

The role of mitochondria in ageing and carcinogenesis

M. A. Birch-Machin

Dermatological Sciences, School of Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

Summary

Mitochondria can perform multiple cellular functions including energy production, cell proliferation and apoptosis. These organelles contain their own genetic material, mitochondrial DNA (mtDNA), which is maternally inherited. Although much smaller than the nuclear genome, mtDNA is equally important, as it has been hypothesized to play a crucial role in ageing and carcinogenesis. This is partly due to the fact that mitochondria represent the major site for the generation of cellular oxidative stress and play a key role in mediating programmed cell death (apoptosis). Damage to mtDNA is therefore an important contributor to human ageing, cancer and neurodegenerative diseases. The most relevant footprints of mtDNA damage are point mutations of single bases, or deletions of the 16.5-kb mitochondrial genome. This review will focus on the key roles of mitochondrial function and mtDNA in oxidative stress production and as a mediator of apoptosis, and on the use of mtDNA as a biomarker of sun exposure. This will be related to the contribution of mitochondria and mtDNA in the ageing process and cancer, with a specific focus on human skin. In conclusion, it is likely that the interplay between nuclear and mitochondrial genes may hold the final understanding of the mitochondrial role in these disease processes.

Mitochondria and mitochondrial DNA rearrangements

Collectively, mitochondria generate approximately 90% of cellular energy by the process of oxidative phosphorylation. This multisubunit pathway results from the complementation of both the nuclear and the mitochondrial genome. Alongside the 3 billion bp nuclear genome, each human cell therefore contains hundreds to several thousand copies of the 16.5-kb human mitochondrial genome, which incidentally exhibits a maternal pattern of inheritance.¹ This closed circular genome encodes 13 polypeptides of the respiratory chain complexes, as well as 22 transfer RNAs and 2 ribosomal RNAs used in mitochondrial protein synthesis (Fig. 1).

The complete mitochondrial DNA (mtDNA) sequence was determined in 1981 and re-sequenced in 1999.¹ A growing collection of reported mtDNA mutations and rearrangements has been associated with muscle and neurodegenerative diseases, a proportion of which exhibit skin manifestations.² The mtDNA has the capacity to form a mixture of both wild type and mutant mtDNA genotypes within a cell, a phenomenon known as heteroplasmy. This is important because cellular dysfunction usually occurs when the ratio of mutated to wild-type mtDNA exceeds a threshold level.²

Mitochondria and oxidative stress

Approximately 90% of the oxygen consumed within a eukaryote is used in mitochondrial respiration, and therefore the metabolic rate of a cell and indeed tissue is related to mitochondrial function. Incomplete oxygen reduction within the mitochondrial respiratory chain can lead to the formation of the superoxide radical, the first molecule in the pathway responsible for the production of reactive oxygen species (ROS)^{3,4} (Fig. 2).

Correspondence: Professor Mark A. Birch-Machin, Dermatological Sciences, School of Clinical and Laboratory Sciences and Leech Building, Medical School, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK.

E-mail: m.a.birch-machin@newcastle.ac.uk

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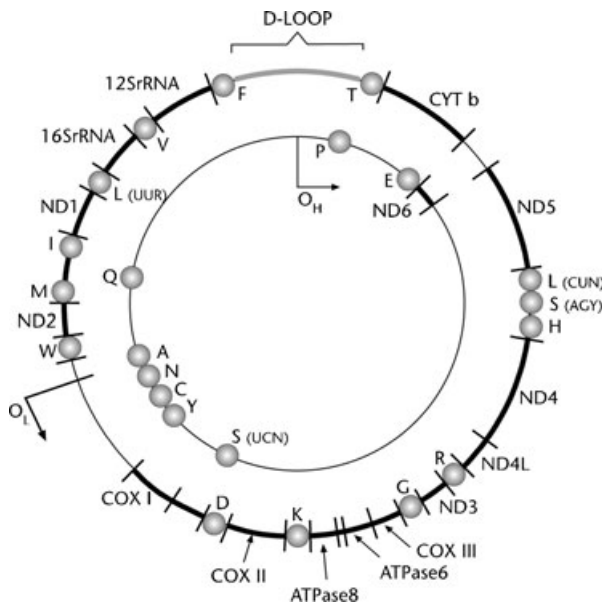


