Mitochondrial DNA damage and atherosclerosis

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Abstract

Mitochondria are the cellular powerhouses, fuelling metabolic processes through their generation of ATP. However we now recognise that these organelles also have pivotal roles in the generation of reactive oxygen species (ROS) and the regulation of cell death, inflammation and metabolism. Mitochondrial dysfunction therefore leads to oxidative stress, cell death, inflammation and altered metabolism, which are all key processes in atherosclerosis. Recent evidence indicates that mitochondrial DNA (mtDNA) damage is present and promotes atherosclerosis through mitochondrial dysfunction. In this review we discuss the role and mechanisms linking mtDNA damage and atherosclerosis, and identify areas of mitochondrial biology that may yield potential therapeutic targets.

Keywords: mitochondria, atherosclerosis, inflammation

Abbreviations

AMPK	AMP activated protein kinase
cGAS	cyclic GMP-AMP synthase
DAMP	damage-associated molecular pattern
Drp1	dynamin-related protein 1
FGF21	fibroblast growth factor 21
IFN	interferon
MOMP	mitochondrial outer membrane permeabilisation
MPTP	mitochondrial permeability transition pore
mtDNA	mitochondrial DNA
NFκβ	nuclear factor kappa beta
Opa1	optic atrophy 1
PAMP	pathogen-associated molecular pattern
ROS	reactive oxygen species
STING	stimulator of interferon genes
TFAM	mitochondrial transcription factor A
TLR	toll-like receptor
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
VSMC	vascular smooth muscle cell

Article

Atherosclerosis- a mitochondrial disease?

Whilst significant advances in medical and surgical treatment have been made, atherosclerosis remains the leading cause of death in the western world. Atherosclerosis is characterised by fatty plaque formation in the arteries- the large conduit vessels carrying vital oxygen and nutrients to the tissues. These plaques can rupture, potentially leading to vessel occlusion and clinical sequelae such as heart attacks and strokes [1]. With such an impact on health, the need remains to characterise the underlying pathological processes and to find new therapeutic targets. Recent work highlights that mitochondrial DNA (mtDNA) damage and dysfunction promote atherosclerosis and thus opens new avenues for therapeutic interventions.

The atherosclerotic plaque forms at sites of endothelial dysfunction, often where there is disturbed flow and altered shear stress [2]. Circulating lipids are taken up into the vessel intima, thereby initiating the early lesion- the fatty streak. The lipids undergo oxidative modification by enzymes and reactive oxygen species (ROS), promoting endothelial dysfunction. The expression of endothelial adhesion molecules recruits monocytes, where they become macrophages that engulf lipids to form foam cells. Whilst the scavenging of oxidised lipids is initially protective, an inflammatory response is triggered, leading to cell death and the development of the plaque necrotic core. Vascular smooth muscle cells (VSMCs) proliferate and/or migrate to the intima, where they secrete the collagen and extra-cellular matrix that forms the protective fibrous cap [1](Figure 1).

The fibrous cap is an important barrier, separating the highly thrombogenic core from the circulating blood. However inflammation, degradative enzymes and cell death can compromise the cap, leading to plaque rupture [3]. Once the core is exposed, thrombus forms which may occlude the vessel or embolise, leading to ischaemic complications such as heart attacks and strokes. Oxidative stress, cell death and inflammation are therefore key processes in atherogenesis [4, 5], all of which may be caused by mtDNA

damage and dysfunction [6, 7]. In this review the role and mechanisms of mtDNA and atherosclerosis will be discussed.

