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Mitochondrial anomalies: driver to age associated degenerative human ailments

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1. ABSTRACT

Mitochondria play a fundamental role in regulating a variety of complex metabolic processes to maintain adequate energy balance for cellular existence. To orchestrate these functions, an undisturbed mitochondrial dynamics is imperative through a set of tightly guided mechanisms. Interference in key signature processes by several genetic, epigenetic and age-linked factors triggers mitochondrial dysfunction through decrease in mitochondrial biogenesis, reduced mitochondrial content, aberrant mtDNA mutations, increased oxidative stress, deficient mitophagy, energy dysfunction, decrease in anti oxidant defense and impaired calcium homeostasis. Mitochondrial dysfunction is widely implicated in origin and development of various age associated degenerative human ailments including metabolic syndromes, cardiovascular diseases, cancer, diabetes and neurodegenerative disorders. The present review revisits the mitochondrial anomalies involved in

aetiology of different human diseases and also highlights the translational significance of nano-vectors aimed for selective mitochondrial engineering which might pave way for development of novel therapeutics.

2. INTRODUCTION

Mitochondrion, often referred as the power house of cell, is a semi-autonomous organelle which plays a central role in Adenosine triphosphate (ATP) generation through oxidative phosphorylation. The process mainly involves two major steps i.e. oxidation of nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide ($FADH_2$) and phosphorylation of adenosine diphosphate (ADP) to form ATP. Oxidation of NADH/$FADH_2$ in the electron transport chain (ETC) generates essential proton motive force needed to pump electrons from inner membrane to the inter membrane spaces. The movement of electrons mainly relies on the standard redox potential and certain defined complexes (I, II & III) of ETC. However, under abnormal physiological conditions when excess electrons are provided to mitochondrial respiratory chain, the possibility of generating reactive oxygen species (ROS) increases (1).

Eukaryotic mitochondria are organelles derived from symbiotic incorporation of alpha-proteobacteria into ancient archaea species. However, in the process of evolutionary transformation the ability to synthesize most of the proteins encoded by the primitive bacterial DNA were severely abrogated (2). Apart from a small circular polycystronic 16 Kb mtDNA controlling the synthesis of about 67 proteins, including 13 polypeptides of the ETC; the rest were relocated into nuclear genome architecture. During this regulatory transitional stage of adaptability between high and low oxidative rates, mitochondria conserved a few bacterial phenotypic characteristics and acquired many exciting functions. Strikingly, mitochondria incorporated different cell signaling pathways to become a central modulator of cell fate, proliferation, cell cycle arrest, and apoptosis by regulating energy production and intermediary metabolism (3). Cellular proliferation mainly depends on metabolic and energy status of cell, both regulated by mitochondria, and that controls growth, development, senescence and ageing by modulating cell cycle (2, 4).

The number of mitochondria in every cell of a person's body varies from a few to hundreds and is mainly maternally derived. As the zygotic cell grow up, due to the effect of different exogenous and endogenous factors, its physiology changes, which may range from genetic modifications, structural changes to altered or lost function. These changes accumulate with time and are acquired by cells with every somatic cell division, irrespective of whether advantageous or not. Notably, mitochondrial morphology during development is precisely controlled by regulated rates of organelle fusion or fission. This results in interconnected (fusion) or fragmented (fission) mitochondria, thereby, affecting different mitochondrial functions. Various diseases occur during ageing in high energy demanding tissue/ organs like neurons, heart and skeletal muscles are reported to be linked with altered mitochondrial function (Figure 1). Mitochondrial dysfunction includes decrease in mitochondrial biogenesis, reduced mitochondrial DNA content, aberrant mtDNA mutations, increased oxidative stress, deficient mitophagy, energy dysfunction, decrease in anti oxidant defense and impaired calcium homeostasis. All these with varied intensity and frequency causes several cellular and organ faults leading to wide spectrum of dissimilar disorders including metabolic syndromes, cardiovascular diseases, cancer, diabetes and neurodegenerative disorders (5-11). Apart from these, there is also a stretched list of diseases which are directly or indirectly linked to mitochondrial dysfunction, however, consequences of these impairments are mainly attributed to several contentious genetic, epigenetic and cellular factors (Figure 2).

