. Most of the oxygen that is consumed is reduced to water through four consecutive one-electron reductions. During this process, a small proportion of the oxygen molecules (1–2%) are converted to superoxide anion radicals. Initially, it was believed that there were two main sites of superoxide production in the respiratory chain, Complex I and Complex IV (1). However, recent studies have identified at least nine submitochondrial reactive oxygen species (ROS) generating sites (2). The superoxide that is formed then dismutates either spontaneously or enzymatically through the action of superoxide dismutase to form hydrogen peroxide (3). Hydrogen peroxide then can diffuse throughout the cell and decompose to form noxious hydroxyl radicals, which can injure the cell through interactions with macromolecules. Because of these processes, mitochondria are a major source for physiological or endogenous production of ROS. Although much attention has been focused on the harmful effects of ROS, it now has become apparent that mitochondrially-generated ROS also are involved in the regulation of intracellular signal transduction pathways leading to cellular activities such as proliferation (4). In addition to supplying ATP, mitochondria are required for numerous other cellular functions including biosynthesis of heme, cholesterol and phospholipids (5). Also, mitochondria play a major role in initiating the process of apoptosis (6).

Mitochondria are organelles which, according to the endosymbiosis theory, evolved from purpurbacteria approximately 1.5 billion years ago (7,8). One of the features of mitochondria is that they have their own genome. Mitochondria replicate and transcribe their DNA semiautonomously. Like nuclear DNA, mitochondrial DNA (mtDNA) is constantly exposed to DNA damaging agents. Regarding the repair of mtDNA, the prevailing concept for many years was that mtDNA molecules suffering an excess of damage would simply be degraded to be replaced by newly generated successors copied from undamaged genomes. However, evidence now clearly shows that mitochondria contain the machinery to repair the damage to their genomes caused by certain endogenous or exogenous damaging agents. The link between mtDNA damage and repair to aging, neurodegeneration, and carcinogenesis-associated processes is the subject of this review.

*This research was supported by National Institutes of Health Grants ES03456, ES05865, NS047208, and AG19602.

Mitochondrial genome

Mitochondrial DNA comprises 0.1–2% of the total DNA in most mammalian cells. There are several very unique features of the mitochondrial genome. First, in humans, it is a circular negatively supercoiled double-stranded molecule that is 16,569 bp long. It encodes two rRNAs, 22 tRNAs and 13 polypeptides, of which seven are components of Complex I (NADH dehydrogenase), three are components of Complex IV (cytochrome c oxidase), two are subunits of Complex V (ATP synthase) and cytochrome b (a subunit of Complex III) (11). The rest of the polypeptides of the ETC complexes, including all the subunits of Complex II (succinate dehydrogenase), as well as approximately 1500 other proteins which function in mitochondria are encoded by nuclear genes, synthesized in the cytosol and imported into mitochondria through various protein import systems. Second, genetic information is not distributed equally on the two mtDNA strands. The two mtDNA strands can be separated by denaturing cesium chloride gradient centrifugation (9). Most of the information is encoded in the heavy (purine-rich) strand. The light (pyrimidine-rich) strand contains genetic information for only one polypeptide and 8 tRNAs. Third, mitochondrial genes have no introns and intergenic sequences are absent or limited to a few bases. Some genes overlap and in some instances, termination codons are not encoded (10). Fourth, the inheritance of mtDNA is almost exclusively maternal, although some important exceptions have been reported (11–13). Fifth, mitochondria do not have histones. However, mammalian mtDNA is organized in nucleoids, which can be seen under the microscope as punctuate structures containing mtDNA and proteins which localize to the matrix surface of the mitochondrial inner membrane. Mitochondrial DNA binding proteins such as transcriptional factor A (TFAM), mtSSB (mitochondrial single stranded DNA-binding protein) and Twinkle have been shown to colocalize with mtDNA in intramitochondrial foci in live cells (14). The molecular structure of the nucleoid has only partially been elucidated for yeast and much less is known about its mammalian counterpart.