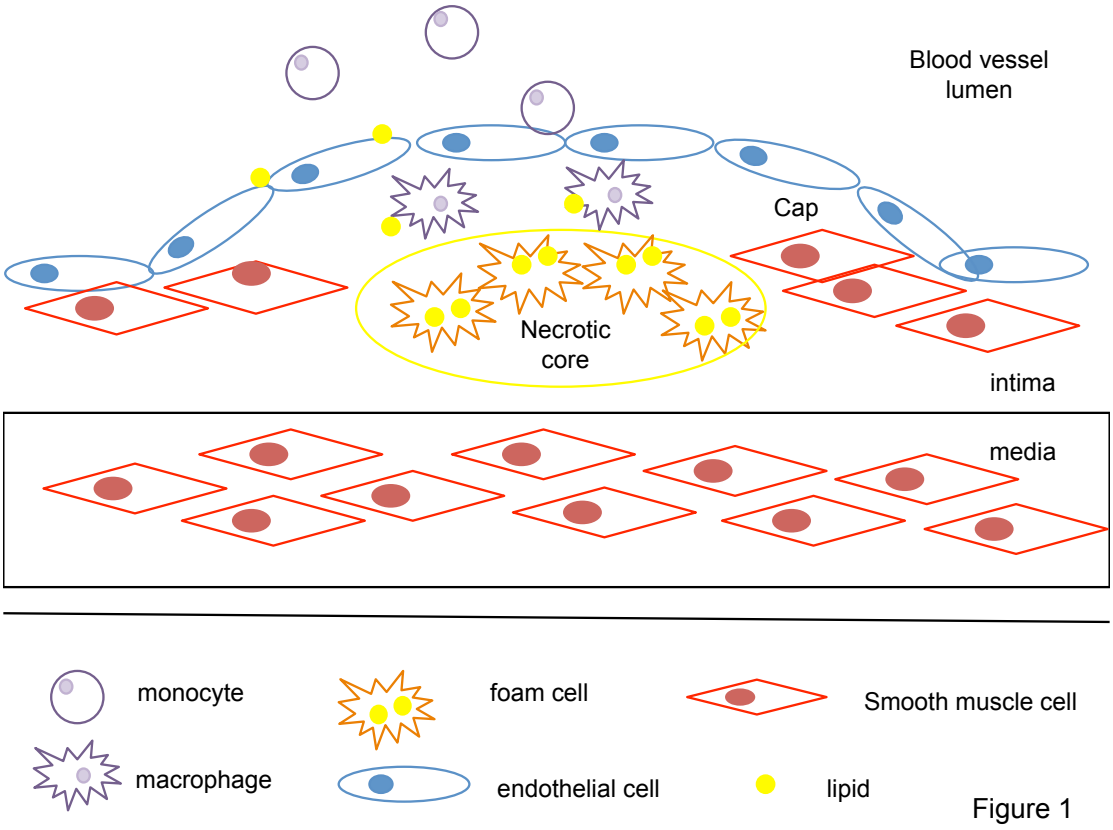

Figure 1. Atherosclerotic plaque formation

Circulating lipids are taken up into the vessel intima, where they become oxidised and promote endothelial dysfunction. Monocytes are recruited and become macrophages that engulf lipids to form foam cells. An inflammatory response is triggered, leading to cell death and the development of the plaque necrotic core. Vascular smooth muscle cells (VSMCs) proliferate and/or migrate to the intima, where they secrete the collagen and extra-cellular matrix that forms the protective fibrous cap.

Mitochondrial DNA damage and atherosclerosis

Mitochondria are the powerhouses of the cell, responsible for generating ATP through oxidative phosphorylation, with ROS formed as a by-product [8]. Except for the nucleus mitochondria are also the only source of DNA within the cell. Human mtDNA exists as a 16569 bp loop, encoding 13 respiratory chain polypeptides, together with transfer and ribosomal RNAs [9].

mtDNA is particularly vulnerable to oxidative damage, as it lies close to the site of ROS production and lacks protection from histones. mtDNA damage may also be increased in atherosclerosis as conditions that promote disease, such as hyperglycaemia and smoking, also increase ROS [10]. Replication error is another source of mtDNA defects. Mutations can occur in early life and become abundant through clonal expansion. It has been proposed that sufficient mtDNA damage can then impair respiratory chain function, and eventually compromise cellular function promoting ageing and disease [11].

There is now evidence that mtDNA damage is not only present but can also promote atherosclerosis. Early studies identified mtDNA damage in the aortas, hearts, and circulating leukocytes of patients with atherosclerosis [12-14]. Furthermore, mtDNA damage is an early event in atherogenesis [14], and mtDNA damage and mitochondrial dysfunction were both demonstrated in a mouse model of atherosclerosis and metabolic syndrome [15]. Although the latter study suggested a causative role for mtDNA damage in atherosclerosis, the differing effects of mtDNA and nuclear DNA damage could not be distinguished [15].

