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Mitochondrial dysfunction in aging: Much progress but many unresolved questions

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ABSTRACT

The free radical theory of aging is almost 60 years old. As mitochondria are the principle source of intracellular reactive oxygen species (ROS), this hypothesis suggested a central role for the mitochondrion in normal mammalian aging. In recent years, however, much work has questioned the importance of mitochondrial ROS in driving aging. Conversely new evidence points to other facets of mitochondrial dysfunction which may nevertheless suggest the mitochondrion retains a critical role at the center of a complex web of processes leading to cellular and organismal aging.

1. Introduction

Understanding the basis of human aging such that we might ultimately slow its course is one of the great biomedical challenges for the 21st century. Age is the most important risk factor for most of the common diseases. Although our knowledge of the aging process remains far from complete, most biogerontologists would now agree that aging starts with molecular damage, leading to cell, tissue and ultimately organ dysfunction [1,2]. This intrinsic aging process is seen as forming a 'tapestry' upon which the diseases of older age may appear. The opposing views would be that aging is simply the net result of accumulating chronic diseases, or that aging and chronic disease are parallel but unrelated processes. Perhaps the best known and most long-standing hypothesis to explain aging is the free radical theory, which proposes a central role for the mitochondrion as the principle source of intracellular reactive oxygen species (ROS) leading to mitochondrial DNA (mtDNA) mutations [3]. Somatic (acquired) mtDNA mutations have been extensively reported in normal human aging, particularly in post-mitotic tissue such as skeletal muscle and neurons, but also in replicative tissue such as the colonic crypt, and somatic mtDNA mutations are also well-described in age-associated neurodegenerative diseases [4–16]. Corresponding declines in mitochondrial function with age are also well described. However, these observations do not necessarily imply a causal relationship between mitochondrial dysfunction and human aging. In recent years the mitochondrion has once against assumed a pre-eminent role in aging research, driven in part by the development of an important mouse model [17,18]. Ironically, much of the recent work has cast doubt on the mitochondrial free radical theory of aging, but at the same time, important steps forward have been made in better understanding the nature of mitochondrial aging. Particularly important amongst these advances have been an increased awareness of the origin and natural history of mitochondrial mtDNA mutations in aging, and an increased ability to link the mitochondrion with other cellular pathways of aging. As a result we are now arriving at a more nuanced and complex understanding of mitochondrial aging, which will hopefully offer a better chance of effective intervention over the next decades. Nevertheless there remain a number of unresolved controversies and contradictory observations within the field. As such in this introductory review we will consider some recent advances in the field, framed here as a number of the more important unresolved questions.

1.1. Mitochondrial DNA mutations and aging: oxidative damage or replication error?

Mitochondria are ubiquitous intracellular organelles, present in almost all mammalian cells. Their primary role is of adenosine triphosphaste (ATP, the main source of intracellular energy) production through oxidative phosphorylation. Mitochondria contain their own small 16.5 kb circular chromosome of DNA encoding several key proteins of the mitochondrial respiratory chain [19]. However the majority of the >1000 predicted mitochondrially targeted proteins are encoded by the nuclear genome. The mitochondrial respiratory chain comprises 5 multi-subunit complexes, the last of which being ATP synthase. Electrons are exchanged down the chain at increasing reduction potentials from complexes I through IV, allowing the shuttling of protons across the mitochondrial membrane creating a proton gradient (membrane potential). Proton flux through the ATP synthase then provides the energy which drives ATP synthesis. Some premature electron leak inevitably occurs at the respiratory chain, resulting in the generation

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of superoxide radicals. Specifically, complexes I and III are reported to be the major sources of ROS [20]. Partial uncoupling (inefficiency) of the respiratory chain allows some proton leak, that is, movement of protons back into the mitochondrial matrix space that does not occur *via* ATP synthase. This makes the respiratory chain less efficient, and physiologically is used for thermogenesis in brown fat. It has been previously assumed that uncoupling might result in increased oxidative damage. States of marked uncoupling are highly deleterious and are associated with increased ROS. However, mild uncoupling in fact significantly reduces ROS production. It has been suggested (albeit controversially) that mtDNA subhaplogroups associated with mild uncoupling may have been selected for their increased thermogenesis in cold climates [21], but may also confer a longevity advantage due to decreased ROS. The mutation rate of the mitochondrial genome is estimated to be ~15× that of the nuclear genome. This observation arises from several considerations: 1) the mitochondrial genome is located on the inner mitochondrial membrane, adjacent to the respiratory chain, which is the major source of intracellular ROS production; 2) the mitochondrial genome lacks protective histones; 3) the DNA repair mechanisms are limited compared with the nuclear genome. It was therefore long assumed that ROS was the major source of somatic (acquired) mtDNA mutations in aging [22,23]. The mitochondrial theory of aging goes on to postulate that the accumulation of mtDNA mutations will lead to abnormalities of mitochondrial respiratory chain proteins, causing partial uncoupling of the respiratory chain. This in turn will lead to further increased ROS and more mtDNA mutations. Such a 'vicious cycle' hypothesis would predict an exponential rather than linear trajectory of increasing mtDNA mutation burden, as the initial mutations would provoke a further mutational 'burst'. In fact, however, recent studies suggest that mtDNA mutational burden may not significantly increase at all during human aging, suggesting that a model based on ROS does not properly explain the natural history of mtDNA mutations over the human life-course [24,25].