Another important piece of information about mtDNA is that this genome is totally dependent upon nuclear-encoded proteins for its maintenance and transcription. Regarding replication, mtDNA replicates throughout the lifespan of an organism in both proliferating and post-mitotic cells in order to maintain a constant supply of genetic material so that mitochondria can undergo continuous turnover. The mean lifetime of a mtDNA molecule has been estimated to be 2 and 4 weeks in rat liver and brain cells respectively (15). Replication of mtDNA is conducted by DNA polymerase gamma (DNA pol γ). Among the 16 known eukaryotic DNA polymerases, DNA pol γ is the only one to have been detected in mammalian mitochondria (16). According to the strand-asymmetric model, mtDNA replication occurs bi-directionally, being initiated at two spatially and temporally distinct origins of replications OH and OL, for the heavy and light strands respectively (17). However, this paradigm recently has been challenged, and evidence exists which suggests the presence of conventional duplex mtDNA replication intermediates, indicative of coupled leading and lagging-strand DNA synthesis (18).

Mitochondrial DNA damage and repair

Mitochondrial DNA, just like its counterpart in the nucleus, is constantly exposed to damaging agents such as ionizing radiation, environmental toxins, as well as many therapeutic drugs. Mitochondrial DNA suffers damage from toxic chemicals to a greater extent than does nuclear DNA (19). Because the mitochondrial membrane potential generates a negative charge on the matrix-side of the inner membrane, lipophilic cations tend to accumulate in mitochondria, specifically in mitochondrial membranes. Mitochondria import lipophilic cations from the cytosol and concentrate them up to 1000-fold (20). Many drugs and biologically toxic chemicals are lipophilic and have positive charges and are thus concentrated in mitochondria and are a threat to mitochondrial components. For example, mtDNA is modified by alkylating agents at least 10-fold more than is nuclear DNA (nDNA) (19).

Mitochondrial DNA is vulnerable to oxidative damage from both exogenous and endogenous ROS due to its proximity to the ETC, and to the lack of protective histones (21). Oxidative damage to mtDNA may be in the form of base modifications, abasic sites and various other types of lesions (22). One of the most studied lesions is 8-oxoguanine (8-oxoG), which is formed by a variety of oxidative treatments. 8-oxoG has been found to be a mutagenic lesion. Mispairing of 8-oxoG with adenine results in a G-C to T-A transversion during the subsequent round of replication. Early studies, using HPLC-electrochemical detection of the 8-oxoG, described 16-fold higher levels of 8-oxoG in mtDNA than in nDNA (23). Further studies revealed that 8-oxoG accumulates with age in nDNA and to a greater extent in mtDNA (24, 21). However, others reported that the damage in mitochondria was overestimated, and its level is comparable to that of nuclear DNA (25,26). In more definitive studies, Yakes and Van Houten employing the method of quantitative extended-length PCR (QXL-PCR) showed that mtDNA damage is more extensive and persists longer than nDNA damage in human cells following oxidative stress (27).

Initially, it was thought that DNA repair mechanisms were either non-existent or very inefficient in mitochondria and that damaged DNA molecules were simply degraded, and undamaged copies served as templates for new mtDNA synthesis. Such views were based on early experiments showing that UV-induced pyrimidine dimers were not repaired in mtDNA (28). However, subsequent studies have demonstrated that certain types of damage to DNA bases resulting from deamination, simple alkylation and oxidation can be efficiently repaired in mitochondria (reviewed in 29–31). Thus, the major DNA repair mechanism acting in mitochondria is base excision repair (BER) (29–34). In general, BER starts with recognition and removal of a damaged or inappropriate base by a DNA glycosylase that cleaves the N-glycosylic bond between the base and the sugar. Many glycosylases have an associated lyase activity that breaks the phosphodiester backbone 3′ to the abasic site. Apurinic/apyrimidinic (AP) endonuclease then cleaves the 5′ phosphodiester bond leaving a one-nucleotide gap. For glycosylases, like those that remove uracil, without an associated lyase activity, the action of AP endonuclease generates a deoxyribose-phosphate moiety which must be removed by the lyase activity in DNA polymerase γ (35). In both cases the resulting 3′-hydroxyl moiety can be extended by DNA polymerase γ and DNA ligase activity completes the repair event by joining the free DNA ends.