A useful model to examine the effects of extensive mtDNA damage is the mutator mouse. Polymerase gamma is the mitochondrial DNA polymerase, uniquely responsible for replicating the mitochondrial genome. Mutator mice express aspartate instead of alanine in the exonuclease domain, which impairs the enzyme's proof-reading activity resulting in extensive mtDNA mutations [16, 17]. Mutator mice that were also deficient in apolipoprotein E showed that mtDNA defects promote mitochondrial dysfunction,

atherosclerosis and plaque vulnerability. Furthermore, mtDNA lesions were associated with high-risk lesions in humans [6].

Collectively we now have evidence that mtDNA damage is present and promotes atherosclerosis, and that the findings may be relevant to human disease. We will now review the potential mechanisms linking mtDNA damage and atherosclerosis.

mtDNA damage and ROS

Harman first suggested the free radical theory of aging more than 40 years ago [18] . He proposed that a vicious cycle of ROS-induced mtDNA damage, leading to mitochondrial dysfunction and the generation of more damaging ROS, could lead to aging and death. The theory has been expanded to also include disease, and is particularly attractive when considering atherosclerosis where oxidative stress appears to be a key mediator.

Increased ROS are present in the vessel wall at all stages of atherosclerosis, and can lead to modification of DNA, protein and lipids [19]. Indeed oxidative DNA damage has been observed in plaque VSMCs, with upregulation of the DNA damage response [20]. Furthermore, mitochondrial DNA damage precedes and correlates with plaque development [14, 21]. Lipids, too, are a target of ROS and lipid oxidation is well-recognised as an important event in atherogenesis [22].

Further support for the role of ROS in atherosclerosis comes from *in vivo* models. A decrease in the antioxidant enzymes superoxide dismutase or glutathione peroxidase increases atherosclerosis [14, 23]. Moreover the expression of catalase in macrophage mitochondria has a protective effect, decreasing plaque formation [21].

Taken together, this evidence suggests that ROS are present and have a causative role in atherosclerosis. However it is uncertain whether increased ROS are necessary to mediate the effects of mtDNA damage. When first described, the mutator mouse showed no increase in ROS, despite extensive

mtDNA defects [17, 24]. More recently, whilst increased mtDNA damage, atherosclerosis and plaque vulnerability were all observed, still no change in ROS was seen [6]. The latter study also benefited from using a novel *in vivo* ROS probe- MitoB- that is specifically targeted to the mitochondria where it binds to hydrogen peroxide [25]. This work therefore casts doubt on whether increased ROS are required to mediate the effects of mtDNA damage in aging and atherosclerosis.

However, other work does suggest a role for ROS in the mutator mouse, with overexpression of mitochondria-targeted catalase attenuating cardiomyopathy [26]. The differing findings of these studies may be reconciled by a recent observation. MitoB was used to assess ROS in mutator mice, and whilst young mice showed no increase in ROS, aged mice did show an increase [7]. This would suggest that chronic, extensive mtDNA damage is necessary for increased ROS to occur.

These findings are important when considering anti-oxidants as therapeutics, and may account for the lack of benefit seen so far in atherosclerosis studies. For example, in a mouse model, the mitochondrial targeted MitoQ ameliorated features of the metabolic syndrome but had no impact on atherosclerosis development [27]. Furthermore in humans, a meta-analysis of trials involving a variety of vitamins and anti-oxidants has also shown no benefit [28]. Why no benefit has been seen remains unclear but may be due to the timing or targeting of therapies. It may be that anti-oxidant therapies need to be given in the early stages of disease and/or for a prolonged period to be effective. The trials also involved systemic administration of anti-oxidants [28] but perhaps targeting the therapies to the specific cell types involved would be more beneficial. We may also need to select the appropriate patient population, as potentially only those who are deficient in anti-oxidants would benefit from treatment.

The timing or targeting of therapies may explain why a beneficial effect of anti-oxidants in human atherosclerosis is yet to be proven. An alternative explanation is that ROS have positive effects, which are negated by

antioxidants. Undoubtedly ROS can cause damaging oxidative modifications, but they are also involved in signalling pathways. For example, mitochondrial ROS may trigger a pattern of gene expression that promotes survival and indeed increased mitochondrial ROS production extended lifespan in a worm model [29, 30].

