Mitochondrion 13 (2013) 444–453

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Stressed out mitochondria: The role of mitochondria in ageing and cancer focussing on strategies and opportunities in human skin

Asif S. Tulah, Mark A. Birch-Machin *

Dermatological Sciences, Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom

ARTICLE INFO

Available online 27 November 2012

Keywords:
Electron transport chain
Infrared radiation
Mitochondrial DNA
Reactive oxygen species
Ultraviolet radiation

ABSTRACT

Mitochondrial DNA damage has been used as a successful and unique biomarker of tissue stress. A valuable example of this is sun damage in human skin which leads to ageing and skin cancer. The skin is constantly exposed to the harmful effects of sunlight, such as ultraviolet radiation, which causes it to age with observable characteristic features as well as clinical precancerous lesions and skin cancer. Formation of free radicals by the sun's harmful rays which contribute to oxidative stress has been linked to the induction of deletions and mutations in the mitochondrial DNA. These markers of mitochondrial DNA damage have been proposed to contribute to the mechanisms of ageing in many tissues including skin and are associated with many diseases including cancer. In this article we highlight the role of this important organelle in ageing and cancer with particular emphasis on experimental strategies in the skin.

1. Introduction to ageing

Ageing is the gradual impairment of normal biological function; where a cell's ability to maintain normal operations is reduced under basal conditions or conditions of stress. (Finkel et al., 2007). Ageing occurs naturally throughout life and it is clear that multiple genes and the environment are likely to contribute to the processes involved (Herskind et al., 1996). Many theories have been put forward to explain chronological (or intrinsic) ageing and one such is the 'disposable soma theory'. This states that due to the competition for finite resources available in the cell, an organism will strike a balance between maintenance of the soma by repairing damage to the genome and processes which aid reproduction of the organism (Kirkwood, 1977). This decline in biological function is the result of defects in metabolic pathways necessary for biological function. Whilst damage can occur at the protein, RNA or DNA level (Pacifici and Davies, 1991), studies have focussed on DNA damage as the protein and RNA population in a cell is constantly turned over. A damaged genome can affect entire cell integrity, which supports theories that focus on DNA damage as an ageing mechanism (Gensler and Bernstein, 1981). In mammalian cells, as well as the nuclear genome there is also a smaller mitochondrial genome housed in this cellular organelle (Anderson et al., 1981).

The free radical theory of ageing (FRTA) states that organisms age due to the buildup of free radicals over time because these atoms or molecules with an unpaired electron in their outermost shell are unstable and damage cells contributing to ageing (Beckman and Ames, 1998; Harman, 1956, 2003). Oxidative damage is the most common form of damage caused by free radicals which are a byproduct of the intracellular processes such as the electron transport chain (ETC). The enzyme superoxide dismutase forms the free radical superoxide (O_2^-), but other important reactive oxygen species (ROS) include hydrogen peroxide (H_2O_2) and the hydroxyl ion (OH^-) (Droge, 2002). The production of intracellular ROS mostly occurs in the mitochondria through the ETC, linking the FRTA with the mitochondria (Hansford et al., 1997).

2. Mitochondrial function and oxidative stress and ageing in the skin

Mitochondria are cellular organelles 1–2 μm in length and 0.5–1 μm in diameter and play a central role in adenosine triphosphate (ATP) synthesis and production of cellular ROS (Boveris et al., 1972). They are

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; ADP, adenosine diphosphate; ATP, adenosine triphosphate; bp, base pairs; CPD, cyclobutane pyrimidine dimer; D-loop, displacement loop; DNA, deoxyribonucleic acid; EPR, electroparamagnetic resonance; ERK1/2, extracellular signal-regulated kinase 1/2; ETC, electron transport chain; $FADH_2$, reduced flavin adenine dinucleotide; FOXO, forkhead box protein (a family of transcription factors); FRTA, free radical theory of ageing; H^+, proton; H_2O_2, hydrogen peroxide; IRA, infrared A radiation; IRB, infrared B radiation; IRC, infrared C radiation; MED, minimal erythema dose; MMP1, matrix metalloproteinase 1; MMPs, matrix metalloproteinases; mtDNA, mitochondrial DNA; NADH, reduced nicotinamide adenine dinucleotide; Nrf2, nuclear factor-like 2 (transcription factor); O_H, origin of heavy strand replication; O_L, origin of light strand replication; O_2, oxygen; $O_2^{\bullet-}$, superoxide anion; $OH^{\bullet-}$, hydroxyl radical; PCR, polymerase chain reaction; PI(3)K, phosphor-inositide 3-kinase; P_H, promoter for initiation of heavy strand transcription; P_L, promoter for initiation of light strand transcription; RNA, ribonucleic acid; ROS, reactive oxygen species; SED, standard erythema dose; TTFA, thenoyl trifluoroacetate; UVA, ultraviolet A radiation wavelength 320–400 nm; UVB, ultraviolet B radiation wavelength 280–320 nm; UVC, ultraviolet C radiation wavelength 110–280 nm; UVR, ultraviolet radiation.