Mitochondrial BER has been demonstrated by numerous groups and shown to target oxidatively modified DNA bases, such as 8-oxoG and thymine glycol (29–34,36,37). DNA repair enzymes isolated from mitochondria include several types of damage-specific DNA glycosylases (36–39), AP endonuclease (40), and DNA ligase (41,42). DNA glycosylases that are expressed as nuclear and mitochondrial isoforms encoded by the same gene, include uracil DNA-glycosylase (UDG) (43), and 8-oxoG DNA glycosylase/AP lyase (OGG) (44). MUTYH (homologue of the E. coli MutY glycosylase), NTH (thymine glycol glycosylase) and OGG have been demonstrated to localize to mitochondria in rat neurons (45) and human cells (44, 46,47). Recently, it was shown that mitochondrial BER proteins are not freely soluble, but strongly associated with an inner membrane-containing particulate fraction (48). In addition to BER, there is some evidence that mitochondria possess mismatch repair activities (49); homologous recombination (50); and non-homologous end joining (51). Comprehensive lists of the repair proteins that have been isolated from mitochondria have been included in several recent reviews (52–54). However, the significance of these mtDNA repair mechanisms is still a fertile area for investigation.

Mitochondrial DNA repair and aging

There has been a long-standing debate as to whether aging is a process of damage accumulation or an ordered, programmed process that is determined by changes in gene regulation.

Accumulation of oxidative damage is the basis of Harman's free-radical theory of aging (55, 56). One of the main sources for ROS in the cell is oxidative phosphorylation within mitochondria, so that the free-radical theory of aging is essentially a mitochondrial theory of aging. Because mtDNA is extremely vulnerable to oxidative stress, mtDNA mutations accumulate progressively during life and are directly responsible for a measurable deficiency in cellular oxidative phosphorylation activity, leading to enhanced ROS production. In turn, increased ROS production results in progressive mtDNA damage and mutagenesis, thus causing a "vicious cycle" of exponentially increasing oxidative damage and cellular dysfunction, which ultimately culminates in death.

Numerous studies have shown that mtDNA accumulates oxidative damage in an age-dependent manner in skeletal muscle (57–59), cardiac muscle (60–62), brain (63) and liver (64). Specifically, the increase in 8-oxoG levels in mtDNA with age appears to be a general phenomenon and has been reported by de Souza-Pinto et. al (65) and Hudson et. al. (66). The steady-state concentration of 8-oxoG in mtDNA, but not in nDNA, was shown to be inversely correlated with maximum lifespan in the heart and brain of mammals. Slowly aging mammals show lower 8-oxoG levels in mtDNA than rapidly aging ones (67). Persistence of this damage would be expected to cause a high mutation rate in mtDNA. Thus, it is not surprising that mutations, including deletions, duplications, and point mutations, have been found to accumulate in mtDNA in a variety of tissues during aging in humans, monkeys, and rodents (68–73) and cause a mosaic pattern of respiratory chain deficiency in pre- and post-mitotic tissues. The most frequent and best characterized age-associated mtDNA mutation is a 4977-bp deletion also called the "common deletion". Point mutations in the control/D-loop region of human mtDNA accumulate in an age-dependent manner, and age-related large rearrangements of mtDNA have also reported (68). Some of these aging-associated mtDNA mutations were originally observed in the affected tissues of patients with mitochondrial diseases. It has been established that many of these mtDNA mutations start to occur after the mid-thirties and they accumulate with age. The proportions of mutated mtDNA in aging human tissues rarely exceed 1%. To explain how this apparent low level of mutations can be functionally relevant in aging there are debates in the literature concerning mosaic patterns of mtDNA segregation, clonal expansion of many individual mtDNA point mutations, as well as the premise that methodological pitfalls may lead to misinterpretation of some of the results (68,74–77). A definitive explanation remains elusive.

Early studies from our laboratory showed defective repair of oxidative damage in the mitochondrial DNA from a Xeroderma pigmentosum group A cell line (78) and in a Down's syndrome patient's fibroblasts (79). Both of these diseases display some features of accelerated aging. Other authors (80) reported that mitochondrial repair of 8-oxoG is deficient in Cockayne syndrome group B; another segmental premature aging syndrome in humans. Concurrently, several studies have indicated that OGG1 activity in liver and heart mitochondrial extracts from old rats is higher compared with extracts from young animals (65,81). One possible explanation for this apparent disparity was presented in the work of Szczesny et.al. (82). This study investigated the subcellular localization of OGG1 and demonstrated that although *in vitro* OGG1 activity rises in aged hepatocytes most of this enzyme is stuck to the outer membrane of mitochondria and could not translocate to the mitochondrial matrix (82). It also was reported that overall mitochondrial BER activities in rat cerebral cortices gradually decline with age, reaching 80% lower activity in 30-month old rats, compared with 17-day old embryos (87). This decline was attributed to a decreased expression of repair enzymes such as OGG1 and DNA pol γ (83). A very recent publication on age-dependent alterations in mitochondrial and nuclear BER activities in five brain regions of 6-month and 18-month old mice demonstrated a significant age-dependent decrease in uracil, 8-oxoG and 5-OH-C incision activities in mitochondria from all brain regions, whereas variable patterns of changes were seen in nuclei (84).