Collectively, the current evidence indicates that oxidative stress is present in atherosclerosis, and likely promotes disease. However increased ROS are not necessarily required for mtDNA damage to lead to increased atherosclerosis.

mtDNA damage and cell death

MtDNA damage not only promotes oxidative stress but also regulates cell death, which has a key role in atherosclerosis development. Acute VSMC apoptosis leads to thinning of the fibrous cap, an increase of the necrotic core, and intimal inflammation –all features of the vulnerable lesion [31]. If VSMC apoptosis is chronic, increased plaque development and calcification result [5]. Macrophage apoptosis is also important in atherogenesis. Although protective in the early stage, in advanced lesions macrophage apoptosis causes expansion of the necrotic core [32, 33]. Furthermore, phagocytic clearance of apoptotic cells is impaired as plaques develop, promoting secondary necrosis and inflammation [34].

Mitochondria are well positioned to regulate cell death, as they are the only part of the cell that contains cytochrome c under normal physiological conditions. Cytochrome c not only forms part of the electron transport chain but is also a critical signal for apoptosis. Apoptotic signals converge on the Bcl2 proteins Bax and Bak, which oligomerise to promote mitochondrial outer membrane permeabilisation (MOMP). Bax and bak also activate OMA1, a mitochondrial inner membrane protease, that works together with MOMP to allow the release of cytochrome c [35]. Cytochrome c then binds to Apaf-1, leading to caspase activation and apoptosis execution [36](Figure 2).

Mitochondria also regulate apoptosis through their dynamics [37] . The dynamic mitochondrial network undergoes constant fission and fusion to

shape mitochondrial number and mass. Mitochondrial fission accompanies MOMP [38], and the fission protein dynamin-related protein 1 (Drp1) stimulates bax oligomerisation through membrane tethering; overexpression of a mutated form of Drp1 delays oligomerisation and cell death [39]. Another regulator of apoptosis is the fusion protein optic atrophy 1 (Opa1) that is located on the inner mitochondrial membrane. Opa1 controls mitochondrial cristae shape and, by keeping the cristae junctions tight, limits cytochrome c release [40].

Cytochrome C release may also result from mitochondrial permeability transition pore (MPTP) opening. The exact composition of the MPTP remains debated, but cyclophilin D and ATP synthase appear to be components [41-43]. Mitochondrial dysfunction results in conditions such as increased ROS and decreased mitochondrial membrane potential that promote MPTP opening and thus apoptosis. When the MPTP opens, equilibration of ions between the mitochondrial matrix and cytosol occurs. The resultant swelling exerts pressure on the outer membrane, leading to rupture and release of cytochrome c. Whilst transient MPTP opening may allow apoptosis, if prolonged opening occurs, collapse of oxidative phosphorylation and necrosis ensue [44](Figure 2).

Mitochondria tightly regulate cell death, and disruption to their structure or function can promote apoptosis. Indeed apoptosis is seen in mutator mice, and is increased in VSMCs, macrophages, and plaques [6]. mtDNA damage therefore promotes apoptosis, and thus can promote plaque formation and vulnerability.

