Mitochondrial Medicine
A New Era in Medicine Opens New Windows and Brings New Challenges

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Socrates: . . . And he who is most skilled in preventing or escaping from a disease is best able to create one?
Polemarchus: True
Socrates: And is he the best guard of a camp who is best able to steal a march upon the enemy?
Polemarchus: Certainly
Socrates: Then he who is a good keeper of everything is also a good thief?
Polemarchus: That I suppose is to be inferred
—Plato, The Republic

Reading Plato one might not be surprised by the apparent paradox in the ability of mitochondria to promote both life and death: On the one hand, they are the major producers of energy in the cell, and on the other hand, they can steal the life of their host “at will.” Mitochondria can sense the supply of fuel (they are important O2 sensors) and via the production of mediators (reactive O2 species [ROS]), communicate with critical effectors in the cell (like redox-sensitive ion channels in the membrane or the nucleus).1 In response to the needs of the cell, mitochondria produce both energy (ie, ATP) and heat, allowing the organism to work and keep a stable core temperature, facilitating optimal adaptation of eukaryotic cells to their environment. If the mitochondria cannot provide optimal adaptation to the metabolic needs and the environment, they have the ability to induce cell death (apoptosis). As strategic regulators of life and death, mitochondria are deeply involved in human disease, but the depth and breadth of mitochondria-based diseases is only now starting to be recognized.2

Metabolism and apoptosis converge in mitochondria and are both recognized as playing a major role in disease. Primary metabolic abnormalities are now recognized in pulmonary and systemic vascular disease, and the apoptosis resistance seen in proliferative vascular remodeling might have a mitochondrial basis.3,4 Metabolic abnormalities are prominent in myocardial hypertrophy, and increased mitochondrial-dependent apoptosis plays an important role in dilated cardiomyopathies.5 In cancer, the apoptosis resistance might have a mitochondrial basis,2,6 an idea first proposed by Warburg 80 years ago.7 In contrast, increased mitochondria-dependent apoptosis underlies the pathology of neurodegenerative diseases.2 Mitochondrial medicine is extending into common and serious diseases, beyond the narrow field of rare primary mitochondrial diseases. Mitochondrial genetics8 is leaving anthropology labs and is now used by epidemiology research teams.

Mitochondrial Membrane Potential: A Master Regulator of Life and Death

Mitochondria oxidize dietary hydrogen (carbohydrates and fats) with O2 to generate heat and ATP. Electrons donated from NADH and FADH2 (products of Krebs cycle) to complexes I and III of the electron transport chain (ETC), flow down a redox-gradient to complex IV and finally to O2 to give H2O (respiration). As electrons flow down the ETC, H+ from complexes I, III, and IV is pumped out of the mitochondrial matrix, creating a very negative potential, $\Delta \Psi_m$ ($\geq -240$ mV). In fact, mitochondria are the most negatively charged organelles in the cell. The stored energy in $\Delta \Psi_m$ is used to synthesize ATP: As protons flow back into the matrix through a proton channel in complex V, ADP and Pi are bound, forming ATP (oxidative phosphorylation). Matrix ATP is then exchanged for cytosolic ADP.

ATP Versus Heat Versus ROS

The efficiency by which dietary calories are converted to ATP versus heat is determined by the coupling efficiency between respiration and oxidative phosphorylation. If the ETC is efficient at pumping H+ out of the matrix (high $\Delta \Psi_m$) and the ATP synthesis is efficient at converting the H+ reentry into ATP, then the mitochondria will generate maximum ATP and minimum heat per calorie consumed (coupled mitochondria). If the efficiency of H+ pumping is reduced (low $\Delta \Psi_m$) then each calorie will produce less ATP and more heat (uncoupled mitochondria). Maintenance of a stable core temperature despite changing environmental conditions is critical, and in that sense $\Delta \Psi_m$, by determining the coupling efficiency, facilitates adjustment to the environment.8

In coupled mitochondria, in the presence of excess calories and low cytoplasmic ADP (due to less exercise), complex V stops bringing H+ back in the matrix and thus $\Delta \Psi_m$ increases; eventually the very negative potential prevents further H+ pumping and the ETC is stalled. As electrons keep coming in, they cannot be oxidized and interact with molecular O2, increasing ROS production. In contrast, decreased calories and high levels of ADP because of exercise result in H+ return into the matrix, decreasing the $\Delta \Psi_m$ and ROS.
production. Could this explain the fact that low calorie intake and exercise have been associated with longevity and less cancer or cardiovascular disease (Figure, A)?

