Mitochondrial DNA damage and atherosclerosis

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Keywords: mitochondria, atherosclerosis, inflammation

Abstract

Mitochondria are often regarded as the cellular powerhouses through their ability to generate ATP, the universal fuel for metabolic processes. However, in recent years mitochondria have been recognised as critical regulators of cell death, inflammation, metabolism and generation of reactive oxygen species (ROS). Thus, mitochondrial dysfunction directly promotes cell death, inflammation and oxidative stress, and alters metabolism. These are key processes in atherosclerosis and there is now evidence that mitochondrial DNA (mtDNA) damage leads to mitochondrial dysfunction and promotes atherosclerosis directly. In this review we discuss the recent evidence for and mechanisms linking mtDNA defects and atherosclerosis, and suggest areas of mitochondrial biology that are potential therapeutic targets.

Atherosclerosis- a mitochondrial disease?

Despite pharmacological and surgical treatment options, atherosclerosis remains the leading cause of death in the western world. The disease affects arteries and is characterised by the formation of fatty plaques. These plaques can rupture, potentially leading to vessel occlusion and clinical manifestations such as heart attacks and strokes[1]. With such morbidity and mortality the need remains to identify the underlying disease mechanisms and to find new therapeutic targets. Recent evidence highlights a role for mtDNA damage and dysfunction in atherogenesis.

The atherosclerotic plaque forms when lipid accumulates at sites of endothelial damage and dysfunction. The lipids are susceptible to oxidative modification by reactive oxygen species (ROS, see Glossary) and act as inflammatory stimuli, attracting leukocytes to the site. Monocytes therefore migrate into the developing plaque and differentiate into macrophages that engulf the oxidised lipids. Inflammatory factors released by macrophages stimulate the migration and/or proliferation of vascular smooth muscle cells (VSMCs) [1]. VSMCs are important for plaque stability, as they secrete the extra-cellular matrix that forms a fibrous cap. However, VSMC death, fibrous cap thinning and subsequent plaque vulnerability may be induced by inflammation [2].

Inflammation, cell death and oxidative stress are therefore key processes driving plaque development and transition to a vulnerable plaque phenotype [3, 4]. As mitochondrial dysfunction can promote these pro-atherogenic processes, mitochondrial damage has been implicated in atherogenesis, including damage to mitochondrial DNA (mtDNA) [5]. However, the finding that mtDNA defects can

directly promote atherogenesis and plaque vulnerability has only recently been demonstrated [6]. Furthermore, emerging work has identified multiple mechanisms linking mitochondria and inflammation [7], whilst the role of the vicious circle of mtDNA damage leading to mitochondrial dysfunction and oxidative stress leading to further mtDNA damage remains under debate [8]**.** In this review we will discuss the recent evidence of the role and mechanisms of mtDNA damage and atherosclerosis.

Mitochondrial DNA damage and atherosclerosis

Mitochondria generate ATP through oxidative phosphorylation within the respiratory chain, where electron transport is coupled with the production of ATP from ADP. However, ROS are formed as a by-product of the respiratory chain making mitochondria the major source of cellular ROS [9] **(Figure 1)**. The respiratory complexes are composed of both nuclear and mtDNA encoded subunits, meaning that their formation requires careful coordination of the two genomes [10]. Mitochondria are unique as they are the only source of DNA within a cell apart from the nucleus. The human mitochondrial genome exists as a 16569 bp loop, coding for ribosomal and transfer RNAs, in addition to 13 respiratory chain subunits [10]. However mtDNA is vulnerable to damage, partly because it lies close to the site of ROS production and also because it lacks protection by histones. It has been proposed that mtDNA damage can impair respiratory chain function, and eventually compromise cellular function promoting ageing and disease [11].

mtDNA lesions are found in circulating cells and hearts of patients with coronary artery disease, suggesting that mtDNA damage may contribute towards atherosclerosis development [12, 13]. mtDNA damage is an early event in

atherogenesis [5], and recent work shows that both mtDNA damage and mitochondrial dysfunction are present in a model of atherosclerosis and metabolic syndrome [14]. However, although this study suggested that mtDNA damage promotes both atherosclerosis and the metabolic syndrome, the effects of mtDNA damage could not be separated from those of nuclear DNA damage.