* Corresponding author at: Dermatological Sciences, Institute of Cellular Medicine, Medical School, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom. Tel.: +44 191 222 5841; fax: +44 191 222 7179.

E-mail address: mark.birch-machin@newcastle.ac.uk (M.A. Birch-Machin).

1567-7249/$ – see front matter © 2012 Elsevier B.V. and Mitochondria Research Society. All rights reserved.
http://dx.doi.org/10.1016/j.mito.2012.11.007

thought to have formed billions of years ago through a symbiotic relationship involving an alpha-protobacterium and a methanogenic archaebacterium (Gray et al., 1999). Analysis of mitochondrial genome sequences from different organisms provides support for this hypothesis. The organelle is encapsulated by the outer membrane and also contains an inner membrane with proteins needed for the redox reactions of oxidative phosphorylation. The inner membrane is folded to form the cristae space which increases the surface area and so the ATP generating capacity. The matrix is the space within the inner membrane (Palade, 1953; Perkins and Frey, 2000).

Each cell can contain 100–1000 mitochondria depending on its metabolic demands. This organelle is responsible for the production of approximately 90% of the cell's energy. Energy in the form of ATP is the required currency for the cell and this is converted from nutrients by a process called respiration (Brown, 1992). Cellular respiration can be both aerobic (oxygen requiring) and anaerobic. Using oxygen for the complete oxidation of these organics generates far more energy than anaerobic respiration. Useful compounds from the food we ingest, such as glucose, enters glycolysis (which is anaerobic) where the glucose molecule is oxidised and split to form two pyruvate molecules with the net production of two ATP molecules and two NADH molecules. NADH is an electron carrier (or donor) that can take these electrons to the ETC where more energy is generated (Birch-Machin and Turnbull, 2001). The next stage involves the Krebs cycle which produces more NADH and $FADH_2$ molecules (another electron donor). Most of these NADH and $FADH_2$ molecules generated are transported to the mitochondria were they can enter the ETC.

The inner mitochondrial membrane is the site where oxidative phosphorylation occurs. This reaction involves four protein complexes, ubiquinone and cytochrome *c* which make up the ETC (Wilson et al., 1974). Electrons from NADH and $FADH_2$ (donors) are combined with O_2 (carrier) during oxidative phosphorylation, with the transfer of H^+ (a proton) across the membrane (Fig. 1). The electrochemical proton gradient formed from these reactions generates energy used to drive the conversion of ADP to ATP. The mechanism of coupling electron transport to ATP generation is called chemiosmotic coupling (Mitchell, 1961). Transferring the electrons from these carriers to molecular oxygen requires energy, which needs to be produced gradually by the passage of electrons through the ETC carriers (Papa et al., 2012). Electrons from NADH enter complex I where they are transferred to flavin mononucleotide and then to coenzyme Q (ubiquinone). This carries the electrons through the membrane to complex III, where they are transferred to cytochrome *b* and then to cytochrome *c*. Cytochrome *c* then carries the electrons to complex IV where they are finally transferred to molecular O_2. Complex II receives electrons from succinate (a derivative of the citric acid cycle) which are then transferred to $FADH_2$ (rather than NADH), then to coenzyme Q before passing to complex III and complex IV in the normal way. Throughout this process the energy formed by the passage of electrons through the complexes is coupled to the synthesis of ATP (conversion of ADP to ATP) (Fig. 1).