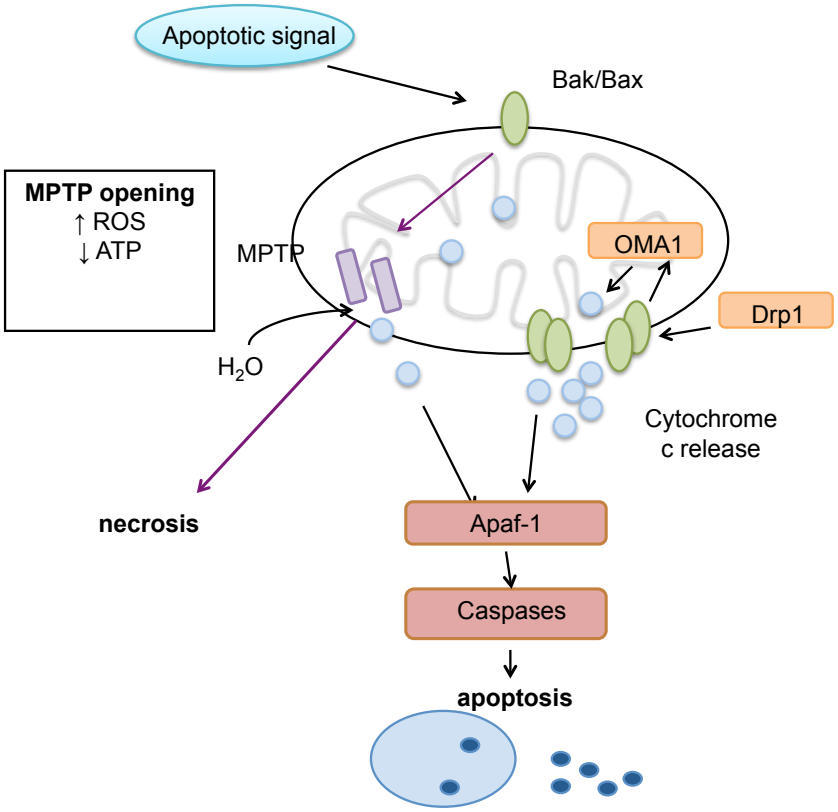

Figure 2

Figure 2. Mitochondrial regulation of cell death

Apoptotic signals converge on the Bcl2 proteins Bax and Bak, which oligomerise to promote mitochondrial outer membrane permeabilisation (MOMP); the fission protein dynamin-related protein 1(Drp1) also stimulates bax oligomerisation. Bax and bak activate OMA1, a mitochondrial inner membrane protease, that works together with MOMP to allow the release of cytochrome c. Cytochrome c then binds to Apaf-1, leading to caspase activation and apoptosis execution. Apoptosis can be amplified by mitochondrial permeability transition pore (MPTP) opening, which allows entry of water and solutes. The resultant swelling promotes rupture and release of cytochrome c, but prolonged MPTP opening collapses oxidative phosphorylation with ensuing necrosis. Mitochondrial dysfunction leads to conditions that favour MPTP opening, including ATP depletion and increased reactive oxygen species (ROS).

mtDNA damage and inflammation

Inflammation drives atherosclerosis with inflammatory cells present in both the plaque and the thrombus that forms upon plaque rupture. Increasingly we recognise that mitochondria not only generate ATP and ROS but also have a key role in the immune response.

The innate immune response provides rapid detection and protection against dangerous stimuli. Toll-like receptors (TLRs) act as pattern recognition receptors, sensing a broad range of pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs). Like the DNA of their proto bacteria predecessors, mtDNA contains significant amounts of unmethylated CpG islands that act as DAMPs. These inflammatory mtDNA motifs stimulate TLR9, leading to nuclear factor kappa beta (NFκβ) activation and transcription of inflammatory cytokines [45](Figure 3). Although mtDNA can be circulating, it may also come from damaged mitochondria that have escaped from autophagy [45, 46].

In addition to activating TLR9, mtDNA also elicits anti-viral immune responses. In the absence of mitochondrial transcription factor A (TFAM) mtDNA is released to the cytosol where it engages the DNA sensor cyclic GMP-AMP synthase (cGAS). Downstream stimulator of interferon genes (STING) signalling is promoted, increasing the expression of type 1 interferons and other interferon-stimulated genes [47](Figure 3). Interestingly apoptotic caspase activation can attenuate this signalling pathway [48], demonstrating that mitochondria regulate the intertwined pathways of cell death and inflammation.

Interferon signalling not only serves a protective anti-viral response, but is also implicated in atherogenesis and plaque vulnerability. The type 1 interferons include interferon α and β (IFN α and IFNβ), which affect both VSMCs and macrophages. IFNα increases TNF-related apoptosis-inducing ligand (TRAIL) on T cells, promoting VSMC death [49]. IFNβ promotes macrophage-endothelial cell adhesion and leukocyte recruitment to atherosclerosis-prone sites. Furthermore IFNβ treatment has *in vivo* effects,

increasing plaque formation in a mouse model of atherosclerosis and indeed, increased type 1 IFN signalling has been confirmed in ruptured human plaques [50].