To directly study the effects of mtDNA lesions, recent studies have utilized mice with extensive mtDNA damage, such as the mutator mice. These mice express mtDNA polymerase with impaired proof-reading ability that introduces point mutations and deletions into mtDNA [15, 16]. The mice show an aged phenotype, with kyphosis, anaemia and weight loss. Mutator mice that were also deficient for apolipoprotein E showed that mtDNA defects promote atherosclerosis and plaque vulnerability, and can do so without any increase in ROS. In contrast, VSMC and monocyte apoptosis was increased, and monocytes showed a pro-inflammatory profile. Furthermore, mtDNA lesions were associated with high-risk atherosclerotic plaques in humans [6]. Taken together, we now have evidence that mtDNA defects can have a causal role in atherosclerosis, that the effects can be independent of oxidative stress, and that the findings may be relevant to human disease.

Mechanisms of mtDNA damage

Although mtDNA damage has been identified in human atherosclerotic disease, these studies did not examine the underlying cause. One possibility is that mtDNA undergoes cumulative, oxidative damage from ROS generated by the nearby respiratory chain [11] **(Figure 1)**. This hypothesis appears plausible given that atherosclerotic risk factors are associated with increased oxidative stress. For

example, hyperglycaemia increases superoxide production whilst cigarette smoking promotes the reduction of oxygen to form ROS [17]. Interestingly mtDNA defects in circulating cells can be associated with diabetes, although a causal role could not be proven [6].

Apart from oxidative damage, replication errors are another source of mtDNA defects. Lesions such as point mutations can form in early life, and undergo clonal expansion to reach a threshold level where mitochondrial function is impaired [11]. mtDNA lesions may persist as mitochondria have a reduced capacity for DNA damage repair; whilst mitochondrial base excision repair has been well described, nucleotide excision repair is lacking [10].

Inflammation - a link between mitochondria and atherosclerosis

Once mtDNA defects are present, they can result in decreased respiratory subunit formation, impaired mitochondrial respiration and reduced ATP content [18]. Furthermore, cellular phenotype is also altered as mtDNA defects and dysfunction result in a pro-inflammatory profile that likely promotes plaque vulnerability [6]. Thinning of the fibrous cap and increased necrotic core area (features of vulnerable plaque) were observed in apolipoprotein E deficient recipients of mutator mouse bone marrow. Isolated monocytes from mutator mice show mtDNA damage and increased release of tumour necrosis factor alpha (TNFα) and interleukin 1β (IL1β) [6]. These findings are consistent with the growing body of work identifying multiple mechanisms linking mitochondria and inflammation, a key atherogenic process.

How do mitochondria regulate inflammation?

The innate immune system has developed to detect and protect against dangerous stimuli. Toll-like receptors (TLRs) are a key part of detection, and recognise a broad range of pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs). Similar to bacterial genomes, mtDNA has significant amounts of unmethylated DNA as CpG islands. Recent work has shown that mtDNA, with its inflammatory motifs, can act as a DAMP to directly activate the immune response through activation of TLR9 [19]. mtDNA can be circulating or, for example, come from damaged mitochondria that have escaped from autophagy [19, 20]. Activation of TLR9 leads to increased nuclear factor kappa beta (NFκβ) signaling and pro-inflammatory cytokine expression **(Figure 2)**.

