Mitochondrial Genetics and Sepsis

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Introduction

Mitochondria are intracellular organelles that generate the principal source of cellular energy in the form of adenosine triphosphate (ATP). In a highly efficient process, mitochondria convert both carbohydrate and fat into high-energy phosphate compounds by a series of intermediate steps involving electron transfer. Emerging data implicate mitochondrial damage and dysfunction as critical factors in the pathogenesis of sepsis.

Oxidative Phosphorylation and ATP Generation

Mitochondria are present in all nucleated cells. They possess a smooth outer membrane and a convoluted inner membrane, the folds of which are termed cristae. Together the two membranes create an intermembrane space.

The primary role of mitochondria is to produce cellular energy, in the form of ATP, by a process called oxidative phosphorylation. The five multi-subunit enzyme complexes (I-V) of the oxidative phosphorylation system are located within the mitochondrial inner membrane (Fig. 1). Each complex itself is composed of multiple subunits; complex I is the largest with over 40 subunits. During oxidative phosphor-

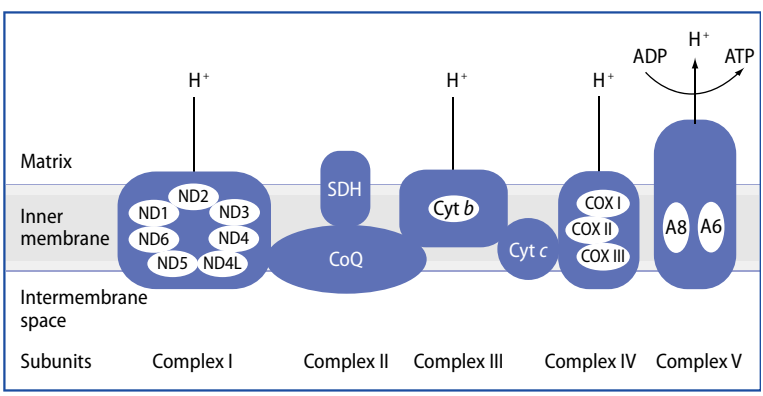

Fig. 1. Diagrammatic representation of the mitochondrial respiratory chain showing the major components: complexes I (NADH dehydrogenase), II (succinate dehydrogenase and coenzyme Q10), III (cytochrome c reductase), IV (cytochrome c oxidase) and V (ATP synthase) and the genes encoded by each complex.

ylation, electrons are transferred down these redox enzyme complexes, known as the electron transport chain. Through this process, energy is released and is used to transport protons out through the inner mitochondrial membrane into the intermembranous space. This produces an electrochemical gradient that is utilized by complex V as a source of energy to condense adenosine diphosphate (ADP) to make ATP.

An efficient oxidative phosphorylation system producing ATP is said to be tightly coupled. Alternatively, if the mitochondria are less efficient at generating ATP they are partially uncoupled and generate heat. Therefore, the mitochondrial oxidative phosphorylation system must be tightly regulated to generate a balance between ATP generation for energy and heat to maintain body temperature. Uncoupling proteins (UCP) are members of the mitochondrial transport carrier family [1]. UCP1 promotes the leakage of protons through the mitochondrial inner membrane. This uncouples ATP production from substrate oxidation, leading to increased oxygen consumption and ultimately heat production.

Reactive Oxygen Species

The mitochondrial electron transport chain is one of the pathways that produces oxidants and free radicals in the body. Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are toxic by-products of cellular metabolism. At complex I and III of the mitochondrial electron transport chain, electrons may leak to molecular oxygen, causing the production of superoxide. Approximately 1–2 % of the oxygen consumed by mitochondria is converted to ROS. As ROS function as cellular messengers, they can broadly influence gene expression, cell proliferation, energy metabolism and mitochondrial biogenesis.

Mitochondria are the major intracellular source of ROS. It is known that ROS production from mitochondria increases with age [2]. Excessive ROS production can impair mitochondrial function and influence cell viability. Production of ROS is associated with damage to DNA, lipids and proteins, cellular proliferation, the expression of UCP, and the maintenance of mitochondrial membrane potential [3]. Mitochondria can increase the synthesis of ROS during hypoxia which triggers an increase in oxygen delivery to the cell and glycolytic metabolism [4].

