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SPOTLIGHT REVIEW

Mitochondria in vascular disease

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Abstract Mitochondria are often regarded as the powerhouse of the cell by generating the ultimate energy transfer molecule, ATP, which is required for a multitude of cellular processes. However, the role of mitochondria goes beyond their capacity to create molecular fuel, to include the generation of reactive oxygen species, the regulation of calcium, and activation of cell death. Mitochondrial dysfunction is part of both normal and premature ageing, but can contribute to inflammation, cell senescence, and apoptosis. Cardiovascular disease, and in particular atherosclerosis, is characterized by DNA damage, inflammation, cell senescence, and apoptosis. Increasing evidence indicates that mitochondrial damage and dysfunction also occur in atherosclerosis and may contribute to the multiple pathological processes underlying the disease. This review summarizes the normal role of mitochondria, the causes and consequences of mitochondrial dysfunction, and the evidence for mitochondrial damage and dysfunction in vascular disease. Finally, we highlight areas of mitochondrial biology that may have therapeutic targets in vascular disease.

Keywords Atherosclerosis • Mitochondria • DNA damage • Reactive oxygen species

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1. Introduction

Cardiovascular disease remains the leading cause of death in the Western World with two-thirds of mortality attributable to atherosclerosis. Atherosclerosis predominantly affects the large and medium-sized arteries, usually presenting from the fifth decade and accounting for over 1 million premature European deaths every year.[1] The atherosclerotic plaque is a multicellular lesion comprising vascular smooth muscle cells (VSMCs), monocyte/macrophages, T lymphocytes, and other inflammatory cells, in addition to intra- and extracellular lipid and cellular debris. Elevated circulating lipids, such as low-density lipoproteins (LDL), are a significant risk factor associated with increased plaque burden.[2] The migration of LDL into the vessel wall with subsequent oxidation and subsequent endothelial dysfunction are key processes initiating atherogenesis. LDL oxidation may occur through the action of intracellular lipoxygenases or be the result of reactive oxygen species (ROS).[3,4] Plaques often develop at regions of low shear stress at sites linked to endothelial dysfunction. Loss of the endothelium is implicated in leucocyte recruitment, adhesion, and migration and plaque development. However, VSMCs and monocyte/macrophages become the dominant cell types as the lesion advances. While early vascular lesions may be characterized by intimal hyperplasia and VSMC proliferation, mature lesions are characterized by a paucity of cells, premature cellular senescence, and increased apoptosis. The plaque environment has increased ROS levels and DNA damage, which may create elevated bioenergetic demands and also promote cell senescence and apoptosis. Together, inflammation, cell death, and senescence lead to the formation of vulnerable lesions. The rupture of vulnerable plaques exposes the prothrombotic core to the circulation. Platelets then aggregate to form thrombi that can lead to arterial occlusion,[5,6] manifesting as heart attacks, or emboli, manifesting as strokes.

2. Mitochondria

Mitochondria are double membrane organelles, contained within the cytoplasmic compartment of all eukaryotic cells. As well as the nucleus, mitochondria are a source of DNA within a cell. The mitochondrial 16 kb genome encodes 13 polypeptides of the respiratory chain while the remaining 79 polypeptides are nuclear-encoded. These polypeptides combine to create the respiratory complexes required for the transport of electrons through the respiratory chain and the generation of ATP.

Coordination between the nuclear and mitochondrial genomes requires a high degree of fidelity. As well as the respiratory chain polypeptides, over 1000 other nuclear-encoded proteins, such as those of the Krebs [tricarboxylic (TCA)] cycle and those required for the formation of protein channels, are required to shuttle into the mitochondria.[7] Protein translocation through mitochondrial membranes

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involves the unfolding and ratcheting of the polypeptides, mediated by motor and chaperone proteins such as hsp70. Once in the matrix the proteins refold to their correct conformation, although the exact mechanism remains unclear.[8]

3. Mitochondrial function and dysfunction

3.1 Oxidative phosphorylation

Mitochondria mediate oxidative phosphorylation (OXPHOS) via the transfer of electrons through multimeric complexes to produce ATP (*Figure 1*). Complexes I, II, III, and IV form the electron transport chain (ETC) on the inner mitochondrial membrane. NADH and succinate produced in the Krebs cycle donate electrons (e^-) to Complexes I (NADH dehydrogenase) and II (succinate dehydrogenase). These electrons are transferred to ubiquinone (Q) and then delivered to Complex III (ubiquinol: cytochrome *c* oxidoreductase). The electrons flow to Complex IV (cytochrome *c* oxidase) via cytochrome *c* (C) and finally to the terminal accepter oxygen, producing water. As the electrons are transferred, protons (H^+) are pumped to the intermembrane space to create a gradient and the mitochondrial membrane potential. ATP synthase (Complex V) couples proton flow down this gradient to the synthesis of ATP, which is then available to fuel cellular function.