Mitochondria also regulate cytokine release by affecting post-translational modification **(Figure 2)**. Emerging work has identified a key role for mitochondrial dysfunction in the activation of the NLRP3 inflammasome, a multi-protein complex composed of NLRP3, ASC and caspase 1. A number of stimuli may act as endogenous danger signals to activate NLRP3, such as low intracellular potassium concentration, bacterial toxins such as nigericin, and indeed cholesterol crystals [21], which may be particularly relevant in atherosclerosis. Upon activation NLRP3 redistributes from the endoplasmic reticulum (ER) to co-localise with its adapter ASC at perinuclear ER-mitochondrial clusters [22]. Recently NLRP3 was shown to be recruited to mitochondria, an action mediated by the mitochondria-associated adaptor molecule MAVS [23]. Once the inflammasome is activated, active caspase 1 assembles which cleaves pro-IL1β into its mature form.

Initial work linking mitochondrial dysfunction and inflammasome activation showed that the accumulation of dysfunctional, ROS-generating mitochondria activates NLRP3 [22]. Mitochondrial dysfunction also reduces mitochondrial membrane potential and increases mitochondrial permeability transition pore (MPTP) opening. mtDNA is released into the cytosol, where it directly binds with and activates the NLRP3 inflammasome [24, 25]. Mitochondria can also signal danger through cardiolipin, a lipid usually found in the inner mitochondrial membrane. In dysfunctional mitochondria cardiolipin localises to the outer mitochondrial membrane, where it recruits, binds to, and activates NRLP3 [26, 27].

Mitochondrial dysfunction also promotes inflammasome activation through altered levels of the coenzyme nicotinamide adenine dinucleotide (NAD+). The ratio of NAD/NADH indicates cellular nutrient and energy status, with an increased ratio in starvation, and decreased levels with mitochondrial dysfunction [28]. Changes in the ratio of NAD/NADH are sensed by the NAD-dependent enzymes deacetylases, sirtuins. Decreased NAD+ concentration during mitochondrial dysfunction leads to reduced Sirtuin 2 activity and the accumulation of acetylated α-tubulin at the perinuclear region. The change in acetylated tubulin increases co-localisation of NLRP3 with ASC and hence inflammasome activation [28].

Mitochondria are therefore a site of NRLP3 inflammasome activation, and when dysfunctional can activate NLRP3 through ROS, mtDNA release, cardiolipin and altering NAD/NADH **(Figure 2)**. mtDNA damage may therefore result in mitochondrial dysfunction, and lead to increased IL1β through the above mechanisms to promote atherosclerosis. However, recent data highlights that IL1α is also important in

atherogenesis, and perhaps even more so than IL1β [29]. In mice deficient for low-density lipoprotein receptor (LDLR), bone marrow transplantation from IL1α deficient, but not IL1β-deficient donors led to a significant reduction in atherosclerosis. IL1α is therefore an important inducer of vascular inflammation and, similar to IL1β, its secretion is increased by mitochondrial dysfunction. The dietary fatty acid oleic acid accumulates in mouse atherosclerotic plaques and selectively stimulates macrophage IL1α production. Mitochondrial uncoupling induced by oleic acid triggers intracellular calcium fluxes that activate the protease calpain. Active calpain then cleaves pro-IL1α into its mature form [29]. In contrast with IL1β secretion, oleic acid induced IL1α secretion is independent of the NLRP3 inflammasome [29].

Collectively this work suggests that mitochondrial dysfunction can promote inflammation through upregulation of cytokine expression and inflammasome-dependent and -independent pathways. Metabolic stress such as cholesterol crystals and fatty acids may be sensed by mitochondria in atherogenesis, leading to an inflammatory response that drives plaque development. This response is amplified if there is underlying mtDNA damage and dysfunction.

Oxidative stress, mtDNA damage and atherosclerosis

Although inflammation is an important link between mtDNA damage, dysfunction and atherosclerosis, oxidative stress may also mediate the effects of mtDNA defects. As suggested in the mitochondrial/free-radical theory of ageing, a vicious cycle can exist where ROS production leads to mtDNA damage and impaired respiratory chain function; this results in increased ROS generation, fuelling the cycle [8].