In many animal models, including yeast, nematodes, fruit flies, and mice, caloric restriction has been show to be a very effective environmental method of increasing lifespan. Calorie restricted rodents have a longer lifespan than their *ad libitum* fed counterparts (85). A recent human trial showed that caloric restriction resulted in the expected reduction in body weight and blood insulin (86). Molecular analysis of this study revealed that caloric restriction and exercise increased the expression of genes involved in nutrient sensing and mitochondrial biogenesis. Additionally, there was an increase in mitochondrial mass. Concurrently, caloric restriction decreased markers of oxidative stress. The authors suggest that caloric restriction induces biogenesis of "efficient" mitochondria as an adaptive mechanism, which in turn lowers oxidative stress (86). Dietary restriction has also been shown to be a modulator of membrane lipid peroxidation and cytosolic antioxidant status (87). Caloric restriction has been reported to lower ROS generation in brain mitochondria (88,89) and prevent age-dependent accumulation of 8-oxoG in rat brain (90,91). Calorie restricted mice were used to investigate whether lifespan extension is associated with changes in mitochondrial BER activities (92). The most significant effect of caloric restriction was a reduction in uracil-initiated BER activity in brain and kidney mitochondria, while this activity in liver was maintained or perhaps slightly enhanced. The authors propose that mitochondrial polymerase γ might be involved in regulation of mitochondrial BER activity in calorie restricted mice (92). Since polymerase γ is the only known polymerase in mitochondria and is responsible for both repair and replication, it is reasonable to expect that it is involved in aging associated repair of mtDNA damage. Trifunovic et.al, recently developed knock-in mice that express a proof-reading deficient version of DNA pol γ and studied these mice for mtDNA mutations and mitochondrial age-related changes (93). They observed a three to five-fold increase in the level of point mutations and deletions in mtDNA. Clinically, these mutant mice developed progeroid symptoms such as reduced lifespan, weight loss, reduced subcuntaneous fat, osteoporosis, alopecia, kyphosis, and reduced fertility (93). Another group (94) confirmed these observations but could not detect elevated levels of ROS in their knock-out mice. However, they did find increased apoptosis in tissues with rapid cellular turnover. They suggested that the aging phenotype in these mice could be due to apoptosis, which in this case could come from decreased maintenance of mtDNA through restricted repair of replicating mtDNA (94). These findings suggest a causative link between mtDNA mutations and aging phenotypic changes in mammals and provide further proof for the mitochondrial theory of aging. However, a recent study in mice using a sensitive assay to measure the *in vivo* rate of change of the mitochondrial genome at a single-base pair level challenges this theory (95). Although the authors observed an 11-fold increase in mitochondrial point mutations with age, they reported that a mitochondrial mutator mouse was able to sustain a 500-fold higher mutation burden than normal mice, without any obvious features of rapidly accelerated aging (95).

Generally, all observations, mentioned above, suggest that modifications in mtDNA repair may contribute to the accumulation of DNA damage associated with aging.

Mitochondrial DNA repair in neurodegeneration and cancer

In the 1980s it was discovered that mutations in mtDNA could be pathogenic. Since that time a number of human diseases have been linked to mutations in the mitochondrial genome. Among them are common pathological states, including premature aging, cancer, diabetes mellitus, and neurodegenerative disorders. The recent release of the Mitomap (Human Mitochondrial Genome Database) lists almost 200 pathogenic point mutations, single nucleotide deletions and insertions (http://mitomap.org.). In contrast to diseases caused by mutations in nDNA, mutations in mtDNA might not be fully expressed. This is because each mammalian cell contains 100 to 1000 mitochondria, each of which carries 2 to 10 copies of mtDNA. If all of the mtDNA in a cell has a single sequence (whether mutated or normal) the cell is homoplasmic. If some of the mtDNA copies have a normal base sequence and others

have a mutated sequence the cell is heteroplasmic. With mitochondrial diseases the concept of threshold burden has been proposed. This means that when the percentage of mutated genomes rises above a certain level, noticeable symptoms will be observed. When the percentage of mutated DNA far exceeds this threshold then an acute (and sometimes lethal) condition will be observed (96). Many mitochondrial diseases have a delayed onset and progressive course and they result in many of the same clinical manifestations that are observed in age-related diseases.