3. GENETIC FACTORS

Mitochondrial proteins are encoded by both nuclear and mitochondrialgenes. Out of approximately 1200 distinct proteins of mitochondrial proteome, only a few are encoded by mitochondrial DNA (mtDNA) (12) and therefore depends on nuclear proteins for proper organization. The typical mitochondrial gene compliment includes 13 protein subunits of theoxidative phosphorylation (OXPHOS) complex of which seven are components of complex I (NADH dehydrogenase), three are components of complex IV (cytochrome c oxidase), two are subunits of Complex V (ATP synthase) and cytochrome b (a subunit of Complex III). The oxidative capacity of mitochondriais determined by the expression level of OXPHOS subunits andby the number and size of mitochondria. Unlike any other organelle, mitochondria have their own DNA consisting of 16,569 base pairs, a closed circular molecule. Each molecule contains 37 genes encoding, two for large and small rRNA (12S rRNA and 16S rRNA), 22 tRNA, and 13 key protein subunits as mentioned above. Since mtDNA has limited coding capacity, nuclear genes make a major contribution to mitochondrial architecture, metabolic systems and biogenesis. Due to lack of histones and limited mtDNA repair machinery, mitochondrialgenes are more proximal to the ROS source and therefore, it has been assumed that the mitochondrial genome is moresusceptible to various mutagenic stressors. In addition, the mitochondrial genome constitutesonly coding sequences, whereas nuclear DNA (nDNA) contains non-codingsequences. As a consequence of these features, the mtDNA has a much higher mutation rate than does nuclear DNA. Recently, it has been suggested that maternal ageing associated long-term risks (because of exclusive maternal inheritance of mitochondria) can cause various

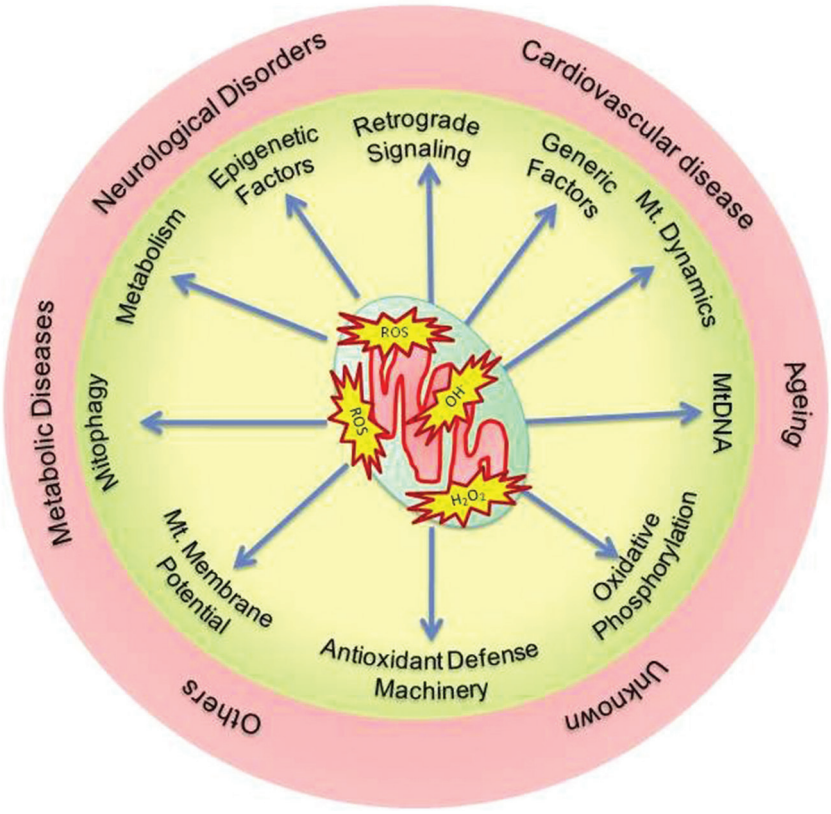

Figure 1. Figure showing different disorders linked with excessive generation of mitochondrial ROS. Perturbed mitochondrial function and ROS generation causes disturbances at cellular and sub-cellular level which further leads to different diseases.