3.2 Reactive oxygen species

ROS are produced as a by-product of the respiratory chain, making the mitochondria the major source of cellular ROS.[9] The leakage of electrons from the ETC, predominantly at Complexes I and III, leads to the partial reduction of oxygen.[10] Superoxide ($O_2^{\bullet -}$) is produced, which matrix manganese superoxide dismutase (MnSOD) or CuZnSOD in the intermembrane space convert into hydrogen peroxide (H_2O_2). H_2O_2 can then be fully reduced to water by antioxidant enzymes, such as glutathione peroxidase (GPX) or catalase.[11] GPX uses reduced glutathione (GSH) to catalyse the reduction in H_2O_2, and the resulting oxidized glutathione (GSSG) is restored to GSH by glutathione reductase (GR) (*Figure 1*). While catalase can also eliminate H_2O_2, it is only present in mitochondria from the heart and liver. BH3 homology proteins such as Bcl-2 have been suggested to also mediate an antioxidant role. For example, Bcl-2 has been shown to increase the expression of SOD. However, others have shown that Bcl-2 is initially pro-oxidant and there is up-regulation of the antioxidant defences in response.[12]

The mitochondrial antioxidant systems are important, because if H_2O_2 is not reduced to water, it can generate the dangerous hydroxyl radical. Superoxide can also combine with nitric oxide to produce highly reactive products such as peroxynitrite ($OONO^-$).[13] ROS can have deleterious effects on cellular function, through the modification of DNA, proteins, and lipids as described below. However, ROS also have important physiological roles, probably the most recognized of which is in the defence against infectious pathogens. Through the respiratory burst, phagocytes are capable of generating high levels of superoxide and hydrogen peroxide, to help with the clearance of microbes.[14] Beyond immune defence, the role of ROS also extends to signal transduction and second-messenger generation. For example, the hydroxyl radical activates guanylate cyclase, leading to the production of cGMP.[15] This is important for regulating vascular

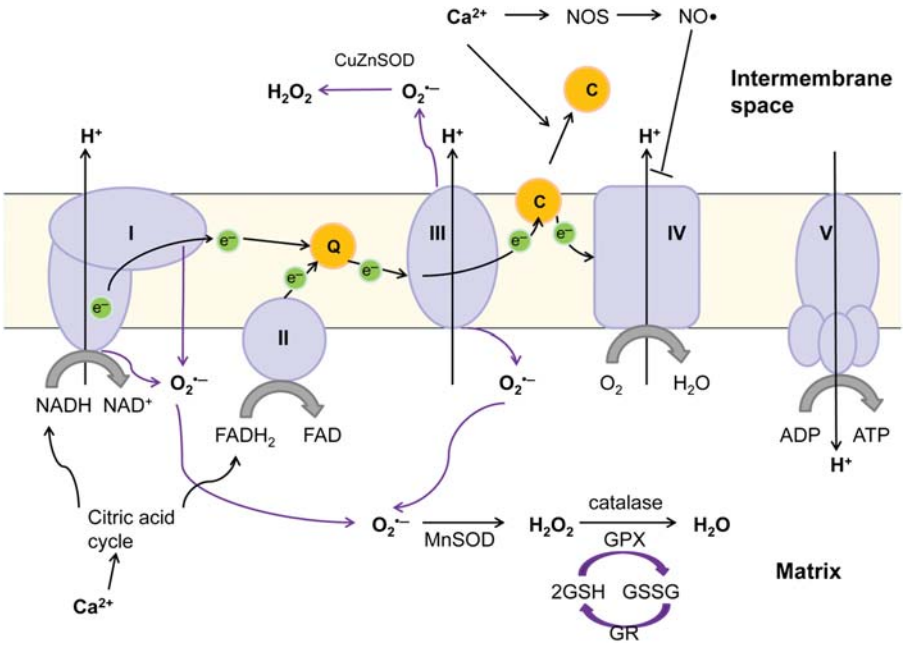

Figure 1 OXPHOS, superoxide production, and antioxidant pathways in mitochondria. NADH and $FADH_2$ supply high-energy electrons (e^-) from metabolic substrates. Electrons pass through the ETC and reduce molecular oxygen to form water at Complex IV. Complex V uses the proton gradient achieved to convert ADP to ATP. Superoxide $O_2^{\bullet -}$ is formed at Complexes I and III and is dismutated to H_2O_2 by matrix MnSOD or CuZnSOD in the intermembrane space. H_2O_2 can then be fully reduced to water by GPX or catalase. GPX uses reduced GSH to catalyse the reduction in H_2O_2, and the resulting GSSG is restored to GSH by GR. Ca^{2+} influences ROS production by promoting citric acid cycle activity, increasing the loss of cytochrome *c* and stimulating NOS. The $NO^{\bullet}$ generated inhibits respiration at Complex IV, enhancing ROS production.

tone, with cGMP mediating vascular smooth muscle relaxation, and hence vasodilation.[16] ROS have also been implicated in mitogenic signalling. In particular, superoxide increases mitogen-activated protein kinase activity and stimulates VSMC proliferation.[17,18] However, frequent exposure to ROS can cause cell death,[18] such that regulation of their levels is crucial to cellular homeostasis.