Neurodegeneration

Mitochondrial oxidative stress and accumulation of the mtDNA mutations are believed to be particularly devastating to post-mitotic, terminally differentiated cells such as neurons. Mitochondria are central components of synapses, where they provide the energy required for synaptic activities (97). Damage to mtDNA could potentially result in bioenergetics dysfunction and consequently aberrant nerve function. Neurodegenerative diseases are associated with a progressive loss of neurons through apoptosis and/or necrosis. An accumulation of mutations and deletions in mtDNA with corresponding defects in energy metabolism have been found in Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (98–100). As might be expected, these mutations have been correlated with an increase in oxidative damage in the brain. Elevated levels of 8-oxoG have been found in the cortex of ALS patients (101), as well as in mitochondria in the substantia nigra of PD patients (102). Although DNA damage is elevated in both nuclear and mitochondrial DNA in AD brains, mtDNA in AD brains was shown to contain between 3- to 10-fold higher levels of oxidized bases than nDNA (103). Several studies regarding BER activity in neurodegenerative disorders showed increased expression of AP endonuclease 1 (APE1) in AD cortex extracts (104); lower activity of OGG1 in nuclear extracts from AD hippocampal gyri and parahippocampal gyri (105); and increased APE1 level in the nuclear fraction in ALS motor cortex (106). A recent publication reports significant BER deficiencies in brains of AD patients due to limited DNA base damage processing by DNA glycosylases and reduced DNA synthesis capacity by DNA polymerase β (107). Meanwhile, far less is known about how neurodegeneration is associated with alteration in the mtDNA repair pathways.

Studies of whole brain regions do not differentiate between neurons and glial cells. However, evidence from studies using cells in culture suggests that there are cell-specific differences in mtDNA repair capacity between neurons and glial cells. Treating primary rat cultures of astrocytes, oligodendrocytes, and microglia with methylnitrosourea, an alkylating agent, does not alter the amount of initial mtDNA damage, but the repair efficiency was significantly decreased in oligodendrocytes and microglia compared with astrocytes (108). Moreover, the induction of apoptosis correlated with this decrease. These studies were the first to demonstrate a cell-specific difference in repair of mtDNA damage in cells from the central nervous system (CNS), and indicated that this difference correlated with the induction of programmed cell death (108). In a similar study, Hollensworth et al. showed that after exposure to oxidative DNA damage, oligodendrocytes and microglia accumulated more mtDNA damage, and they repaired the damage less efficiently than astrocytes (109). The differential susceptibility of glial cell types to oxidative damage and apoptosis did not appear related to cellular antioxidant capacity, because astrocytes had lower total glutathione content and superoxide dismutase (SOD) activity than did oligodendrocytes and microglia (109). In a subsequent study primary cerebellar granule cells were used to determine if mitochondrial DNA repair efficiencies correlated with oxidative stress-induced apoptosis in neuronal cells (110). Primary cerebellar granule cells had increased basal levels of glutathione and APE1 and were more sensitive to oxidative stress, resulting in less efficient repair of oxidative mtDNA lesions when compared with astrocytes. Of interest, however, is that the glycosylase and APE1 activities in the neurons

were significantly higher with a reduction in polymerase γ activity, suggesting that the granule cells have an imbalance in the mitochondrial BER pathway. It is this imbalance which leads to the observed increase in sensitivity to oxidative stress (110). This evidence provides a link between neuronal mtDNA repair capacity and oxidative stress-related neurodegeneration.

The importance of mitochondrial BER pathways in the development of neurodegenerative disorders was shown in an *in vivo* study examining expression of the DNA repair enzymes in transgenic mice carrying a mutant SOD1 gene, an animal model of ALS (111). The authors observed no changes in mitochondrial OGG1 activity, but down-regulated polymerase γ activity in mitochondria as well as upregulated nuclear OGG1 activity in spinal motor neurons in presymptomatic transgenic mice. They assumed that the early and selective impairment of DNA repair enzymes in mitochondria of spinal motor neurons makes them more vulnerable to oxidative stress, leading to the accumulation of DNA mutations and finally cell death in this animal model of ALS (111). Additionally, a previous report suggested the impairment of mtDNA repair enzymes in human ALS cases (112).