Oxidative stress and damage by ROS can cause damage to bases and sugar phosphates in addition to single- or double-strand breaks in mitochondrial DNA (mtDNA). This ultimately leads to the occurrence of somatic mtDNA mutations. The accumulation of these mutations can result in dysfunction of the respiratory chain. In theory, this can lead to increased ROS production and further mtDNA mutations [5].

Apoptosis

Mitochondria are critical for cellular homeostasis. However, they also play an important role in regulating programmed cell death, or apoptosis (as opposed to death by necrosis which is not programmed). Apoptosis is triggered by both intrinsic and extrinsic pathways. Oxidative stress can induce the intrinsic pathway of apoptosis. The mitochondrial regulation of apoptosis occurs during the initiation and regulation of the intrinsic pathway. Lethal agents cause the mitochondria to release cyto-

chrome c and other pro-apoptotic proteins into the cytosol. Here they induce or amplify the activation of apoptotic caspases [6]. Mitochondrial oxidative stress has been implicated in cell death. As cellular metabolism relies on ATP production from mitochondria, any damage e.g., from ROS, affecting respiratory chain function may influence cell viability.

Mitochondria and Sepsis

A considerable body of evidence has accumulated implicating mitochondrial dysfunction in sepsis (reviewed in [7]). The multiple organ dysfunction that characterizes sepsis is poorly understood but is accompanied by impaired cellular oxygen uptake despite adequate tissue perfusion. It is thought that cellular oxygen consumption is impaired primarily at the level of the mitochondrion, ultimately leading to a decrease in energy production.

Several of the inflammatory mediators released during sepsis have been shown to inhibit oxidative phosphorylation [8]. Ultra-structural changes in mitochondria have also been observed in animals with sepsis [9] and in patients who died in critical care with multiple organ failure (MOF) [10]. Subtle defects of complex I and ATP depletion have been observed in the skeletal muscle of septic patients who subsequently die [11].

It is possible that mitochondrial damage in critical illness leads to increased electron leakage and ROS generation. The intense inflammatory response during sepsis also results in increased inducible nitric oxide (NO) synthase (iNOS) activity and reactive nitrogen species production. NO is thought to play an important role in sepsis patients as it has an inhibitory effect on electron transport chain complexes, thus leading to the generation of electrons through relative blockade of the respiratory chain [12]. However, depending upon the concentration and release time of NO, studies have demonstrated that low levels of NO stimulate mitochondrial proliferation during sepsis [13].

Mitochondrial Genetics

Mitochondria probably originated as free-living bacteria-like organisms. At some point in evolution they were engulfed by primitive, nucleated, anaerobic cells and became symbiotic [14]. They contain their own DNA (mtDNA) which is present in high copy number in human cells. mtDNA is a small circular double stranded molecule, 16.5 kb in length (**Fig. 2**). It consists of a heavy (purine rich) and light chain (pyrimidine rich). The 37 genes of the mitochondrial genome encode 13 essential components of the oxidative phosphorylation system, as well as two ribosomal RNAs and 22 transfer RNAs [15]. Nuclear genes code for the remaining and majority of the mitochondrial respiratory chain proteins. They are translated by cytosolic ribosomes with a mitochondrial targeting sequence that directs them into the mitochondrion.

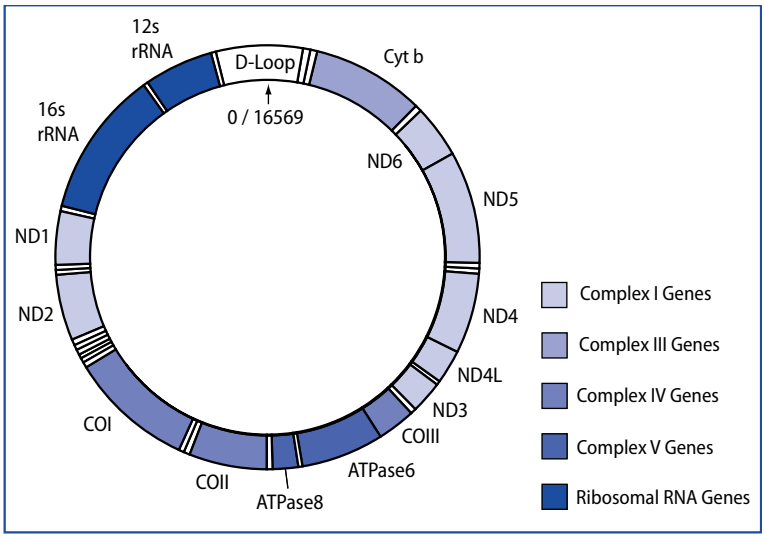

Fig. 2. The human mitochondrial DNA (mtDNA) genome demonstrating the location of the complex genes