Mitochondria regulate inflammatory cytokines through altering their expression and also by triggering post-translational modification and activation. Oxidised mtDNA binds and activates the NLRP3 inflammasome, a multi-protein complex composed of NLRP3, ASC and caspase 1 [51]. NLRP3 responds to danger stimuli, including low intracellular potassium concentration, bacterial toxins such as nigericin, and cholesterol crystals [52]. Once activated, NLRP3 colocalises with its adapter protein ASC at perinuclear ER-mitochondrial clusters [53]. Active caspase 1 then assembles to cleave pro-IL1β to its mature form (Figure 3).

Dysfunctional mitochondria not only activate NLRP3 through mtDNA but also through ROS generation [53] and cardiolipin. Although cardiolipin is usually a mitochondrial inner membrane lipid, it localises to the outer membrane in dysfunctional mitochondria. Cardiolipin can then recruit and activate NLRP3 [54, 55]. NAD/NADH levels indicate cellular nutrient and energy status and can also signal danger. In mitochondrial dysfunction NAD/NADH levels fall, and are sensed by NAD-dependent enzymes such as the sirtuin deacetylases. Sirtuin 2 activity is decreased and α-tubulin accumulates, promoting the co-localisation of NLRP3 with ASC and therefore inflammasome activation [56].

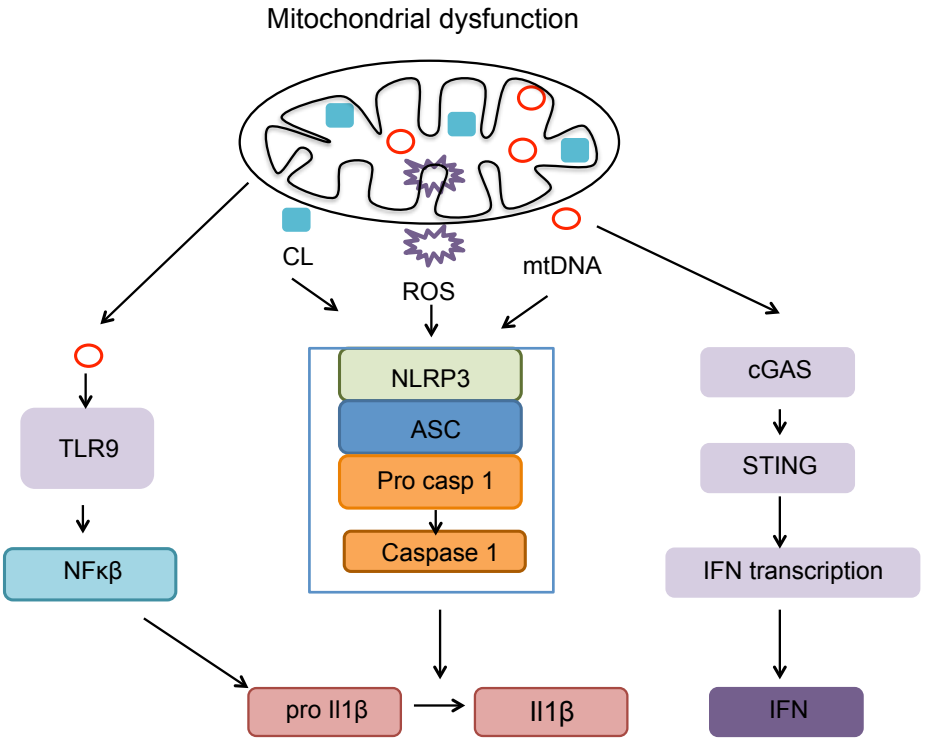

Figure 3

Figure 3. Mitochondrial DNA, dysfunction and inflammation

Mitochondria regulate inflammatory signalling, through shaping of both gene expression and post-translational modification. Mitochondrial DNA (mtDNA) contains inflammatory motifs that activate TLR9, leading to NFκβ activation and transcription of inflammatory cytokines such as pro-IL1β. Pro-IL1β is cleaved by caspase 1 to generate mature, active IL1β. Caspase 1 itself is regulated by the NLRP3 inflammasome, a multi-protein complex composed of NLRP3, ASC and pro-caspase 1. NLRP3 responds to signals generated by mitochondrial dysfunction- ROS, cardiolipin externalisation, and cytosolic mtDNA. Mt DNA also triggers interferon (IFN) expression, through engaging cyclic GMP-AMP synthase (cGAS) and promoting stimulator of interferon genes (STING) signalling.