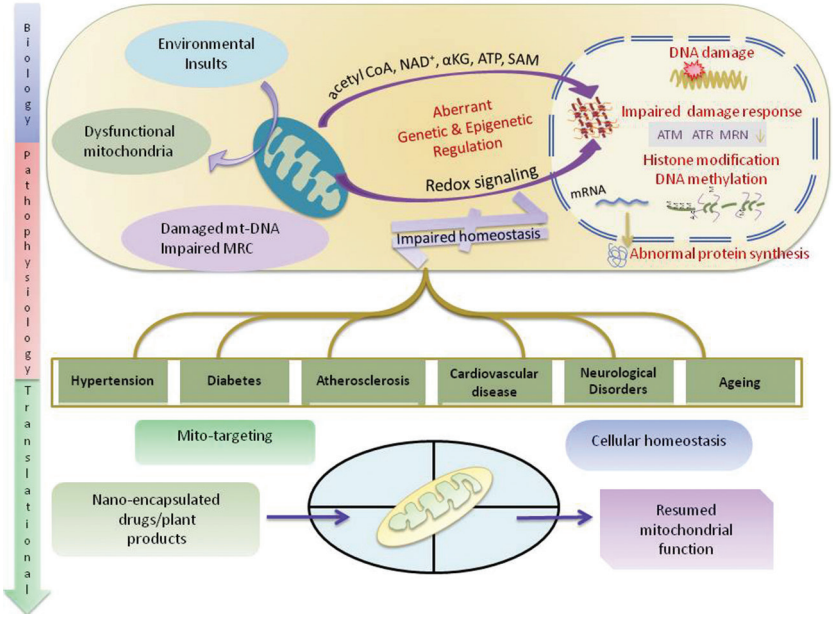

Figure 2. A diagrammatic representation of biology, pathophysiology and translational perspective of mitochondria induced disorders. Dysfunctional mitochondria disrupt various genetic and epigenetic regulatory mechanisms which may lead to different disorders. A suggested strategy of mitochondrial targeting using nanoparticles may be helpful to resume proper functioning of dysfunctional mitochondria.

difficulties in the descendants that could be due to the transmission of damaged mtDNA that commonly arise in later life (13-16). All these studies have now made clear that genetic predisposition could lead to dysfunction of the mitochondrial respiratory chain and may gives rise to heterogeneous group of mitochondrial disorders.

4. EPIGENETIC DETERMINANTS

Epigenetics is the sequence of information heritable during cell division other than the DNA sequence itself. There is a growing body of literature which suggests a possible role of different epigenetic factors in the complex gene-environment interplay. Since mitochondrial metabolism plays an essential role in regulation of substrates for epigenomic machinery, it is evident that any alterations in mitochondrial functions may lead to epigenomic perturbations (17, 18). Mitochondria are house of various cofactors/intermediates that are involved in various nuclear and mitochondrial epigenetic modifications like acetyl Co-A for acetylation, S-adenosyl methionine for methylation, ATP for phosphorylation and alpha-ketoglutarate for demethylation. Epigenetic alteration in mitochondrial function could occur either at local intra-chromosomal domain to suppress mitochondrial regulatory or maintenance genes or at the inter-chromosomal level to disrupt transcriptional factories, resulting in the loss of coordinate regulation of energetic genes (19). Depletion in mtDNA is shown to induce significant changes in methylation pattern of a number of genes (20). It has been also reported that reduction in the mtDNA initiates a Ca^{2+}/Ca dependent signaling cascade and results in epigenetic reprogramming, accompanied by metabolic adaptations and survival advantage (21).

Evidence that mitochondrial dysfunction is associated with epigenomic changes (22-24) can be found for a wide range of classical epigenomic diseases. A recent study has shown that methylation occurs in the mtDNA control region at symmetrical CpG di-nucleotides in a peculiar non-CpG pattern signifying its role in regulating mtDNA replication and/or transcription (25). Moreover, technologic advancement has enabled researchers to know about the numerous key regions concerned with the methylation and regulation of mtDNA gene expression. Key regulators of mitochondrial biogenesis such as nuclear factor-erythroid 2 -related factor (NRF-1) and peroxisome proliferator-activated receptor-gamma co-activator-1alpha (PGC-1alpha) regulate the intra-mitochondrial concentration of DNA methyltransferase 1 (DNMT1) whose translocation in mitochondria occurs through a mature peptide upstream sequence, i.e. mitochondrial targeting pre-peptide sequence (26).

Micro RNAs (miRNA) are another important gene expression regulators and one of the three epigenetic modification factors. Previous studies have shown that miRNAs including miR-30, miR-210 and miR-128a trigger ROS generation and reduces the rate of mitochondrial metabolism (27). It has been also postulated that mitochondria associated miRNAs modulate the expression of genes associated with apoptosis, cell proliferation and differentiation (28). In addition, their expression also regulates the process of DNA methylation and histone modification (29). Hence, it can be postulated that interrelated complex nature of epigenetic regulations acquire modifications in response to environmental factors including ROS and changes endogenous molecules like mtDNA and nDNA, methylation pattern and histone modifications.