The balance between ROS generation and the antioxidant activity of the cell controls cellular oxidative status. ROS production is influenced by a variety of factors including the mitochondrial metabolic state. For example, stimuli such as hyperglycaemia and leptin (which is involved in the regulation of body weight) can both induce superoxide production.[19,20] Increasing levels of oxygen and decreased electron flow through the ETC are also associated with enhanced ROS generation,[9,21] and calcium is an important regulator of ROS production (reviewed in Kowaltowski *et al.*[22]). While an increase in Ca^{2+} may lead to decreased ROS formation through a transient decrease in the mitochondrial membrane potential,[23] excess Ca^{2+} is associated with oxidative stress.[24] The potential mechanisms of how Ca^{2+} influences ROS production include promoting citric acid cycle activity and increasing the loss of cytochrome *c*.[22] Ca^{2+} can also stimulate nitric oxide synthase (NOS),[25] increasing nitric oxide $NO^{\bullet}$ generation, which inhibits Complex IV.[26] Again ROS formation would be enhanced (*Figure 1*).

As oxidative stress occurs when there is an imbalance between ROS production and the antioxidant defences, it is important that ROS can have a regulatory effect on their own levels. For example, superoxide stimulates uncoupling protein (UCP-1), thereby decreasing the mitochondrial membrane potential and reducing ROS generation.[27,28]

3.3 Calcium

While 99.9% of calcium is deposited in bones, its flux within the body is tightly regulated, with serum calcium levels rarely changing by more than 1%. The role of calcium in the contraction of cardiac, skeletal, and smooth muscle is well established. However, intracellular calcium is also involved in signal transduction pathways by acting as a second messenger, and it is a co-factor for many enzymes.

Mitochondrial calcium uptake occurs via the Ca^{2+} uniporter[29] driven by both the concentration gradient and mitochondrial membrane potential. Importantly, through close apposition with the endoplasmic reticulum (ER) or plasma membrane (PM), mitochondria can be exposed to high-concentration Ca^{2+} microdomains,[30,31] which stimulate Ca^{2+} uptake, allowing mitochondria to sense and modulate cellular Ca^{2+} signalling. Calcium extrusion occurs via the sodium/calcium exchanger to maintain mitochondrial Ca^{2+} levels.[32] In addition, the mitochondrial permeability transition pore (MPTP) also allows calcium efflux. Furthermore, mitochondria can influence cellular Ca^{2+} through their generation of ATP, which is necessary for Ca^{2+}ATPase activity. These transporters are found on the PM and sarcoplasmic reticulum (SR) and help regulate cytosolic Ca^{2+} concentration.[33,34]

4. Mitochondria dysfunction in vascular disease

4.1 Evidence for mitochondrial dysfunction in atherosclerosis

There is increasing evidence that mitochondrial damage and dysfunction occurs in atherosclerosis in both human cells and in animal models. For example, a large 5 kb section of deleted mtDNA is often observed and is termed 'the common mitochondrial deletion'. This occurs at sites of mis-repaired mtDNA damage[35] and is increased in leucocytes of patients with atherosclerosis.[36] ROS exposure increases levels of mtDNA oxidative lesions and reduces mitochondrial protein and ATP production in human VSMCs.[37]

Hyperlipidaemia is a risk factor for atherosclerosis and apolipoprotein E (ApoE) is a component of lipoprotein particles required for their uptake into tissues. Mice deficient for ApoE ($ApoE^{-/-}$) develop hyperlipidaemia and subsequent accelerated atherosclerosis. It has been observed that mitochondrial DNA damage in $ApoE^{-/-}$ mice precedes atherogenesis and the damage is exacerbated by impaired antioxidant activity.[38] Smoke exposure also promotes atherogenesis and aortic mtDNA damage, with an accompanying decrease in cardiac adenine nucleotide transporter (ANT) activity which is important for ATP synthesis.[39] More recently, $ApoE^{-/-}$ mice haploinsufficient for the DNA repair enzyme ataxia telangiectasia mutated (ATM) demonstrated accelerated atherogenesis, increased nuclear and mtDNA damage, and impaired liver mitochondrial Complex I activity.[38,40] Respiratory chain dysfunction is therefore shown to be associated with atherosclerosis development, but as yet, its role as a causal factor in atherogenesis has not been proven.