Mitochondrial Haplogroups

mtDNA is inherited almost exclusively through the maternal lineage, and does not undergo significant recombination. The high mutation rate of mtDNA is due to its lack of protective histones, inefficient DNA repair mechanisms, and exposure to the mutagenic effects of oxygen radicals generated by oxidative phosphorylation [16]. These mutations are completely random and can affect any base in the mitochondrial genome in either the coding or non-coding regions. Therefore, the rate of mtDNA sequence evolution is much higher than that of the average nuclear gene [17]. Consequently a substantial number of mtDNA single nucleotide polymorphisms (SNPs) have accumulated, sequentially along radiating maternal lineages. In turn, these have diverged as human populations, colonizing different geographical regions of the world. These specific SNPs are known as mtDNA haplogroups [18]. Different mtDNA haplogroups are associated with major global ethnic groups [19]. The majority of Europeans belong to one of nine haplogroups: H, J, T, U, K, V, W, I, X, with haplogroup H being the most common (44 %) [20].

The geographical variation observed in mtDNA haplogroups could reflect selection acting on specific lineages. Ruiz-Pesini and colleagues hypothesized that certain mtDNA haplogroups cause a decrease in coupling efficiency, leading to lower ATP generation and increased heat production. These particular haplogroups are thought to have been positively selected for during radiation of modern humans into colder climates [21]. However this hypothesis was later tested using a bioenergetic test on the specific mtDNA variants. The authors found that mitochondria from Arctic haplogroups had similar or increased coupling efficiency when compared to tropical haplogroups [22]. Moreover, a further study observed no evidence for climate-induced geographical varied selection [23].

Table 1. Definitions of common genetic terms

Genetic term	Definition
Adaptive selection	Selection favoring advantageous alleles/traits. This allows advantageous alleles to become prevalent in the natural population
Cybrid	A hybrid cell produced by fusing a cell nucleus with a cell of the same or a different species whose nucleus has been removed
Genetic drift	The process by which gene frequencies are changed by the process of random sampling in a small population
Non-synonymous	A substitution that replaces one codon with another that encodes a different amino acid
Purifying selection	Selection acting against deleterious alleles. This prevents harmful alleles becoming prevalent in the natural population
Recombination	The exchange and rearrangement of DNA
Single nucleotide polymorphism	A change in which a single base in the DNA differs from the usual base at that position
Synonymous	A substitution that replaces one codon with another that encodes the same amino acid

The various influences of random genetic drift, positive selection, or purifying selection that eliminate non-synonymous changes in mtDNA sequence variation remain uncertain (**Table 1**). Kivisild and co-workers produced further evidence for non-random processes affecting the evolution of the human mtDNA encoded proteins [24]. Although functional studies have failed to identify a biochemical consequence of mtDNA haplogroups [25], these effects could be subtle and only be manifest on an evolutionary scale.

A recent study investigated the historical genetic diversity of ancient human mtDNA genotypes and compared them to modern mtDNA genotypes from England, Europe, and the Middle East. The investigators found a higher genetic diversity in the ancient samples. This could be the result of genetic drift, selection, or stochastic processes. Examples include the Black Death in 1347 which led to a loss of 50 % of the European population and the Great Plague of 1665 in which 20 % of the London population died [26]. This work provides circumstantial evidence that major environmental insults, including pandemic infections, have influenced mtDNA evolution.

Haplogroups and Human Disease

Multiple associations have been documented between clinical conditions and mtDNA haplogroups. These include some neurodegenerative diseases, ageing, and, more recently, survival in human sepsis. Analysis of patients with Parkinson's disease demonstrated a 22 % reduction in the risk of development of Parkinson's disease in patients in the UKJT haplogroup cluster [27]. Another study found that European individuals with haplogroup J or K were significantly less likely to develop Parkinson's disease compared to those with haplogroup H [28]. De Benedictis and co-workers found that in a group of healthy centenarians, haplogroup J was significantly overrepresented, therefore linking haplogroup J with longevity [29].