The free radical theory is attractive in the setting of atherosclerosis as oxidative stress appears to be a key part of the disease. Increased ROS are present in the vessel wall at all stages of atherogenesis [30]. ROS can modify DNA, proteins and lipids, with lipid oxidation being an important event in atherogenesis [31]. Oxidative DNA damage has also been observed in VSMCs in human atherosclerotic plaques, associated with upregulation of DNA damage repair proteins [32]. Further evidence supporting the role of ROS in atherosclerosis comes from several murine models. For example, impaired anti-oxidant activity, such as decreased glutathione peroxidase acitivity, promotes atherogenesis whilst decreased superoxide production leads to a reduction of atherosclerosis [5, 33, 34]. Furthermore, macrophage-specific expression of mitochondrial-targeted catalase decreases both mitochondrial oxidative stress and plaque formation [35]. The ectopic expression of catalase also decreases NFκβ pathway activation and plaque monocyte infiltration [35], again highlighting the link between mitochondria, ROS and inflammation.

Overall the evidence from human and mouse studies suggests that oxidative stress is present and promotes atherosclerosis. However, whether increased ROS mediates the effects of mtDNA damage is more uncertain. For example, when first described mutator mice showed no evidence of increased oxidative stress despite extensive mtDNA lesions [16, 36]. In contrast, later work showed that reducing mitochondrial ROS with over-expression of mitochondrial-targeted catalase partially rescued a cardiomyopathy phenotype [37]. One of the difficulties in the field has been the ability to assess ROS accurately *in vivo*, and this may account for differing findings in published studies. The conventional methods of measuring ROS rely on redox-sensitive dyes that are taken up into the cytoplasm or mitochondria. In

atherosclerosis these dyes can lack both sensitivity and specificity, in part because of autofluorescence of both the normal vessel wall and plaque components. In contrast, the mitochondria-targeted ratiometric probe MitoB accumulates within mitochondria, where it reacts with hydrogen peroxide to form MitoP [38]. Quantifying the mitoP/mitoB ratio enables measurement of mitochondrial hydrogen peroxide. MitoB showed no difference in mitochondrial ROS levels in young mutator mice [39] even when crossed with apolipoprotein E deficient mice [6]. This suggested that mtDNA damage and mitochondrial dysfunction do not necessarily lead to increased ROS, but can still promote atherosclerosis. The finding may explain why in clinical studies antioxidants such as Vitamins C and E fail to reduce events in atherosclerosis, and a mitochondria-targeted antioxidant did not affect plaque burden in mice [40].

Collectively, recent studies indicate that prolonged mtDNA damage and dysfunction may lead to increased oxidative stress [39]. However, ROS-independent mechanisms also link mtDNA damage and mitochondrial dysfunction with atherosclerosis. We may therefore need to consider the timing of anti-oxidant therapies and to target other aspects of mitochondrial dysfunction when developing treatments for atherosclerosis.

Cell death, mtDNA damage and atherosclerosis

mtDNA damage not only promotes inflammation and oxidative stress but also regulates cell death, which has a well-recognised role in atherogenesis. Selective VSMC apoptosis accelerates plaque growth [4], and promotes thinning of the fibrous cap, an increase of the necrotic core, and intimal inflammation - all features of the vulnerable plaque [41]. Monocyte/macrophage apoptosis also affects plaque

composition and development, with suppression of circulating monocytes reducing atherogenesis [42], but macrophage apoptosis leading to an expansion of the necrotic core [43].

Cell death therefore influences plaque development and mitochondria have an essential role in the intrinsic apoptotic pathway **(Figure 3)**. Signalling of apoptotic stimuli converges on the Bcl2 proteins Bax and Bak, which form pores leading to mitochondrial outer membrane permeabilisation. The release of pro-apoptotic factors such as cytochrome c leads to activation of the cascade that executes apoptosis [44]. Cytochrome c release may also result from opening of the MPTP. MPTP opening leads to equilibration of the ions between the mitochondrial matrix and cytosol, leading to mitochondrial swelling. Pressure is exerted on the outer mitochondrial membrane that then ruptures, releasing cytochrome c, which binds with the adapter protein apaf 1 and activates caspases. MPTP is an important regulator of cell death as transient opening may allow apoptosis, whilst prolonged opening results in collapse of oxidative phosphorylation and necrosis [45].

mtDNA damage and dysfunction result in decreased ATP and mitochondrial membrane potential, both factors that increase MPTP opening and apoptosis. Indeed, several studies have identified increased apoptosis in mutator mice [16, 18], and we found increased apoptosis in their plaques *in vivo*, and in both VSMCs and monocytes *in vitro* [6]. mtDNA damage and dysfunction therefore increase apoptosis and thus can promote atherosclerosis.