Figure 1 The 16 569 bp human mitochondrial genome. There are 37 genes in mtDNA; 24 (2 ribosomal RNAs and 22 transfer RNAs) are required for mtDNA translation and 13 encode the respiratory chain, which is essential for aerobic metabolism. Both strands of the mtDNA genome can be fully transcribed to produce large polycistrons that are processed to create the transfer RNAs (circles), ribosomal RNAs (12S and 16S rRNA), and mRNAs, which are translated to respiratory chain protein complexes (e.g. COX I-III, ND1-6, CYTb and ATPase 6 and 8). The ATPase 6 gene overlaps the ATPase 8 gene and is encoded on the same processed RNA species. The two strands can be separated by density-gradient centrifugation into a heavy (H) strand and a light (L) strand. Origins for the transcription of both strands and of H-strand replication (O_H) are found in the only major noncoding region, which is also termed the displacement or D-loop. The origin of L-strand replication (O_L) lies outside the D-loop as indicated. A single-letter code is used to represent each transfer RNA gene (codons in brackets denotes changes in codon usage). COX I-III, ND1-6 and ATPase 6/8 refer to genes encoding components of the cytochrome c oxidase (complex IV), the NADH:ubiquinone oxidoreductase (complex I) and the F_0F_1 -ATPase synthase (complex V), respectively, of the respiratory chain. CYTb encodes apocytochrome b of the ubiquinol-cytochrome c oxidoreductase (complex III).

Mitochondrial ROS formation is mainly due to electron leakage naturally occurring at complexes I and III of the respiratory chain (Fig. 3), but recent evidence in human skin cells has postulated the additional contribution by complex II.⁵ Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity and mitochondrial malfunction.^{6,7} As the mitochondrial respiratory chain is a major source of ROS generation, and the naked mtDNA molecule is in close proximity to the source of ROS, the vulnerability of the mtDNA to

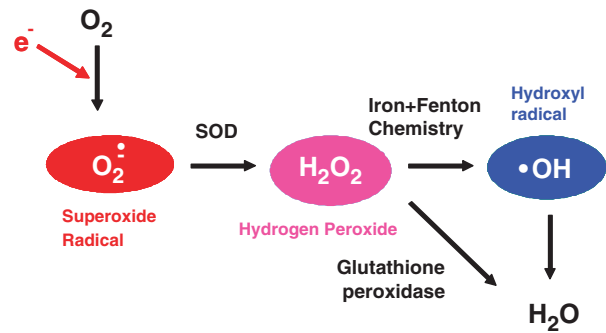


Figure 2 The cascade for the production of oxidative stress. Under physiological conditions, superoxide radicals comprise about 0.1% of the oxygen transformed to reactive oxygen species (ROS). Superoxide radicals can be transformed to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) leading to free hydroxyl radicals ($\cdot OH$) via Fenton chemistry. The hydroxyl ions have a extremely short half-life (< 1 ns) but they are one of the most aggressive forms of ROS. Hydroxyl radicals can react with DNA bases to form oxidative products such as 8-oxo-guanine (8-oxo-G) or with the deoxyribosyl backbone of DNA, leading to strand breaks.