If mitochondrial DNA repair plays a specific role in oxidative stress-induced cell death, the modulation of mtDNA repair efficiency by targeting BER enzymes to mitochondria should enhance cellular defenses of CNS cells. Indeed, targeting hOGG1 to mitochondria of oligodendrocytes enhanced mtDNA repair and protected cells against caspase 9-dependent apoptosis after menadione-induced oxidative stress (113) and cytokines-mediated damage (114). Additionally, when the yeast AP endonuclease Apn1, was expressed in mitochondria of a neuronal cell line derived from rat substantia nigra, it promoted the repair of the oxidative lesions in mtDNA and enhanced the resistance to cell death following oxidative insult (115).

Thus, it can be concluded that mtDNA repair is a critical player in the response of CNS cells to genotoxic insults. Strategies to enhance the DNA repair system in mitochondria may prove useful for retarding the pathogenesis of neurodegenerative diseases.

Cancer

Over 50 years ago Warburg suggested that mitochondria are involved in carcinogenesis through respiration alterations (116). Since then somatic mutations in mitochondrial DNA have been reported in virtually all forms of cancers examined to date (117–119). Mitochondrial DNA mutations often are found in primary tumors but not in surrounding tissues. According to Carew and Huang, the main features of mtDNA mutations common to all tumor types are: (i) the majority of the mutations are base substitutions; (ii) mutations occur in all protein-coding mitochondrial genes; (iii) the D-loop region is the most frequent site of somatic mutations across most tumor types; and (iv) the mutations are homoplasmic in nature (119). Deletions of mtDNA, as well as insertion mutations, also are reported in many kinds of cancer (120). The homoplasmic nature of mutated mtDNA raises the possibility that some mutations are involved in tumorigenesis itself by affecting energy metabolism and/or ROS production. Mitochondrial DNA mutations in tumors also could be due to the effect of aging (93,94). Shidara et al. showed that specific point mutations in mtDNA accelerate growth and reduce apoptosis in a variety of tumors, supporting the notion that some mtDNA mutations in tumors have functional advantages that promote tumor growth (121). Additionally, mitochondria are key players in tumor control by apoptosis. A demonstration of the physiological significance of mtDNA mutations found in cancer was provided recently by studies with transmitochondrial cybrids (“chimeric” cells in which their own mtDNA is destroyed and replaced by mtDNA from other cells). A known pathogenic mtDNA mutation in ATP6 was introduced into PC3 cells, a prostate cancer cell line via cybrid transfer. Injection of the resulting ATP6 mutant cybrids into nude mice resulted in the generation of tumors that were seven times larger than those generated from wild type cybrids (122). In addition, the ATP6 mutant cybrid tumors generated significantly more ROS than their wild type counterparts. These findings are a part of a growing

body of evidence which indicates that cancer cells are under increased intrinsic oxidative stress (123). Since mitochondria are the major source for ROS production in cells, the vulnerability of mtDNA to ROS-mediated damage appears to be a mechanism for amplifying ROS stress in cancer cells. Also, mitochondrial ROS may play a role in carcinogenesis. This notion is supported by the finding that mice that are heterozygous for MnSOD ($MnSOD^{+/-}$) have a 100% increase in tumor incidence which is accompanied by an increase in oxidative damage in nuclear and mtDNA (124).

Proper mtDNA repair mechanisms can protect cells from mutations and increased genomic instability and thus diminish the risk for the development of cancer. Some studies have observed decreased nuclear and mitochondrial hOGG1 expression in human lung cancer (125), and in a mutant rat strain that develops spontaneous hepatocellular tumors (126). The idea of imbalanced mtDNA repair pathways in cancer was tested in several studies examining the effects of over-expression of repair enzymes in mitochondria on cellular response to oxidative and alkylating agents. Previous work from our lab revealed that targeting hOGG1 to mitochondria of a HeLa cancer cell line, which exhibited limited capacity to repair oxidative damage to its mtDNA, resulted in enhanced repair of mtDNA oxidative lesions caused by menadione and, additionally, this augmented repair led to increase cellular viability following oxidative insult (127,128). A recent study from another group confirmed these findings by showing that mitochondrial over-expression of hOGG1 protects HeLa cells from H_2O_2-induced oxidative stress, but fails to defend cells from 4NQO damage, a UV-mimetic agent that induces a wide range of DNA lesions (129). These lesions are mainly repaired through nucleotide excision repair, a mechanism apparently absent in mitochondria. Furthermore, targeting and over-expression of mutant hOGG1 (R229Q mutation of hOGG1, found in human leukemia that impaired hOGG1 activity) to mitochondria resulted in a reduction of both cellular viability and mtDNA integrity after oxidative damage. These authors suggested that such mutations in DNA repair enzymes in cancer may be more detrimental to cellular survival when present in the mitochondria than in nucleus (129). Kim et al. used 4NQO to test mtDNA repair capacity in head and neck carcinomas cell lines (130). They observed mtDNA damage repair deficiencies in two of three cell lines tested in response to 4NQO treatment. Based on these findings, it was suggested that an absence of efficient repair in mitochondria may confer an intrinsic susceptibility to mutation and subsequent carcinogenesis (130).