Although there is evidence that mtDNA defects promote apoptosis, the exact mechanisms of how mitochondria regulate cell death remain unclear. One particular area of debate is the composition and regulation of the MPTP, with only cyclophilin D confirmed as an essential component [46] Although the voltage-dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT) were proposed to form the MPTP, genetic studies have shown that they are not essential components [47]. Recent work now suggests that dimers of the ATP synthase form the MPTP [48], and others have shown novel roles for p53 and Bax in triggering MPTP-mediated necrosis [49, 50]. As p53 and Bax are well-recognised pro-apoptotic signals, regulation of MPTP is clearly important for determining the mode of cell death **(Figure 3)**.

Mitochondrial dynamics

Mitochondria form a dynamic network within the cell, undergoing constant fusion and fission. Mitochondrial dynamics not only regulate mitochondrial morphology and number, but are also a determinant of cell death [51]. Mitochondrial fission occurs in apoptosis, with loss of mitochondrial membrane potential facilitating release of cytochrome c. Although fission is largely mediated by the GTPase dynamin-1-like protein (Drp1), Drp1-independent mechanisms may also exist [52]. Conversely, optic atrophy 1(Opa1) is an inner mitochondrial membrane fusion protein with anti-apoptotic effects. Opa1 controls mitochondrial cristae shape, and reduces cytochrome c release by keeping the cristae junctions tight during apoptosis [53]. These effects are proposed to be independent of mitochondrial fusion, and indeed the relationship between fusion and cell death is more uncertain. Inhibiting fission leads to a fused phenotype, and decreased MPTP opening and subsequent cell

death in cardiomyocytes [54]. In contrast, recent work shows that inhibiting fission potentiates MPTP opening in fibroblasts [49]. The role of mitochondrial dynamics in regulating cell death is therefore complex and remains to be fully understood.

mtDNA damage, altered metabolism and atherosclerosis

Atherosclerosis is not only promoted by local effects on plaque cells, but also by systemic metabolic effects, including hypercholesterolaemia, diabetes and hypertension. It is increasingly recognised that mtDNA defects not only affect mitochondrial and cellular function but also whole organism metabolism [55]. Mitochondria generate ATP using substrates from the Krebs cycle that can be derived from lipids, carbohydrates and proteins. Mitochondria therefore coordinate metabolism and energy production, with energy levels signalled by the AMP/ATP ratio. In mitochondrial dysfunction AMP/ATP increases, activating AMP-activated protein kinase (AMPK), which has multiple effects including inhibition of gluconeogenesis and adipogenesis [56]. Indeed, mutator mice show decreased body weight and reduced subcutaneous fat [15].

Recent studies have shown that mitochondrial dysfunction also disrupts fat metabolism through fibroblast growth factor 21 (FGF21), a hormone that regulates lipolysis [57]. Human and mouse muscle fibres that have defective oxidative phosphorylation release FGF21, leading to mobilisation of lipids from adipose tissue [58, 59]. Such lipolysis is associated with decreased fat mass, but increased serum glycerol and free fatty acids [60]. Similarly, mutator mice that were also deficient for apolipoprotein E showed reduced fat mass but exaggerated pro-atherogenic hypercholesterolaemia [6].