ROS-mediated damage appears to be a mechanism to amplify ROS-stressing cancer cells.⁶ The DNA and membrane damage resulting from increased ROS is further enhanced through combination with reactive nitrogen species, particularly nitric oxide, which is also a key regulator in cutaneous physiology.⁸

Mitochondria and ageing

The free-radical theory proposed by Harman⁹ remains the most vigorous contender to explain the basis of ageing in a wide range of species by postulating that the production of intracellular ROS is the major determinant of life span. Intracellular ROS are primarily generated by the mitochondrial respiratory chain and thus are a prime target for oxidative damage. The mitochondrial theory of ageing predicts that a vicious cycle contributes to the ageing process.¹⁰ This theory proposes that mtDNA mutations caused by ROS accumulate within the cell, leading to impaired respiratory chain proteins, thereby generating more ROS, which in turn causes higher mtDNA mutation rates. Indeed, there are a host of either short- or long-lived organisms that appear to have changes in mitochondrial metabolism, ROS generation or oxidative stress resistance as their primary alteration.¹⁰ Although there are recent data supporting a direct functional role of mtDNA in ageing and photoageing,¹¹ there is still considerable debate as to the type of mtDNA species associated with ageing. For example, the most frequently reported species is the

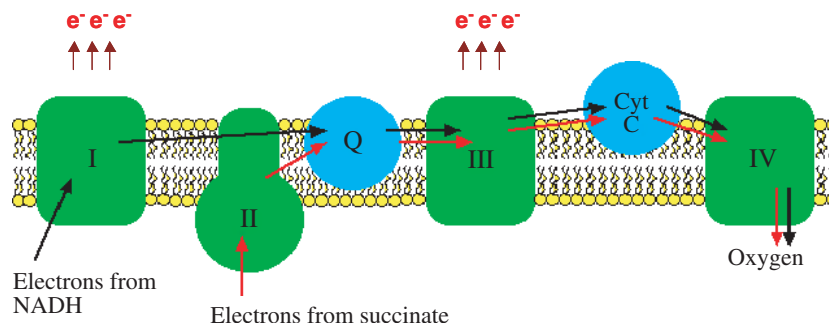


Figure 3 Electron flow within the mitochondrial respiratory chain. Electrons (e^-) derived from the oxidation of succinate (i.e. red arrows) enter the electron transport chain (ETC) at complex II (succinate–ubiquinone oxidoreductase). The black arrows designate the electrons originating from NADH and entering the ETC at complex I (NADH–ubiquinone oxidoreductase). ‘Leakage’ of electrons from the ETC occurs at designated locations, the details of which are described in the text.

4977-bp common deletion, but its significance is still under debate.¹² In addition, there are single somatic mtDNA control region mutations associated with ageing in tissues, including skin, but their functional significance is still unclear.¹³ This process of chronological ageing can of course be accelerated in skin by chronic exposure to ultraviolet radiation (UVR), which has been shown to be associated with a further increase in mtDNA damage as described below.

Mitochondrial DNA as a biomarker of sun exposure

Although the major determinant of nonmelanoma skin cancer (NMSC) is the UVR in sunlight, which induces the DNA damage, it is both the pattern and the cumulative amount of sun exposure that influences the development of NMSC.¹⁴ To determine a reliable marker of cumulative UVR exposure in human skin, several groups have examined the novel idea of using mtDNA rather than nuclear DNA as a biomarker of UV-induced DNA damage.^{2,15,16} Compared with mutation screening of candidate nuclear DNA genes such as *p53*, there are certain advantages to studying mtDNA damage in sun-exposed skin. Firstly, mtDNA is highly susceptible to damage because it is not associated with protective histones. Secondly, it is continually exposed to high levels of ROS generated by oxidative phosphorylation. Thirdly, there is a limited capacity for mtDNA repair, particularly photoproducts such as pyrimidine dimers.^{2,17} Finally, as each cell contains up to several thousand copies of the mtDNA genome, mitochondria can tolerate very high levels (up to 90%) of damaged mtDNA through complementation of the remaining wild-type DNA.¹⁸ Therefore, cells are able to accumulate photodamage in mtDNA without compromising cell

function, a necessary requirement for a reliable and sensitive biosensor. Of the spectrum of mtDNA deletions identified in sun-exposed human skin¹⁸ the major species have been the 4977-bp common deletion and a 3895-bp deletion.^{1,15,19–23} These mtDNA deletions can be also be induced in human skin and cultured skin cells by sublethal repetitive doses of UVR.^{15,21} Apart from deletions, a higher frequency of tandem mtDNA duplications has been observed^{22,23} in sun-exposed human skin.