Obviously, in the case of cancer, researchers are looking for different ways to kill cancer cells. Altering DNA repair mechanisms might be another approach to push cancer cells toward death. Thus, it was shown that the expression of Exonuclease III from E.coli in mitochondria of breast cancer cells diminishes mtDNA repair capacity and cellular viability following oxidative stress (131). Targeting and over-expressing of N-methylpurine DNA glycosylase (MPG) in mitochondria of the breast cancer cell line resulted in increased mtDNA damage following treatment with the alkylating agent methyl methansulfonate, as well as increased apoptosis levels in these cells (132). These findings might have implications in combination therapy approaches; over-expression of mito-MPG and/or MPG could result in increased kill of tumor cells using lower doses of harmful alkylating agents such as temozolomide and cross-linking agents such as cisplatin (132). A recent publication reported that nuclear-targeted MPG (employing adenoviral expression system) sensitized astroglia to methylnitrosourea (133). The authors found that mitochondrial pathways of apoptosis were blocked in astrocytes, which may be responsible, in part, for resistance of astocytomas to chemotherapeutic agents (133).

The other problem yet to be overcome in cancer treatment is how to protect normal cells against harmful effects of chemotherapeutic agents and ionizing radiation treatment. Mitochondrial DNA is a vulnerable target for anticancer drugs. One reason is that the mitochondrial respiratory chain can activate redox-cycling agents and in turn the activated agents produce ROS. Additionally, mitochondria accumulate lipophilic cations, resulting in elevated levels of

mtDNA damage as mentioned above. Therefore, mtDNA repair pathways may be crucial for alleviating some of the side effects of cancer chemotherapy. It was reported that targeting O6-MeG DNA methyltransferase (MGMT) to mitochondria of breast non-tumorigenic epithelial cells protected these cells against alkylation induced cell death (134). Evidence indicates that protection of mtDNA after alkylation is equally as important as protection of nuclear DNA. Cai et al. carried out experiments with hematopoietic cell lines characterized by low repair activity for alkylation DNA damage (135). Transfection with nuclear- or mitochondrial-targeted MGMT generated resistance in these cells against the cytotoxic effects of chemotherapeutic drugs such as 2-chloroethylnitrosourea (BCNU) and temozolomide (TMZ). This effect was more dependent on mitochondrial than on nuclear MGMT, emphasizing the contribution of mtDNA repair mechanisms in cellular resistance to alkylation chemotherapy (135).

Although many associations have been made between mtDNA mutations and cancer, the exact role of mtDNA repair systems in carcinogenesis still remains to be fully elucidated.

Conclusion

The mitochondrial genome has been completely sequenced and mapped for a variety of species including human. Numerous studies have shown that mtDNA is more susceptible to various carcinogens and ROS than nDNA. Damage to mtDNA is implicated in a wide variety of common pathological states, including neurodegenerative diseases and cancer. Additionally, the accumulation of the damage in mitochondrial genomes plays a central role in the mitochondrial theory of aging. The major protective mechanism that cells use to deal with this damage is DNA repair. Mammalian mitochondria clearly posses the ability to repair endogenous damages such as abasic sites and oxidized bases through BER mechanisms. Nevertheless, studies into the mechanisms involved in mtDNA repair are limited compared to the vast amount of information that is in the literature concerning nuclear repair mechanisms. Consequently, many questions remain to be answered. Undoubtedly, future research will address such topics as: a more precise definition of the components involved in mtDNA repair; a better comprehension of how they are regulated; and a more thorough understanding of how they can malfunction to precipitate disease states and how they become altered in aging. This knowledge will be essential for the development of future therapeutic strategies to combat a variety of human pathologies.

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