5. MITOCHONDRIAL BIOGENESIS

Apart from cellular energy demand, number and shape of mitochondria in a particular cell type and in an organ, is regulated by controlled biogenesis upon several stimuli. Mitochondria are originated from pre-existed mitochondria during cell division (30), for instance during muscle formation i.e. myogenesis. Independent studies have shown that different nuclear encoded mitochondrial factors are also involved for proper regulation and co-ordination of mitochondrial gene expression. In addition, stimulation for mitochondrial biogenesis can also be occurring through nitric oxide (NO), ROS, influx of calcium and hypoxia (31).

Mitochondria exist as dynamic networks that often change shape and sub-cellular distribution according to surroundings. This is critical for equal separation of mitochondria at the time of cell division, value control of the mtDNA, metabolic instruction, and reply to apoptotic stimuli (32). The number and morphology of mitochondria within a cell during development, the cell cycle or when challenged with various toxic conditions are controlled by precisely regulated rates of organelle fusion and fission. Studies have suggested that fusion supports interconnected mitochondria, while fission results in mitochondrial fragments. The resulting damage accumulates throughout life thereby generating functionally normal and damaged mitochondria. Abnormal mitochondria undergo mitophagy while normal ones re-enter the cell cycle. Biogenesis of mitochondria requires a co-ordination of nuclear and mtDNA, both of which encode for mitochondria proteins (12). It has been demonstrated earlier that a number of protein factors encoded by nuclear genes are involved in the process of mitochondrial biogenesis. Dysfunction of either biogenesis or fission/fusion of mitochondria is associated with diseases which may be caused due to defects in the nuclear encoded proteins or mitochondrial genome.

Number and size of mitochondria are correlated with oxidative capacity of mitochondria. Due to decreased oxidative capacity, the expression of

mitochondrial gene encoded by both the mitochondrial (cytochrome *c* oxidase 1) and nucleus (succinate dehydrogenase and pyruvatedehy drogenase) genome get reduced, with this production of energy (ATP) also decreases. Mitochondrial fusion, mediated by metofusins (*mfn*) and optic atrophy gene 1 (*opa1*), is more stable and less frequent in less differentiated muscle cell, signifying adaptation of the fusion-fission machinery to the highly organized environment and contractile forces. While in somatic muscle cells there is occurrence of dynamic fusion events and forms stable connections, which are necessary for normal contractile function and are likely targets of disease-causing agents. Fusion dynamically unites skeletal muscle mitochondria and its prolonged loss endangers bioenergetics, furthermore, excitation-contraction coupling provides a potential patho-mechanism basis to different myopathies (33).

Mitochondrial biogenesis is also regulated by different transcription factors including nuclear respiratory factor, NRF-1, NRF-2 and estrogen-related receptor-alpha (ERR-alpha) that function in concert with other activators of PGC-1 family (34). The expression of PGC-1alpha, a major regulator of mitochondrial biogenesis increases with higher cellular ATP demand (35). Studies have shown that PGC-1alpha over-expression leads to mitochondrial proliferation in the adipocytes of heart and myoblasts (34). Expression of PGC-1alpha and mitochondrial biogenesis has been proposed to be regulated by different signaling mechanisms including NO soluble guanylate cyclase, beta-adrenergic/cAMP, calcineurin A, calcium/calmodulin dependent protein kinase, and AMP-activated proteinkinase (AMPK) (35). NRF-1 and NRF-2 are now reported to act as the transcriptional regulators of nuclear genes for the coding of OXPHOS subunits. While in aged cells AMPK activity has been observed to be associated with deficient mitochondrial biogenesis, insulin resistance and impaired lipid metabolism. DNA microarray studies have shown that expression of PGC-1 with regard to mitochondrial biogenesis may be responsible for various metabolic disorders, including type 2 diabetes mellitus (T2DM) and insulin resistance. All these studies suggest that various controllers of mitochondrial biogenesis directly or indirectly influence the regulation of metabolic functioning and disturbances in these regulators may profoundly affect the cellular programming and result in different disorders.