In contrast, recent data have suggested an importance for naturally occurring replication errors in the formation of age-associated mtDNA mutations. The characteristic mtDNA mutation type in post-mitotic tissues (such as muscle and neurons) is the large-scale deletion [26]. Such mutations typically delete several kbs of the mitochondrial genome, and as this is composed almost entirely of coding genes, such mutations are highly likely to have a functional effect. Large-scale deletions have a very characteristic distribution within the 'major arc' of the mitochondrial genome, between the origins of replication. The 5′ and 3′ ends of the deletion are clustered around hotspots associated with homologous repeats [27–29]. The classic example is the 4977 bp 'common deletion' which is associated with 13 bp homologous repeats at each end. The majority of deletions are similarly associated with homologous (or near homologous) repeats. Recent physicochemical modeling suggests that once formed these deleted mtDNA species have inherent stability [27]. The importance of homologous repeats in deletion formation suggests a role for single-stranded DNA (ssDNA) intermediates as these will allow the homologous repeats to anneal. Previously this phenomenon had been thought to arise through the 'strand asynchronous' mechanism of mtDNA replication. More recent data suggest however that double-stranded breaks (DSBs) may be the driving force [30]. These could arise through a variety of processes known to occur naturally including: replication stalling, oxidative damage and UV radiation. Once a DSB has formed, repair of the mtDNA molecule will be attempted by exonuclease activity which initially creates ssDNA. This can then anneal at homologous repeats, leading to the mtDNA deletion. This recent hypothesis however remains controversial and many authors remain in favor of the previous model of slipped mispairing [31].

1.2. Mitochondrial aging and the 'mutator' mouse: proof of causality?

About a decade ago, two very similar mouse models were developed almost simultaneously which have revealed many new insights into mitochondrial aging [17,18]. These mice have a homozygous knock-in mutation ($Polg^{D257A/D275A}$) for an error-prone polymerase gamma (the sole mtDNA polymerase). These mice are referred to as *PolgA*, or colloquially as the 'mutator mice'. They show greatly increased accumulation of somatic mtDNA mutations throughout life, associated with significantly reduced longevity, and a marked progeroid phenotype that recapitulates the vast majority of phenotypic features of normal human aging including: kyphosis, reduced fertility, testicular atrophy, cardiomyopathy, hemopoietic stem cell decline, and frailty.

Prior to the development of the 'mutator' mouse the evidence for a role of mtDNA mutations in aging was largely correlative. That is, although a number of studies had reported somatic mtDNA mutations in aged persons (as described above), it was possible that these were simply a marker of chronological rather than biological age. The mouse models appeared to suggest that mtDNA mutations had a causal role in aging. Closer scrutiny, however, revealed that the true picture was likely to be more complex. Although the homozygous mouse has a clear progeroid phenotype, this is associated with a vastly increased mtDNA mutation rate. The heterozygous mouse has a modestly increased mutation rate, which appears to exceed that seen in an elderly human, but has an apparently normal phenotype [32]. These further observations led some authors to suggest that the 'mutator' mouse could not properly capitulate mtDNA mutations in normal human aging. Whilst this objection has some currency, the model should not however be rejected out of hand [33]. A key further consideration is the great difference in lifespan between humans (>80 years) and mice (~3 years). MtDNA is constantly turned over throughout life, even in non-dividing cells, and to the best of our knowledge the rate of turnover ('half-life') of mtDNA is likely to be very similar in mice and humans. Therefore, the elderly human has experienced vastly more cycles of mtDNA replication the aged mouse. Recent data suggest that cycles of mtDNA replication are likely to play a critical role in the natural history and functional relevance of mtDNA mutations in aging, as is discussed in the following section.

Finally there is some controversy over the types of mutations seen in the 'mutator' mouse, the extent to which these reflect those seen in normal human aging, and which type(s) may drive the phenotype. Linear forms of mtDNA (which are presumably not being properly degraded) seem to be particularly common in the mouse model but are not thought to be an important feature of normal human aging. In contrast 'canonical' deletions occur rather rarely if at all in the 'mutator' mouse [34,35].

1.3. Clonal expansion: the importance of early mutations?

Normal mammalian cells contain multiple copies of the mitochondrial genome, typically hundreds to tens of thousands per cell. Thus any mtDNA mutation will co-exist with the wild-type within a cell, a state known as heteroplasmy. Typically the mutant mtDNA must exceed a heteroplasmy level of ~70% in order to cause a functional defect (although this may vary somewhat between mutation types) [36,37]. A somatic mutation will presumably initially exist as a unique species within a cell. How can it therefore reach a sufficient heteroplasmy level to cause a functional defect? This process is known as clonal expansion, and broadly speaking could either occur selectively (*i.e.* the mutant mtDNA species expands preferentially at the expense of the wild-type), or neutrally. A selective expansion, based on differential size, is plausible for large-scale deletion mutations, and there is some *in vitro* evidence to support its occurrence [38]. A neutral theory of clonal expansion is based simply on the notion that mtDNA is continuously turned over in non-dividing cells (termed 'relaxed replication') [39–41]. By chance, in a minority of cells a mutant mtDNA species will increase to a significant level through random drift. This process was predicted to be slow (progressing over decades), and thus implied a functional importance for mutations arising early in life [42].

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