Nuclear and mitochondrial DNA damage such as 8-oxo-G (an oxidized form of guanine) has been found in human lesions, and recent data suggest that accumulation of this damage precedes atherogenesis and correlates with the extent of disease.[41] Nuclear and mtDNA damage can combine, causing the assembly of faulty respiratory complexes, with resultant respiratory chain dysfunction.[42] While damage to Complex V only affects ATP synthesis, disruption to Complexes I, III, and IV can also decrease the mitochondrial membrane potential. Overall, the reduced energy supply affects cellular activity.[43]

4.2 Causes of mitochondria dysfunction

Mitochondrial dysfunction can be caused by DNA damage which is associated with many of the risk factors for atherosclerosis.[44] For example, smoking can both induce DNA damage and inhibit the rate of DNA repair.[45] Diabetes mellitus is characterized by Islet cell dysfunction and failed DNA repair,[46,47] which is exacerbated by ROS.[48] In addition, hyperlipidaemia is regarded as one of the key driving forces of atherosclerosis, with oxidation of lipoprotein particles associated with increases in DNA damage markers. Importantly, mitochondrial DNA is particularly susceptible to free radical damage. While nuclear DNA is ensconced within protective histones and chromatin, mitochondria lack this protection. Furthermore, mitochondrial DNA is closer to the generator of free radicals, the respiratory chain. Finally, mitochondria rely on more basic DNA repair processes, such as base excision repair (mt-BER), which removes smaller adducts incorporated by alkylation, deamination, or oxidation.[49] However, unlike the nucleus, mitochondria can increase their biogenesis[50] and remove poorly performing mitochondria via mitophagy and the ubiquitin–proteasome system (UPS).

Altered mitochondrial dynamics could be another cause of mitochondrial dysfunction. Mitochondria constantly undergo fission and fusion events, which control their morphology and integrity.[51,52] Fusion allows mixing of the mitochondrial genomes, diluting and so protecting against damaged DNA.[53] Fission is also required for normal mitochondrial function, with impairment leading to decreased respiration.[54] The accumulation of dysfunctional mitochondria may therefore result from changes in mitochondrial dynamics. Whether

this contributes to vascular disease development is an interesting concept, which is yet to be fully explored.

The health of mitochondria is in part regulated by their biogenesis, and PGC-1α (peroxisome proliferator-activated receptor gamma coactivator alpha) is regarded as the master regulator of mitochondrial biogenesis and homeostasis.[55] PGC-1α is a transcriptional coactivator of PPARγ, and together, they regulate genes involved in energy metabolism. Working through cAMP response element-binding proteins and nuclear respiratory factors, they provide a link between extracellular stimuli and regulation of mitochondrial biogenesis. PGC-1α can be regulated through several different mechanisms. Cell stress can increase levels of ROS, which in turn can stimulate PGC-1α.[56] The cell responds by increasing ATP availability, through the transcription of OXPHOS co-regulated genes.[57] The histone deacetylase SIRT1 is also known to bind and activate PGC-1α through deacetylation,[58] and post-translational modifications such as sumoylation are also thought to modify its activity and its degradation through ubiquitin-mediated degradation.[59] Collectively failure of PGC1-α regulation can lead to impaired mitochondrial biogenesis and health and contribute to the disease phenotype.

4.3 Consequences of mitochondrial dysfunction

One theory of the contribution of DNA damage to atherosclerosis is that nuclear and mtDNA code for mutated polypeptides, which are incorporated into the respiratory chain and contribute to defective OXPHOS. Loss of integrity of the respiratory chain, especially at Complex I, is thought to increase ROS and feeds back to further increase DNA damage. The nuclear genome has evolved complex and multiply redundant pathways to effect repair in response to DNA damage[60] (*Figure 2*). Thus, there is constitutive expression of sensor proteins, such as Mediator of DNA damage Checkpoint-1 (MDC1), which keep guard, waiting for a reactively modified DNA nucleotide or base pair.[61] Once faulty DNA is detected, MDC1 is bound and a number of proteins are recruited. Initially, MRE11/RAD-50/NBS-1 (MRN) or 9-1-1 complexes activate ATM kinase and ATM-related kinase by phosphorylation causing dimer dissociation. This can lead to phosphorylation of the checkpoint kinases (CHK1/2).[27,62] These in turn activate effector molecules such as p53, to result in DNA repair, cell cycle arrest, or initiation of apoptosis.[63]

Normal mitochondria can become dysfunctional through DNA damage and disrupted mitochondrial dynamics. Mitochondrial dysfunction manifests as impaired ATP production, increased ROS generation, and calcium dysregulation. These changes are likely to affect all the cell types involved in atherosclerosis, including endothelial cells (ECs), inflammatory leucocytes, and VSMCs. The negative changes in cell physiology promote apoptosis, cell cycle arrest, senescence, altered lipid processing, and inflammation, which are all key processes in the development of vulnerable atherosclerotic plaques.