mtDNA damage and dysfunction can therefore activate NLRP3 through mtDNA, ROS, cardiolipin and altering NAD/NADH levels. Importantly NLRP3 inflammasome activation and increased IL1β release may promote atherosclerosis [52]. IL1α is also important in atherogenesis, and perhaps even more so than IL1β [57]. For example, transplantation of IL1α-deficient bone marrow into mice deficient for low-density lipoprotein receptor (LDLR) significantly reduces atherosclerosis. IL1α therefore induces vascular inflammation and its secretion, like IL1β, is regulated by mitochondrial function. Dietary fatty acids uncouple mitochondria, triggering a calcium flux that activates the calpain protease. Calpain then cleaves pro-IL1α to release its active, mature form [57].

Taken together mitochondria are now recognised as regulators of pro-atherogenic inflammatory signalling, through shaping of both gene expression and post-translational modification. In atherogenesis metabolic stressors such as cholesterol crystals and fatty acids may converge on mitochondria, leading to an inflammatory response that is amplified by underlying mtDNA damage and dysfunction.

mtDNA damage and metabolism

Although cell death and inflammation are important processes in atherogenesis, the systemic metabolic changes of diabetes, hypercholesterolaemia and hypertension are well-known atherosclerosis risk factors. We increasingly recognise that mtDNA defects not only impact upon mitochondrial and cellular function but whole organism metabolism [58] . Mitochondria use substrates from the Krebs cycle, derived from lipids, carbohydrates and proteins, to generate ATP during oxidative phosphorylation. Mitochondria therefore have a critical role in coordinating metabolism and energy production.

MtDNA damage is particularly associated with significant lipid changes. Mutator mice show loss of adipose tissue [16] and hypercholesterolaemia that may promote atherosclerosis [6]. The underlying mechanisms were not fully explored in these papers but mitochondrial dysfunction increases AMP/ATP to

activate AMP-activated protein kinase (AMPK) signalling. AMPK activation has multiple effects, including inhibition of gluconeogenesis and adipogenesis [59] that could lead to reduced adiposity. Alternatively, mitochondrial dysfunction also promotes the release of fibroblast growth factor 21 (FGF21), a hormone that regulates lipolysis [60-62]. Lipids are mobilised from adipose tissue, resulting in decreased fat mass and increased serum glycerol and free fatty acids [63].

In addition to these systemic signalling effects on lipid metabolism, mitochondrial function influences cellular cholesterol transport. Macrophage cholesterol efflux is a step of reverse cholesterol transport, the process where intracellular cholesterol is returned to the liver for clearance [64]. The ATP binding cassette transporters ABCA1 and ABCG1 mediate cholesterol efflux but their ATP dependence subjects them to mitochondrial regulation. Indeed, inhibition of ATP synthase leads to decreased cholesterol efflux capacity, whilst increased mitochondrial respiration promotes cholesterol efflux and reduces plaque formation [65].

Extensive mtDNA damage and dysfunction may therefore have multiple effects on lipid metabolism, including reduced cholesterol efflux, to promote atherosclerosis.

Mitochondria as therapeutic targets in atherosclerosis

MtDNA damage promotes increased ROS, cell death, inflammation and lipid changes that can all drive atherogenesis. This highlights mitochondria as potential therapeutic targets in atherosclerosis, and harnessing pre-existing protective processes may be a promising way to start.

Firstly important anti-oxidant pathways are already present to protect mtDNA and other cellular macromolecules against the damaging effects of mitochondrial ROS. Matrix and inter-membrane superoxide dismutases convert superoxide to hydrogen peroxide, which is then safely reduced to water by catalase or glutathione peroxidase. Augmenting or supplementing

these anti-oxidant processes may reduce oxidative stress and downstream damage.