Mitochondria and apoptosis

One important development has been the recognition that mitochondria play a central role in the regulation of programmed cell death or apoptosis. A number of apoptotic signals converge on mitochondria, such as oligomerization of the apoptotic Bax and Bak proteins, leading to permeabilization of the outer membrane and release of cytochrome c, apoptosis-inducing factors and Smac/DIABLO into the cytoplasm. Cytochrome c binds to adaptor molecules (including apoptosis protease-activating factor 1 and initiator pro-caspase proteins), forming an ‘apoptosome’, which leads to cleavage of pro-caspase-9 to active caspase-9, which can then activate downstream effector caspases (e.g. caspase-3), resulting in apoptosis.²⁴ In certain cell types there is also crosstalk between the death receptor apoptotic pathway and mitochondria. It is now well established that apoptosis is an important mechanism in the therapeutic action of most anticancer drugs,²⁵ and that mitochondria play a key role in this process. Interestingly, the antipsoriatic drug anthralin accumulates in keratinocyte mitochondria, and induces apoptosis through a pathway dependent on respiratory-competent mitochondria.²⁶

Mitochondria and cancer

Mitochondria have been implicated in the carcinogenic process because of their role in apoptosis and other aspects of tumour biology, and also because of their role as a generator of ROS.^{6,27} Many types of human malignancy such as colorectal, liver, breast, pancreatic, lung, prostate, bladder and skin cancer have been shown to harbour somatic mtDNA mutations.^{6,7,27–29} Moreover, sequence variations of mtDNA have been observed in preneoplastic lesions, which suggests mutations early in tumour progression.^{1,27} Three recent studies have identified somatic mtDNA mutations in cutaneous malignant melanoma.^{30–32} However, two of these studies^{30,31} have focused on the noncoding region, which constitutes less than 7% of the mitochondrial genome. Durham *et al.*²⁹ provided the first detailed study of multiple forms of mtDNA damage (including deletions, tandem duplications and point mutations) in NMSC. Somatic heteroplasmic point mutations were identified in addition to clear differences in the distribution of deletions in the tumours compared to perilesional skin. One cautionary note provided by this study and confirmed by subsequent studies^{23,33} is the use of appropriate control tissue, as perilesional skin may also harbour UV-induced mtDNA damage.

Learning points

- Mitochondria can perform multiple cellular functions including energy production, cell proliferation and apoptosis.
- These organelles contain their own genetic material, mitochondrial DNA (mtDNA), which has a maternal pattern of inheritance.
- Mitochondrial DNA defects occur in many different tissues, and mitochondrial disorders are involved in a large number of human disease processes, including cancer and ageing.
- Mitochondria represent the major site for the generation of cellular oxidative stress.
- Mitochondrial DNA can be used as a reliable and sensitive biomarker of cumulative UVR exposure in skin.
- The most relevant footprints of mtDNA damage are point mutations of single bases or deletions of the 16.5-kb mitochondrial genome.
- The mitochondrial theory of ageing predicts that a vicious cycle of reactive oxygen species production contributes to the ageing process.

- Many types of human malignancy such as colorectal, liver, breast, pancreatic, lung, prostate, bladder and skin cancer have been shown to harbour somatic mtDNA mutations.
- It is currently unknown whether the observed mtDNA damage has a primary and causative link to the process of cancer development or if it may simply represent a secondary bystander effect that reflects an underlying nuclear DNA instability.
- The interplay between nuclear and mitochondrial genes requires careful investigation and may hold the key to the final understanding of the mitochondrial role in tumorigenesis.

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