4.3.1 ROS formation

Inhibition of OXPHOS or Complex I deficiency promotes increased production of superoxide and hydrogen peroxide.[64,65] The resulting oxidative stress promotes DNA damage, and oxidative modification of mitochondrial lipids and proteins, altering cellular bioenergetics. For example, cardiolipin located in the inner mitochondrial membrane is needed for electron transfer in Complex I,[66] oxidative damage of cardiolipin reduces Complex I activity.[67] Furthermore, ROS can affect ATP generation by modifying and inhibiting ANT.[68] The

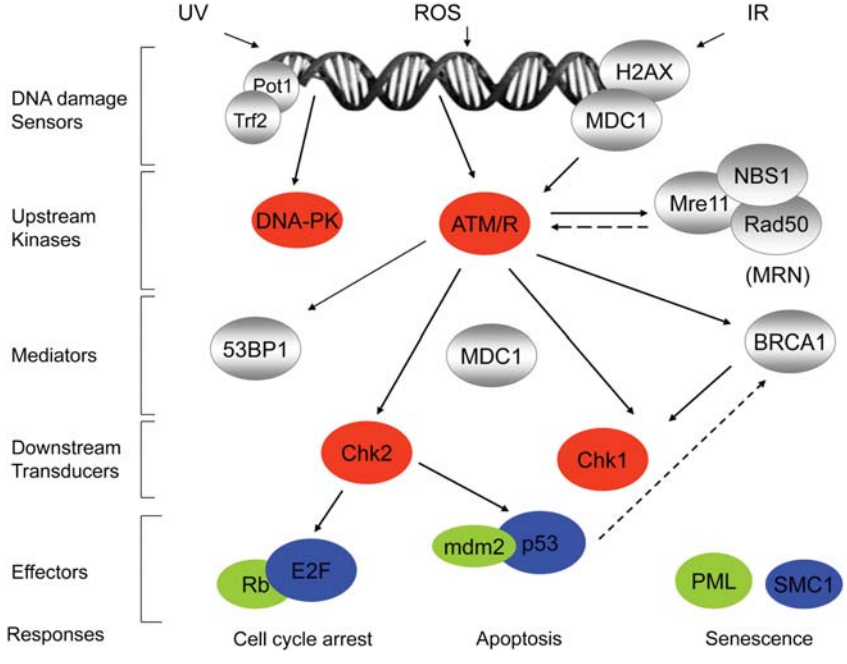

Figure 2 DNA damage repair signalling pathway in response to single- and double-strand breaks. Constitutive expression of sensor proteins such as MDC-1 and H2AX help recruit the MRN complex. PIKK family proteins such as DNApk and ATM are then activated, leading to phosphorylation of the checkpoint kinases CHK1 and CHK2.[27,62] These and other proteins in turn activate effector molecules such as p53, to result in DNA repair, cell cycle arrest, or initiation of apoptosis.[63] A full description of these pathways is provided in the text.

resulting decrease in ATP favours MPTP opening, and indeed, loss of SOD antioxidant capacity leads to early apoptosis in a murine model.[69]

4.3.2 Calcium dysregulation

Cytosolic calcium must be regulated with exquisite sensitivity to maintain cellular homeostasis. While the ER is the traditional cytoplasmic store,[70] recent evidence suggests that mitochondria may be the gatekeepers controlling calcium signalling.[32]

Reduced mitochondrial ATP generation can disrupt cellular Ca^{2+} homeostasis, through impaired PM and SR Ca^{2+} ATPase activity. At the level of the mitochondria, respiratory chain dysfunction leads to loss of the membrane potential, which is required for mitochondrial Ca^{2+} entry via the uniporter.[71] Resultant changes in Ca^{2+} concentration affect respiration, because several intramitochondrial enzymes (pyruvate, oxoglutarate, and sodium isocitrate dehydrogenase) are Ca^{2+}-sensitive.[72] A decrease in their activity leads to reduced substrate provision for OXPHOS, further favouring apoptosis or cellular senescence.

4.3.3 Apoptosis and cellular senescence

Although pro- and anti-apoptotic signalling pathways are complex, broadly apoptosis can occur via two pathways—the receptor-mediated extrinsic pathway or the mitochondrial-dependent intrinsic pathway.

The intrinsic apoptotic pathway is dependent on the MPTP, first proposed by Haworth and Hunter in 1979.[73] While its exact structure still remains uncertain, it has been suggested that the outer membrane voltage-dependent anion channel combines with inner membrane pores, such as the phosphate carrier Pic or ANT[74,75] (*Figure 3*). Although this is debated, nearly all groups confirm the presence of cyclophilin D as an essential component. When the MPTP opens in response to apoptotic stimuli, equilibration of Na^+, K^+, and Ca^{2+} ions between the mitochondrial matrix and cytosol can occur, leading to mitochondrial swelling.[76] There is a subsequent release of factors promoting cell death, including cytochrome *c*, apoptosis-inducing factor, and second mitochondrial activator of caspases (Smac).[77–79] Cytochrome *c* triggers the binding of Apaf-1 with procaspase 9, and the subsequent caspase 3 activation initiates the downstream apoptotic pathway.[80] Furthermore, the process is self-amplifying, with the loss of cytochrome *c* impairing OXPHOS and the antioxidant capacity of the mitochondria. Regulation of MPTP is controlled by calcium flux, ROS, and ATP.[81] As decreased levels of ATP favour pore opening,[76] mitochondrial respiratory chain dysfunction can result in increased apoptosis. Interestingly, recent work has shown that rupture of the outer mitochondrial membrane will not automatically lead to cell death if inner mitochondrial membrane integrity can be maintained. This provides a window of opportunity to rescue and repair damaged mitochondria, maintaining their viability and avoiding the onset of mitochondrial death.[82]