However ROS generation may overwhelm the anti-oxidant defences, resulting in mtDNA damage. Countering this, mtDNA repair pathways, including base excision repair, mismatch repair and possibly homologous recombination [66], can potentially correct these defects and improve mitochondrial function. For example, Twinkle is a mitochondrial helicase and may have a role in mtDNA recombination and repair [67]. Mice over-expressing Twinkle show protection against ROS-induced mtDNA mutations, and improved cardiac function [68]. Targeting mtDNA repair may therefore be a useful pathway to protect against the effects of mtDNA damage.

If mtDNA lesions persist despite the repair systems, mitochondrial dynamics may act to alleviate the damage. Mitochondrial fusion allows sharing of mtDNA and mitochondrial contents, protecting against mtDNA damage. Subsequently, when mitochondrial fusion is impaired multiple defects in mtDNA content, mitochondrial function and tissue function occur [69].

Mitophagy is also a key determinant of mitochondrial health when mtDNA damage exceeds the capacity of the protective anti-oxidant, repair, and fusion processes. Mitophagy is a specialised form of autophagy, where lysosomal degradation clears dysfunctional mitochondria from the cell, recycling components for further use. Dysfunctional mitochondria accumulate Pink1, which phosphorylates ubiquitin, parkin and mitofusin 2 [70-72]. Parkin is subsequently activated and ubiquinates multiple proteins, including VDAC, to mark mitochondria for mitophagy [73]. Although the Pink1/Parkin pathway is well-recognised other activation mechanisms also exist. Pink1 recruits NDP52 and optineurin independently of Parkin to activate mitophagy [74], whilst BNIP3 and Nix proteins can also recruit the phagophore, and thus promote lysosomal fusion [75, 76]. These pathways may be useful in targeting mitophagy, which holds particular promise given that macrophage autophagy has a protective role in advanced atherosclerosis. If macrophage autophagy is

impaired, increased apoptosis and oxidative stress lead to increased plaque area and necrosis [77].

Another way to improve mitochondrial health may be through targeting the sirtuin family of NAD+-dependent protein deacetylases; of the 7 members, 3 (Sirt 3 to 5) are localised to the mitochondria. The sirtuins are vital as metabolic sensors, matching mitochondrial function to nutrient supply [78]. Sirtuins regulate mitochondrial biogenesis, through sirtuin 1 deacetylation and regulatation of peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC1a) [79, 80]. Furthermore sirtuin 3 regulates cellular metabolism [81, 82], and increases oxidative phosphorylation through the deacetylation and activation of complexes I and II [83, 84]. ROS would of course be generated as a by-product, and importantly sirtuins can detoxify ROS, including through sirtuin 3 activation of manganese superoxide dismutase [85, 86]. Targeting sirtuins could therefore promote changes to mitochondrial function, metabolism and ROS generation that could be athero-protective; indeed inhibition of sirtuin 1 increases atherosclerosis [87].

Collectively the evidence indicates that mtDNA damage promotes multiple pro-atherogenic processes. Targeting mitochondria may therefore provide a new strategy in reducing plaque development and vulnerability.

Conclusions and future

Mitochondria exist as a dynamic network that must be able to respond to metabolic demands and generate ATP. Mitochondria are therefore critical for cellular function, yet they have kept some independence, with their own genome and timescale of replication. We now recognise that mitochondria have pivotal roles in the generation of ROS, and the regulation of cell death, inflammation and metabolism. mtDNA damage and mitochondrial dysfunction can disrupt these processes, driving plaque development and vulnerability.

However important questions remain that are yet to be answered. We do not know whether the mtDNA damage actually seen in atherosclerosis results in sufficient dysfunction to be causative. Future work may also identify other

causes of mitochondrial dysfunction in atherosclerosis. The exact role and requirement for mitochondrial ROS in atherosclerosis remains debated, and indeed what determines whether mtDNA damage and mitochondrial dysfunction result in oxidative stress is unclear. Future studies may also identify other mechanisms linking mitochondrial dysfunction and inflammation. For example, does mitochondrial dysfunction lead to other pro-inflammatory transcriptional changes, and do mitochondria affect immune-suppressive signalling? Finally it is important to examine whether protecting against mtDNA damage and mitochondrial dysfunction can reduce atherogenesis or plaque vulnerability.

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