Protection against mtDNA damage and potential therapeutics

Taken together, current evidence indicates that mtDNA damage and dysfunction can promote atherosclerosis through inflammation, oxidative stress, cell death and altered lipid metabolism. Protecting against mtDNA damage and dysfunction is therefore a potential therapeutic strategy, and could utilise the multiple processes that already exist to protect against mtDNA damage. First line is the mitochondrial anti-oxidant system that scavenges ROS generated by the respiratory chain. Matrix and inter-membrane space superoxide dismutases convert superoxide ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2). H_2O_2 is then safely reduced to water by glutathione peroxidase or catalase **(Figure 1)**. However, the balance between ROS generation and antioxidant activity determines the mitochondrial oxidative status, and mtDNA damage may still occur.

If mitochondrial damage develops during disease, then mitochondrial dynamics can have a protective effect. For example, mitochondrial fusion allows mtDNA and substrates to be shared, alleviating the effects of mtDNA damage or substrate deprivation [61]. Indeed, multiple defects in mtDNA copy number, mitochondrial function and tissue function are observed when mitochondrial fusion is impaired [61]. Finally, if the levels of mtDNA damage and dysfunction overwhelm the protective anti-oxidant and fusion processes, mitophagy is an important determinant of mitochondrial health. Mitophagy is a mechanism by which dysfunctional mitochondria are cleared from the cell by lysosomal degradation and components recycled ready for further use. To signal mitophagy dysfunctional mitochondria accumulate PTEN-induced putative kinase 1 (Pink1) on the outer mitochondrial membrane. Parkin, a

component of the E3 ubiquitin ligase complex, is susbsequently recruited by mitofusin 2 and ubiquitinates multiple proteins, including VDAC, to mark mitochondria for autophagic degradation [62-64]. All of these pathways may be useful therapeutic targets in the treatment of atherosclerosis.

Concluding Remarks and Future Perspectives

The dynamic mitochondrial network generates ATP, the universal fuel for metabolic processes. Mitochondria are thus vital for eukaryotic function, yet they retain some independence, with their own genome and time-scale of replication. Mitochondria are also critical regulators of cell death, inflammation, generation of ROS, and metabolism. Recent evidence indicates that mtDNA damage and dysfunction disrupts these processes and can promote atherosclerosis. However many questions remain that need to be answered by future studies (Box 1). Although ROS are associated with both atherosclerosis and mtDNA damage, whether there are other significant causes of mitochondrial dysfunction in atherosclerosis remains to be seen. Future work may also clarify the mechanisms linking mitochondrial dysfunction and inflammation. For example, are there other mitochondrial-derived ligands for the NLRP3 inflammasome in addition to mtDNA and cardiolipin, and does mitochondrial dysfunction promotes other inflammasome-independent pathways? We also do not know what determines whether mtDNA damage and dysfunction leads to oxidative stress. Finally, it is critical to determine whether protecting against mtDNA damage and mitochondrial dysfunction reduces atherogenesis and/or promotes plaque stability in atherosclerosis.

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Glossary box

Apoptosis: a process of programmed cell death, involving caspase activation and degradation of cellular components. The intrinsic apoptotic pathway converges on the proteins Bax and Bak that form pores which mediate mitochondrial outer membrane permeabilisation (MOMP). MOMP releases pro-apoptotic factors such as cytochrome c.

Mitochondrial DNA (mtDNA): a circular loop of DNA found in the mitochondrial matrix. mtDNA encodes 13 of the respiratory chain polypeptides and tRNAs and rRNAs.

Mitochondrial dynamics: mitochondria constantly undergo fission and fusion. Fission is mediated by Drp-1. Mitochondrial fusion is mediated by mitofusins 1 and 2 (located on the outer mitochondrial membrane) and Opa-1, found on the inner mitochondrial membrane.

Mitochondrial Permeability Transition Pore (MPTP): A pore formed on the inner mitochondrial membrane that is regulated by ciclosporin A, and which is permeable to small solutes and ions. MPTP opening is increased in conditions of mitochondrial dysfunction- decreased mitochondrial membrane potential, increased ROS, decreased ATP, and promotes cell death.

Mitophagy: process where dysfunctional mitochondria are recognised and targeted to the autophagy pathway for degradation.