Impaired respiratory chain function can also lead to senescence. Cellular senescence describes the phenomenon where cells have a limited number of replicative divisions before they enter irreversible cell cycle arrest.[83] Alterations in metabolism occur with cellular

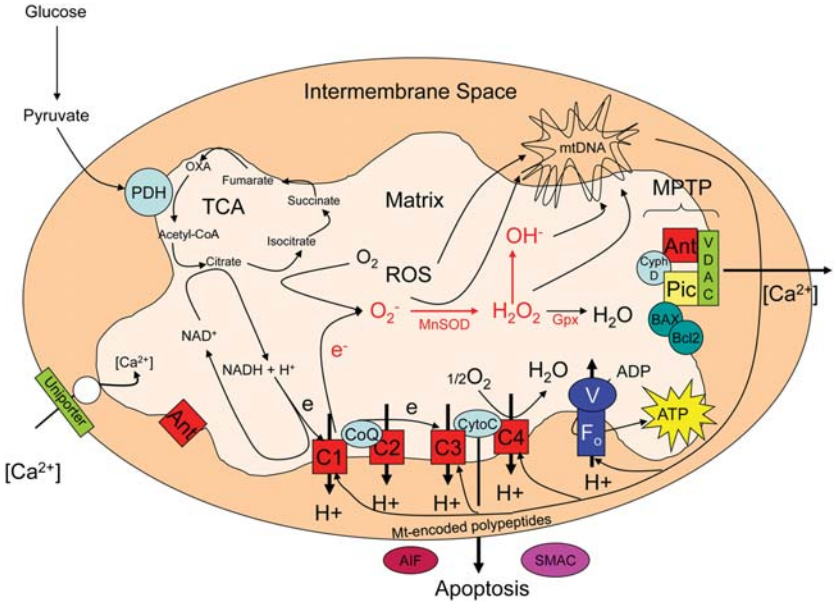

Figure 3 Interconnections between energy generation via OXPHOS through respiratory Complexes C1–C5, ROS production and apoptosis. The TCA cycle regenerates NADH as an electron donor to Complexes C1 and C2. Protons are concomitantly pumped across the intermembrane space and later returned via Complex V to generate ATP from ADP. The mitochondrial genome encodes polypeptides (seven for C1, one for C3) that combine with nuclear variants to create the multimeric complexes. Electrons can become unpaired and leak from passage through the respiratory complexes forming electron-free radicals. In close proximity to molecular oxygen, these form superoxide that can transmute to hydrogen peroxide and hydroxyl radicals. These ROS damage membranes and mtDNA, resulting in faulty complexes that leak more electrons to produce more ROS. Loss of cell homeostasis results in MPTP opening and dysregulation of calcium homeostasis. Inappropriate activation of calcium-dependent enzymes and protein kinase results in MPTP opening, loss of osmotic control, and release of cytochrome *c* to the cytosol and apoptosis (modified from Puddu *et al.*[44]).

senescence, including a significant reduction in ATP levels.[84] A decrease in ATP, and subsequent increase in AMP:ATP, can be sensed by AMP-activated protein kinase (AMPK).[85] AMPK activation inhibits biosynthetic pathways, helping energy conservation, while the decreased expression of proliferative genes, including cyclins A and B, leads to senescence.[86,87] Consistent with this, murine cells with mitochondrial defects have reduced proliferation.[88]

5. Consequences of mitochondrial dysfunction in atherosclerosis

Although direct evidence of causality is lacking, multiple potential consequences of mitochondrial dysfunction occur in atherosclerosis, including inflammation from ROS generation, apoptosis, and senescence, raising the prospect that mitochondrial damage/dysfunction directly promotes these features.

Apoptotic VSMCs, ECs, and macrophages are present in atherosclerotic lesions[89] and affect plaque development and morphology. VSMC apoptosis leads to accelerated plaque growth, with increased calcification and medial degeneration.[90] Furthermore, VSMC apoptosis can result in thinning of the fibrous cap, an increase in the necrotic core, and intimal inflammation.[5] Similarly, macrophages comprise 40–50% of the identified apoptotic cells present,[89] and macrophage apoptosis leads to an expansion of the necrotic core.[6] EC apoptosis is also significant as it would compromise the integrity and function of the vascular endothelial layer. Damage to the endothelium is considered to be an initiating step in atherosclerosis,[91] with LDL uptake and leucocyte adhesion and migration occurring at sites of endothelial dysfunction. EC apoptosis may also be mediated through mitochondrial dysfunction and MPTP activation, and indeed, oxidized LDL induces the mitochondrial apoptotic cascade in vascular ECs.[92] While endothelial mitochondrial dysfunction may not necessarily result in apoptosis, the altered Ca^{2+} handling could also affect ROS and nitric oxide generation, promoting atherosclerosis.[93]