In addition to diet and exercise, molecular mechanisms can directly alter the ΔΨm. Nuclear genes encode for uncoupling proteins (UCPs), channels that conduct $H^+$, and, by being inserted into the mitochondria membranes, can “short circuit” the ΔΨm (ie, the $H^+$ return back into the matrix without being linked to ATP production). This allows the nucleus to regulate mitochondria coupling by increasing the production of UCPs. Alternatively, the ΔΨm can be decreased by “defective” or “leaky” complexes I, III, IV, and V as a result of mutations in the genes encoding subunits of these megacomplexes. Therefore, UCP or a “leaky complex” can result in mitochondria producing more heat, allowing better adjustment to cold climates, and in that sense can be evolutionary beneficial. At the same time, many diseases result directly from such mutations or UCP abnormalities, including atherosclerosis.

### Mitochondrial Transition Pore

The mitochondrial transition pore forms a megachannel that, when open, allows the efflux of proapoptotic mediators to the cytoplasm, activating protein breakdown (ie, apoptosis). The mitochondrial transition pore is both redox- and voltage-dependent, and decrease in ΔΨm promotes apoptosis. In contrast, mitochondrial hyperpolarization (increased ΔΨm) moves the mitochondrial transition pore away from the apoptotic threshold.

Maximal hyperpolarization stalls respiration and oxidative phosphorylation, essentially “shutting off mitochondria” and shifting energy production to the cytoplasm, with glycolysis becoming the primary source of ATP. A glycolytic phenotype is associated with resistance to apoptosis, in part because the “inactive” hyperpolarized mitochondria cannot induce apoptosis. The majority of human cancers are characterized by hyperpolarized mitochondria, and this metabolic remodeling might be the basis of the Warburg effect in cancer.
Intriguingly, the same mitochondrial hyperpolarization is also present in smooth muscle cells from human and animal pulmonary hypertension, explaining the apoptosis resistance that drives the proliferative vascular remodeling in this disease. Thus, ∆Ψm changes are seen in common diseases and can be induced by primary changes in ETC complexes, which could be a result of genetic changes. It is intriguing that the very few selected genes that form the mitochondrial DNA (mtDNA) are the ones encoding for ∆Ψm-relevant complex subunits.

mtDNA: The Protected Holy Grail of a Relationship That Stood the Test of Time

A proto-mitochondrion/bacterion entered eukaryotic cells ≈2 to 3 billion years ago. As the symbiosis matured, many bacterial genes were transferred to the nucleus of the host cell, such that today the maternally inherited mtDNA retains only few genes. Intriguingly, these are the genes coding for critical subunits of the ETC complexes I-III-IV-V, all which are involved in H+ pumping (I-III-IV) or ATP/ADP transport and therefore regulate ∆Ψm. In addition to those, mtDNA retained the genes for their transcription and translation (12S and 16S rRNAs and tRNAs), for a total of 37 genes. The remaining <1500 genes required for mitochondrial function are nucleus encoded, translated on cytosolic ribosomes, and imported into mitochondria through specialized import systems. These genes that are critical for ∆Ψm never left the mitochondria despite the pressure for the nucleus to “take over.” Thus, mtDNA can be seen as an index of ∆Ψm, opening new windows into the many diseases caused by abnormal ATP production (ie, metabolism), apoptosis, or ROS-toxicity (Figure, A).