Electrons can leak from complexes I and III and are free to react with molecules to form free radicals (ROS) (Turrens, 2003). Reacting with a donor such as molecular oxygen forms the superoxide ($O_2^{\bullet -}$), this can be catalysed by superoxide dismutase to form hydrogen peroxide (H_2O_2) which can be processed into the hydroxyl radical ($OH^{\bullet -}$) (Boveris and Chance, 1973). These oxygen radicals can initiate other pathways that lead to the generation of further reactive species such as more radicals, aldehydes, epoxides and hydroperoxides. Whilst the cell does possess antioxidant defence mechanisms, imbalance between this and free radical production causes so called oxidative stress/damage. Severe oxidative stress activates apoptosis or necrosis pathways the buildup of which has been linked to mechanisms in many diseases associated with ageing such as cardiovascular (e.g. atherosclerosis), cancers and neurological (e.g. Parkinson's and Alzheimer's disease) (Selvaraju et al., 2012). Free radicals in these disorders are proposed to be detrimental to cells by causing oxidative damage at the protein level by modifying amino acids which affects the function of the polypeptide (Butterfield et al., 1998) and the RNA or DNA level involving hydroxyl radical chemical modifications of DNA which are mutagenic contributing to cancers (Mates et al., 1999) and ROS DNA damage which activates apoptosis pathways (Kamata and Hirata, 1999). Diseases such as these (associated with free radical damage) are linked to ageing because the changes caused by the unfavourable action of radicals over time are deviated from the normal pattern of ageing and contribute greatly to the development of these disorders which are observed during the ageing process (Harman, 1988).

The skin is exposed to UVR, ionising radiation and the action of environmental insults such as drugs, cosmetic products and food substances (to name a few) form ROS in amounts that the antioxidant defences in the skin cannot tolerate. The breakdown of these defences and ROS build-up lead to skin disorders such as cutaneous neoplasia and allergic conditions (Briganti and Picardo, 2003). NF-κB and AP-1 are activated by ROS and increased cytokine production and production of second messengers contribute to disorders such as psoriasis and acne (Okayama, 2005).

As we age structural changes in the skin, in part due to the action of free radicals, affect this organ's ability to function. Decreased numbers of keratinocytes and fibroblasts with resulting decreases in

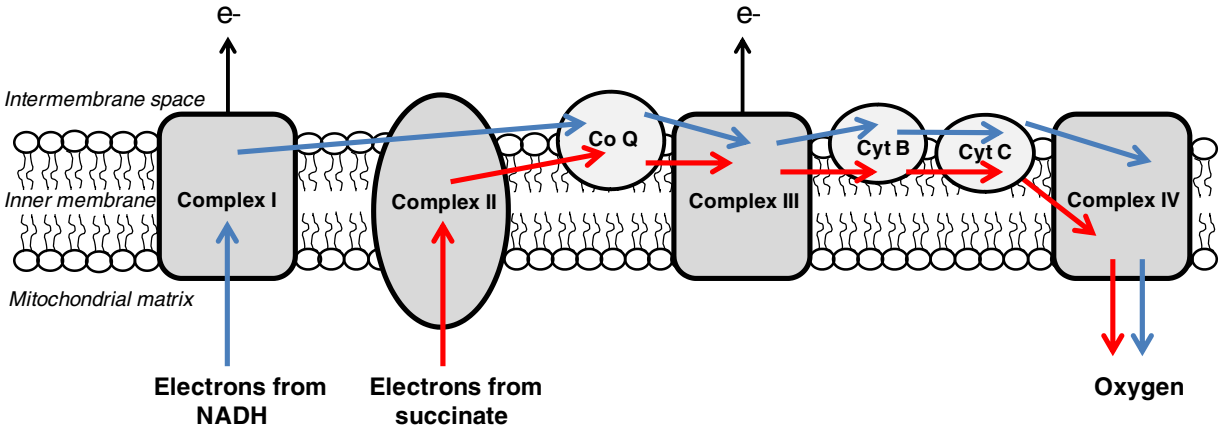

Fig. 1. The electron transport chain. A schematic representation of the mitochondrial electron transport chain (ETC). NADH electrons enter complex I where they are transferred to flavin and then ubiquinone or coenzyme Q (CoQ). Electrons from succinate enter complex II before being transferred to CoQ. This carries the electrons to complex III (through the membrane) where they are then transferred to cytochrome *b* (Cyt B) and cytochrome *c* (Cyt C). Cyt C transfers the electrons to complex IV where they are transferred to molecular oxygen. Energy is formed by the passage of electrons through the complexes. Electrons which leak from complexes I and III contribute to free radical generation (in the form of reactive oxygen species) are able to cause cellular damage.