Cell senescence has also been demonstrated in atherosclerosis. For example, VSMCs derived from human plaques show a senescent phenotype in culture[94,95] and express markers of senescence, including senescence-associated β-galactosidase and p21.[96] Senescent VSMCs have a decreased response to β-adrenergic receptor stimulation, which could increase vascular tone and blood pressure.[97] Furthermore, elastase production is increased by both senescent cells and in atherosclerosis,[98] promoting the breakdown of extracellular matrix and a decrease in vascular compliance. Importantly, the senescence of VSMCs could potentially contribute to inefficient plaque repair, with resulting plaque instability.[99]

While there is increasing evidence of mitochondrial dysfunction in atherosclerosis, it is not clear whether this is a consequence of the disease or that both atherosclerosis and mitochondrial dysfunction share common causes. For example, mitochondria evolve from normal to dysfunctional throughout their lifespan. The risk factors for atherosclerosis may accelerate this process, by directly affecting mitochondrial DNA and proteins, but by also changing their biogenesis and degradation. Similarly, it is unclear whether these effects are mediated entirely through ROS generation, or other effects from mitochondrial damage/dysfunction. These questions will not be answered until we can selectively promote or inhibit mitochondrial damage/dysfunction separate from ROS production and investigate the lifecycle of mitochondria during the development of atherosclerosis.

6. Mitochondrial dysfunction in other vascular diseases

Mitochondrial damage and dysfunction may also have a role in other vascular diseases, such as hypertension, stroke, heart failure, and cardiac ischaemia/reperfusion injury.[100,101] Global levels of hypertension are estimated at over 1 billion people worldwide,[102] and if untreated, the condition predisposes to increased cardiovascular-related mortality. However, hypertension has a complex aetiology with over 50 genes postulated to be involved,[103] some of which are also involved with mitochondrial homeostasis. For example, the angiotensin receptor AT-1 is a frequent target of drug intervention, and both drugs such as Losartan (AT-II antagonist) and the receptor itself may regulate free radical generation at the level of the mitochondria.[104] The AT-1 receptor has recently been shown to be sequestered to mitochondria and may influence signalling.[105] Mitochondrial dysfunction has also been seen in the microvasculature of stroke patients, which can be reduced by Losartan and mitochondrial-specific antioxidants such as Mito-TEMPO.[106] This suggests that not only is mitochondrial dysfunction leading to increased generation of ROS, which promotes these pathologies, but that targeted therapies may mediate protection.

As in the vasculature, an intact endothelium (endocardium) is required for normal cardiac physiology, as it is vital for substrate supply, provides mediators such as nitric oxide, and trophic support for cardiac myocytes.[93] Disruption to endothelial function has been documented in heart failure[107,108] and mitochondrial dysfunction may have a contributory role. For example, TNFα is increased in advanced heart failure[109] and can increase mitochondrial ROS formation in ECs.[110] The ROS produced can compromise endothelial function through oxidative damage, and through interacting with, and so decreasing the bioavailability of nitric oxide.[111]

Mitochondrial ROS production may also contribute to the endothelial dysfunction observed in ischaemia–reperfusion injury following restoration of coronary blood flow.[112] Ischaemia–reperfusion results in damage to the endothelium, with apoptosis of capillary ECs and decreased endothelium-dependent relaxation of coronary arteries.[113,114] Increased respiratory chain ROS generation may contribute to this endothelial dysfunction, as hypoxia–reoxygenation increases respiratory chain ROS production, which can then trigger interleukin-6 secretion and increased EC permeability.[115]

7. Mitochondria as targets for treatment in vascular disease

From the above discussion, it is apparent that prevention or reversal of mitochondrial damage/dysfunction may represent a target in vascular disease. Indeed, it is highly likely that the current therapeutics reduce mitochondrial damage/dysfunction as part of their mode of action. For example, the HMG-CoA reductase inhibitors (Statins), proven as a successful treatment of atherosclerosis,[116] capable of inducing lesion regression,[117] also reduce oxidative DNA damage, in part by accelerating DNA repair.[118] Statins also improve mitochondria biogenesis via PGC1-α and reduced ROS.[119]