Mitochondrial Genetics: Beyond Mendel

In contrast to nDNA, mutations in mtDNA accumulate sequentially, in part because of the strict maternal inheritance and the high mutation rate in mtDNA. The history of human mtDNA mutations can be tracked back to their last common ancestor, 150 thousand to 200 thousand years ago, and can be reconstructed as a single sequential mutational tree (Figure, B). Intriguingly, tree branches were found to correlate significantly with region-specific mtDNA polymorphisms and the geographic origin of indigenous populations. These mtDNA variants are called haplotypes, and groups of related haplotypes forming a tree branch are called haplogroups. The 4 oldest haplogroups L0–1 to 2–3 are found in Africa. Haplogroups M and N branched out of L3 in northern Africa ≈65 thousand years ago. As Europe was populated ≈50 thousand years ago, many haplogroups arose from N, including H (≈45% of European haplogroups)-T-U-V-W-X-I-J-Uk. In Asia, M and N branched out to A-B-F-C-p-G and others. Mixtures of B-A-C-D and X populations moved from Siberia and the Arctic Circle to the New World. Fascinating evidence now exists that the haplotypes found in populations residing in northern cold climates were promoting less-coupled mitochondrial function, generating more heat than ATP, thus facilitating adjustment to these environments.

Each cell hosts hundreds of mitochondria and copies of mtDNA. When an mtDNA mutation arises, a mixed population of mtDNAs is generated, a state known as heteroplasm. At cell division, mutant and normal mtDNAs are randomly distributed into daughter cells, drifting toward homoplasmic mutant, wild-type, or intermediate states. Therefore, the cellular phenotype produced, in contrast to the binary Mendelian genetics, is the net sum of these mixed states.® Deliberate mtDNA mutations resulting in abnormal mitochondria (less ATP, more ROS) can be amplified in a cell because the nucleus senses the lack of energy and commands the production of more (abnormal) mitochondria. Eventually, a critical threshold of ROS production is reached and apoptosis is induced, leading to disease. Because of genetic recombination, biparental inheritance of genes allows for species-wide spread of deleterious mutations that offer a replication advantage; perhaps in an effort to prevent this, the “fragile” mtDNA is now, after selection pressures, transmitted only maternally.

In this issue of Circulation, several European mtDNA haplogroups are shown to have no association with any cardiovascular or neoplastic disease or mortality. The Copenhagen City Heart Study provided blood and complete clinical data for 9254 patients, followed up for up to 25 years. In contrast to some associations with certain diseases shown by previous small case-control studies, the authors performed appropriate analysis, including multiple comparisons, that showed a lack of any associations. This is a well-done prospective study, and the authors need to be commended for their enormous effort, particularly in light of the negative results. Were the results expected? At first sight, given the importance of mitochondria in multiple aspects of health and disease, these results might appear surprising. However, a more careful approach suggests otherwise.

The haplogroups studied by the authors are associated with critical points in migrations of humans and were likely driven by natural selection, allowing optimal adjustments to the environment. At large, these mutations were beneficial or adaptive. In that sense, they should not be expected to be markers of severe diseases. As can be seen in the tree in the Figure (B), several mutations have since been tracked beyond these early branching points, which suggests that the mutations are unlikely to be driven by environmental stimuli. Potentially deleterious mutations should be expected to be found later on in evolution, toward the periphery of the mutation tree. To detect disease associations with such mutations in large populations is not an easy task and requires creative polymerase chain reaction approaches to overcome the unique challenges of mitochondrial genetics, like heteroplasm. Because of different mitotic rates and metabolic demands among different tissues, the degree of heteroplasm varies among tissues, and that of circulating blood cells might be different from that of the myocardium in a patient with heart disease.

In addition, the effort to identify a specific mutation as an independent risk factor for disease is based on the assumption that this is not related to other risk factors of the disease. This lack of relationship can be assumed in Mendelian genetics but is more challenging in mitochondrial genetics and in multifactorial diseases. For example, it is the accumulation of mtDNA mutations that increases with age that determines the disease phenotype, and therefore mitochondria-related diseases are often diseases of old age (cardiovascular or neuro-
degenerative diseases). Therefore, the ability of 1 mtDNA mutation to directly cause or facilitate a disease needs to be separated from simply its accumulation with age. Even then, the phenotype cannot be predicted by the presence of a mutation without knowing the degree of heteroplasmy. At this point, difficulties in the establishment of mtDNA mutations in animal models make genotype–phenotype associations in human diseases even more difficult.

Studies like this should inspire us for the effort, with the meticulous approach followed by the investigators and their courage to report a negative finding, which often enjoys less enthusiastic response than “typical” positive studies. However, like all good science, the study offers more questions and challenges for the future than answers, a necessary process for the momentum that the new mitochondrial medicine needs.

Disclosures
None.

References

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