A longitudinal clinical and genetic study of 150 patients with severe sepsis demonstrated that on admission to intensive care, the haplogroup distribution was similar to a large age-matched control group from the same region. However, survival in the haplogroup H patients was significantly better than other patients at 28 days, hospital discharge and six month follow-up [30]. Examination of mtDNA haplogroups in severe sepsis in the Chinese Han population revealed that haplogroup R, one of the three main haplogroups of the Han population, predicts a survival advantage [31].

Haplogroups and Mitochondrial Function

Evidence has emerged, over recent years, to link different mtDNA haplogroups with alterations in the phenotypic expression of mitochondrial activity. Leber hereditary optic neuropathy (LHON) is a rare cause of inherited blindness primarily due to three pathogenic mtDNA mutations (11778G>A, 14484T>C and 3460G>A). The penetrance of the 11778G>A and 14484T>C mutations is markedly influenced by the background mtDNA haplogroup [32]. Sperm motility is strongly dependent upon ATP supplied by oxidative phosphorylation activity. Haplogroup H has been associated with increased sperm motility and the T haplogroup with reduced motility [33]. A further study illustrates that several sublineages of haplogroup U were associated with differences in sperm motility and vitality [34]. However, the mechanism by which the haplogroup exerts its functional effect upon mitochondria in sperm function is unclear. Differences in haplogroup distributions have been revealed between long distance runners and sprinters in a study of elite Finnish athletes [35], but the number of subjects was small, and this result must be confirmed before firm conclusions can be drawn.

The haplotypes associated with decreased survival in sepsis [30] could have a direct effect on ATP levels, leading to reversible cellular dysfunction, reduced respiratory chain function with increased levels of ROS, and ultimately cellular apoptosis; uncoupling of mitochondria could result in increased heat generation. mtDNA SNPs could also influence phenotypic variation in humans. However, a study using a cybrid cellular model did not reveal any significant respiratory defect in haplogroups J or T cybrids [36]. A more recent investigation studied the bioenergetic capacities and coupling efficiencies of mitochondria in transmitochondrial cybrids [25]. These cybrids harbored mitochondria with either haplogroup H or haplogroup T, with identical nuclear backgrounds. At the mitochondrial and cellular level, the results demonstrated no significant bioenergetic differences in these mitochondria. Therefore, the mtDNA haplogroup could affect mitochondrial proliferation or signaling but it does not appear to affect coupling efficiency in cells [25]. However, the effect could be too subtle to be detected by conventional techniques, only becoming manifest at times of extreme stress. An investigation into oxidative phosphorylation performance and ROS production in mouse cells with different mtDNA variants demonstrated that mtDNA haplotypes produce different levels of ROS and their growth in galactose is affected [37]. Further work is needed in this area.

Mitochondrial Biogenesis

Mitochondrial biogenesis involves multiple transcriptional regulation pathways that require the expression of nuclear and mitochondrial genes. The number of mtDNA molecules doubles in every cell cycle, under normal physiological conditions. When

conditions change, the mtDNA copy number can be altered according to the energy need of the cell. Animal models of sepsis have demonstrated depletion in the number of heart [38] and liver [39] mitochondria. Mitochondrial dysfunction in sepsis is associated with a decrease in mitochondrial number. This depletion could be associated with a decrease in oxidative phosphorylation activity and impaired oxygen use or hypoxia which is associated with sepsis. In a recent short term survival study, it was found that mtDNA copy number was low when compared to controls. In recovering patients, the copy number increased over time compared to those that did not survive [40].

Data suggest a role for mitochondrial biogenesis in the response to inflammatory conditions. A recent study found that mitochondrial biogenesis can restore both mitochondrial number and oxidative metabolism, after selective damage to the organelles [41].

Conclusion

There is strong evidence that changes in mitochondrial function occur in human sepsis. The underlying cause/s of these changes remains uncertain but a failure of adequate energy production is plausible. mtDNA is a small, plasmid-like structure which is inherited almost exclusively down the maternal line. The mitochondrial genome contains a number of common single base variations which together define a set of inherited haplogroups. These haplogroups may influence mitochondrial function, particularly at times of intense cellular stress. The frequency of these haplogroups may have been influenced by natural selection due to infectious disease. Their diversity may, therefore, explain some of the variation in outcome from severe infection.

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