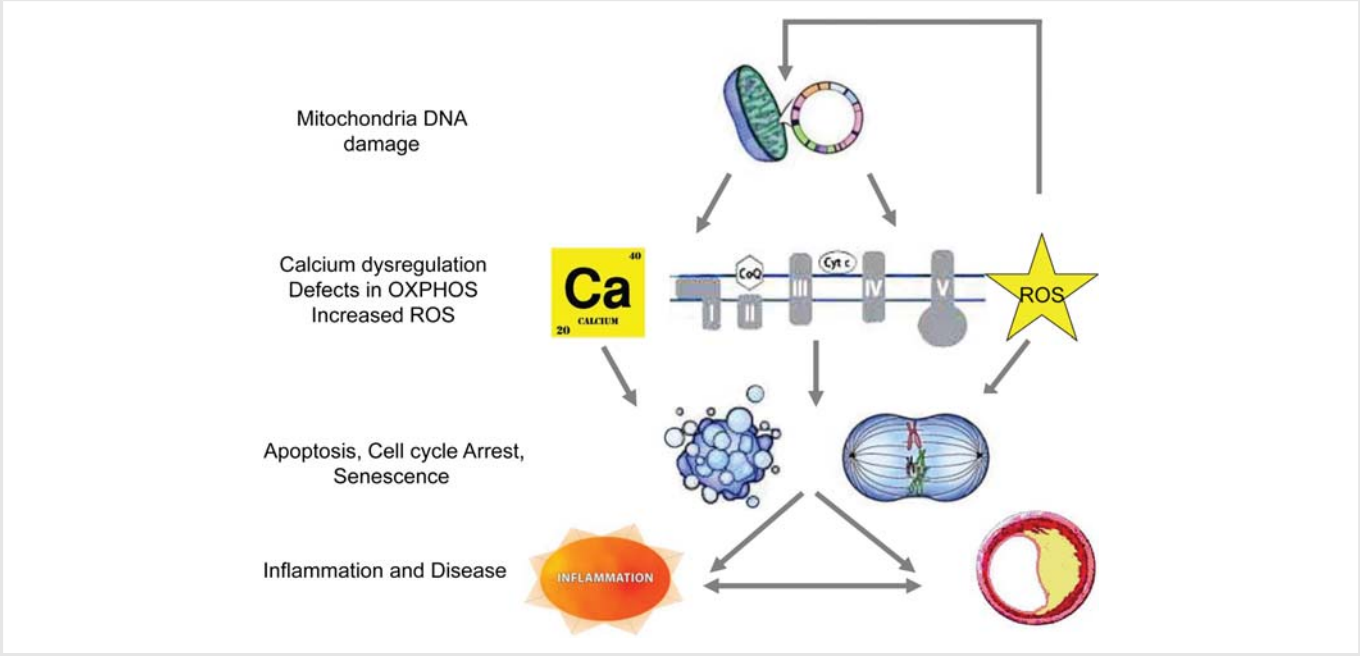

Figure 4 Summary of interactions between mitochondrial DNA damage/dysfunction and disease.

In contrast, it is more difficult to establish a role for specific treatments whose primary target is the mitochondria. However, given that ROS have a critical role in atherosclerosis development, recent advances in targeting antioxidants may be an effective strategy. An example would be an agent such as MitoQ, a targeted ubiquinone moiety that accumulates in mitochondria and decreases oxidative damage.[120] Although MitoQ improves cardiac hypertrophy[121] and ischaemic–reperfusion injury,[122] its potential role in human atherosclerosis remains undetermined.[123]

An alternative therapeutic approach is via intervention in the recycling of damaged mitochondria. Normal mammalian mitochondria are targeted for recycling by the UPS. Proteins such as Parkin are recognized by E3-ubiquitin ligase and are expressed on the outer mitochondrial membrane. These proteins tag the organelle for mitochondria-associated degradation or mitochondrial autophagy, now termed mitophagy.[124] It has been suggested that proteasomal ageing limits mitochondrial capacity to recycle and may provide a fruitful area of intervention.[125,126]

In addition to drug therapy, lifestyle interventions may also be beneficial in reducing the effects of mitochondrial damage/dysfunction, as suggested by knockout mouse models. For example, mice lacking DNA proofreading activity have mitochondrial dysfunction, an accelerated ageing phenotype, and multiorgan pathologies.[127] Recent work suggests that endurance exercise can confer a partial rescue of the pathology with reduced apoptosis in multiple tissues.[128] Increased mitochondrial biogenesis, decreased mtDNA damage, and improved respiratory chain capacity were demonstrated.[129] Similarly, calorie restriction has been shown to reduce mitochondria respiratory chain activity and ROS generation.[130] The reduced ROS production may be the result of decreased substrate availability or be due to Akt activation. Akt is a pro-survival kinase, mediating activation of eNOS.[131] eNOS is known to increase mitochondrial biogenesis, which is predicted to reduce ROS production. SIRT3, a mitochondria histone deacetylase, regulates fatty acid catabolism and ketogenesis during fasting, which is also implicated in the control of ROS generation.[132]

8. Conclusions

There is increasing evidence that mitochondrial damage/dysfunction occurs both in normal ageing and in atherosclerosis. Mitochondrial dysfunction can result in impaired OXPHOS, increased ROS generation, and calcium dysregulation. These effects promote apoptosis and senescence, which are key processes in the development of vulnerable atherosclerotic plaques (*Figure 4*). Mitochondrial dysfunction also has key metabolic effects, whose systemic manifestations may also promote atherosclerosis. Mitochondrial damage/dysfunction is thus a target for therapeutic intervention by targeted medicines or lifestyle changes.

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