6. MITOPHAGY

Mitophagy is an evolutionary conserved pro-survival mechanism that maintains mitochondrial turnover by selective elimination of the damaged mitochondria. Although the process was first observed in rat hepatocytes, the molecular machinery and regulatory pathways were best documented in yeast. However, in mammalian cells, it broadly involves three distinct (yet interconnected) pathways. Type 1 (linked with mitochondrial fission and requires adaptor proteins like mitochondrial outer membrane protein Uth1or Atg32, Microtubule-associated protein-1 LC3); type II (linked with mitochondrial depolarization and proteins DJ-1, Pink1 and parkin); and type III which involves transfer of mitochondria-derived vesicles to the lysosomes through multi-vesicular bodies (36, 37). Deficiencies of mitophagy are closely associated with chronic human ailments such as cancer, Parkinson's, hepatic dysfunction, heart failure, renal impairments, cardiomyopathy and ageing (36, 38). Although mutation in genes involved in the mitophagic pathway: *DJ-1, PINK1, PARKIN* in Parkinson's disease; *PARKIN, BNIP3*, *NIX,* GABARAPL1in cancer; Atg5, *BNIP3*, *NIX* in cardiac dysfunction have been recently evidenced in several clinical reports (38-41), exact mechanisms remains largely unknown.

7. REDOX SIGNALING

Approximately, 1-3% of the total electrons flowing through redox gradient leaks and reacts pre-maturely with oxygen at complexes I and III before reaching to complex V thereby generating ROS (42). Extra mitochondrial oxygen consumption can occur by different enzymatic and non-enzymati creactions. It includes NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase, D-amino-oxidase, p450 cytochromes,and proline hydroxylases. It has been estimated that during the normal course of OXPHOS, generation of partially reduced oxygen molecules comprises about 0.4.-4% of the oxygen consumed (43). ROS production is increased when the electron carriers in the initial steps of respiratory chain harbor excess electrons. These excess electrons residing in the electron carriers can be directly donated to the oxygen for generating superoxide anion which can be subsequently converted into H_2O_2 via superoxide dismutases (SOD). Under normal circumstances, H_2O_2 plays an essential role in redox signaling, however, excess H_2O_2 over oxidizes protein thiols, reacts with free metals to form •OH, cause lipid per-oxidation and contribute to cellular oxidative stress.

Normal proliferating cells also exerts "Warburg effect", a phenomenon to avoid excessive ROS production by maintaining redox homeostasis. This help cells to maintain their genomic integrity by altering OXPHOS induced metabolic flux to glycolysis. Disruption in mitochondrial machinery due to alterations in mtDNA results in re-programmed OXPHOS and glycolysis which potentially disturbs Warburg effect and leakage of ROS (44). The rate of ROS production is also affected substantially by the subsequent energy expenditure. The highest rate of ROS production occurs when the ATP demand is low and the proton motive force is high. Excess calorie intake and low energy expenditure can cause high proton-motive force and less ATP demand. Therefore, respiratory chain continues to draw on the

excess calories and electron carriers become maximally occupied with electrons, tending to spill over to O_2. Mitochondria are not only one of the major sources of O_2- and H_2O_2 production in vascular cells but also the major target of cellular ROS. Mitochondrial membranes, proteins and DNA are predominantly susceptible to oxidative damage and therefore cell with altered mitochondrial function are more prone to the improper cellular demise (45).

Generated ROS induces a variety of harmful effects on genetic and epigenetic organization of cells and if un-repairable may lead to different mutagenic events associated with drastic diseases like cancer and neuro-degeneration (46). When compared with nDNA, the phenotypic consequences of these mutageneic events are more common in mtDNA. Due to the lack of introns in mtDNA sequences almost all mutations occurs in the coding sequences thus increasing the severity of the event. In addition, mutagenic events in the only mtDNA non-coding sequence i.e D-loop could have severe functional consequences as it is essential for replication and transcriptional processes. However, living organisms possess a variety of physiological protective mechanisms to counteract the generated oxidative stress and to restore redox balance. There should be a balance between oxidative stress and antioxidants as they are the major controller of the different cell proliferation processes. Despite a well established endogenous antioxidant system comprising of dismutase, catalase,and reduced glutathione, excessive levels of ROS are detrimental and may results in mitochondrial dysfunction (47-49). Oxidative damage to cells that results from an imbalance of ROS over production, particularly from O_2^- and H_2O_2, is associated with a variety of cardiovascular diseases (CVD). Studies have also shown that increased ROS causes impaired mitochondria-nuclear crosstalk in endothelial cells (47) and neutrophils (48). Besides, age-related mitochondrial changes possibly affect different cellular physiological functions concurrently and contribute to progression of extensive range of age-related diseases. These studies made clear that the proper maintenance of redox balance is crucial for normal mitochondrial functioning and any perturbation in this results in oxidative stress mediated mitochondrial dysfunction and related disorders (50).