Mutator mouse: a mouse model of mtDNA disease that has multiple mutations and deletions in the mtDNA.

NLRP3 inflammasome: a complex formed from NLRP3, ASC adapter protein and caspase 1. The active NLRP3 inflammasome activates caspase 1, which cleaves pro-Il1β and pro-Il18 into their mature forms.

Nuclear Factor Kappa Beta (NFκβ): a transcription factor that regulates many genes involved in the immune response, including cytokines and growth factors.

Reactive oxygen species (ROS): reactive oxygen species, primarily superoxide, hydrogen peroxide and the hydroxyl radical. ROS are produced by the respiratory chain when electrons leak away, predominantly at complexes I and III, leading to the partial reduction of oxygen.

Respiratory chain: is composed of respiratory complexes I-IV that mediate electron transfer, reducing oxygen to water.

Vascular smooth muscle cell (VSMC): cells present in the atherosclerotic plaque that secrete the extracellular matrix cap. The cap stabilises the plaque.

Box 1. Outstanding questions

- Apart from mtDNA damage, are there other factors that promote mitochondrial dysfunction and atherosclerosis?
- Are there other pathways linking mitochondrial dysfunction and inflammation. Do other mitochondrial-derived ligands for the NLRP3 exist? Are there additional inflammasome independent pathways?
- What determines whether mtDNA damage and dysfunction leads to oxidative stress?
- Does protecting against mtDNA damage and mitochondrial dysfunction decrease atherosclerosis?

Figure legends

Figure 1. Mitochondrial respiratory chain

The electron transport chain receives electrons (e^-) from NADH and $FADH_2$ and mediates electron transfer from complex I to complex IV, via ubiquinone (Ub) and cytochrome c (C). At complex IV electrons reduce molecular oxygen to form water. As the electrons are transported a proton (H+) gradient is created across the inner mitochondrial membrane (IMM). Complex V (ATP synthase) uses this gradient to convert ADP to ATP. As a by-product of the respiratory chain reactive oxygen species (ROS) are generated. Superoxide (O2•–) is formed at complexes I and III, and is dismutated to hydrogen peroxide (H_2O_2) by matrix manganese superoxide dismutase (MnSOD). H_2O_2 can then be safely reduced to water by catalase or glutathione peroxidase (GPX).

Figure 2. Mechanisms linking mitochondrial dysfunction and inflammation

The NLRP3 inflammasome is a multi-protein complex composed of NLRP3, the adapter protein ASC and pro-caspase 1 (pro casp 1). Once activated caspase 1 cleaves pro-IL1β into its mature form. Dysfunctional mitochondrial generate reactive oxygen species (ROS), externalize cardiolipin (CL) and release mitochondrial DNA (mtDNA), all of which can activate NLRP3. NAD/NADH levels are also decreased, leading to decreased sirtuin 2 activity and the accumulation of α-acetylated tubulin that promotes NLRP3 and ASC colocalisation. mtDNA also has significant amounts of unmethylated DNA as CpG islands, which activate NFκβ signaling and increased cytokine expression through toll-like receptor 9 (TLR9).

Figure 3 Mitochondrial regulation of cell death

Apoptotic stimuli converge on Bax and Bak, which oligomerise to form pores that mediate mitochondrial outer membrane permeabilisation. Cytochrome c is released from the intermembrane space and binds with the adapter protein Apaf-1 to activate caspase 9. The ensuing caspase cascade leads to apoptosis. Apoptosis can be amplified by opening of the mitochondrial permeability transition pore (MPTP), which allows entry of water and solutes (<1.5 kDa). Mitochondrial swelling leads to rupture of the outer mitochondrial membrane and cytochrome c release; however prolonged MPTP opening results in collapse of oxidative phosphorylation and necrosis. MPTP opening is increased in conditions associated with mitochondrial dysfunction including increased reactive oxygen species and depletion of ATP. The pro-apoptotic signals Bax and p53 also promote MPTP opening.