8. MITOCHONDRIAL DISEASES

Epidemiological studies have shown that age is the dominant risk factor for different associated degenerative ailments. However, the molecular mechanisms underlying the increased risk of such diseases that is conferred by ageing remain unclear. Because mitochondria are exclusively maternally inherited, mitochondrial defects in ova, subsequently passes to zygotic cell and descendant cells acquires the defect. A major drawback in the mtDNA replication is the absence of proof reading activity in mtDNA replicating enzyme polymerase gamma, disabling cells to repair the incorrect base pairs. Individuals with pathogenic mtDNA mutations often have a mixture of wild-type and mutated molecules, recognized as heteroplasmy. It has been established that somatic mtDNA mutations during ageing undergo clonal expansion, accumulates and cause a mosaic respiratory chain dysfunction in different tissues. In addition, it is also been assumed that age-associated somatic mtDNA mutations are caused by accumulated damages at some point in the ageing progression (42). As we grow older, accumulated mitochondrial mutations due to endogenous and exogenous influence may result in fatal disorders like cancer (51). The large rearrangements or deletions of the mitochondrial genome and about 200 point mutations, including those in genes encoding proteins for subunits of complex I, III, IV and V, rRNAs and tRNAs, have been linked to a variety of clinical disorders (52). It has been also reported nuclear genes encoding mitochondrial proteins are widely involved in the mechanism of insulin resistance.

Moreover, numbers of standing evidences are available for impaired cross talk between nucleus and mitochondria, leading to prevalence for diseased conditions in later stages. Mice studies have also shown that progressive telomere degeneration leads to p53 mediated repression of PGC-1alpha and PCG-1beta (main regulator of biogenesis of mitochondria) and are therefore linked with mitochondrial dysfunction (53). Xiong *et al*. recently shown that PGC-1alpha plays a potential casual role in cellular senescence induced pathogenesis (54). There is also sufficient data suggesting the role of PGC-1 independent pathways involving sirtuins (seven different types of deacylases) towards depletion of NAD^+ level in nucleus and ageing (55). Thus, genetic factors that are inherited through nuclear or mitochondrial genes accumulate with age and may influence the pathogenesis of the various disorders through functional impairment of mitochondria.

8.1 Metabolic syndromes

Changes in mitochondrial morphology, damaged bioenergetics, enhanced concentration of lipid peroxides, diminished ATP amount and functioning further augment the danger of rising metabolism related disorders (55). Metabolic syndrome is a group of various abnormal metabolic risk factors such as obesity, dyslipidemia, increased blood pressure, increased plasma glucose (pre-diabetes) levels, pro-thrombotic condition, and pro-inflammatory condition (56, 57). These risk factors are suggested to be linked with mitochondrial genome alterations and also increase the frequency of CVDs, such as heart failure, thrombosis, and cardiac arrhythmias. Most of the patients with metabolic syndrome gradually develop T2DM, which not only increases the incidence of CVDs, but also, affect multiple organs causing neuropathies, and so forth. Recently,

a study also correlated possible role of mitochondrial dysfunction in obese and asthma (58, 59). Although the aetiology for metabolic syndromes are complex as these result from intertwining of genetic, environmental and life style factors, but defects or deficiencies in mitochondrial function relating to mitochondrial-associated metabolic diseases is implicated.

8.2 Hypertension

Hypertension has a complex etiology with over 50 genes postulated to be associated with oxidative stress, endothelial dysfunction, increased vascular resistance and altered mitochondrial homeostasis. Increased arterial blood pressure is considered as a main consequence of mitochondrial dysfunction and is consistently seen with atherosclerosis. In addition, earlier studies have shown a positive correlation of hypertension with oxidative stress (60). It has been also reported that in circumstances of metabolic perturbation, increased mitochondrial ROS (mtROS) might generate endothelial dysfunction, probably leading to hypertension (61). In a previous study on rats, a positive association of hypertension with decreased mitochondrial energy and calcium metabolic processes was reported. Over expression of SOD attenuates the increase in blood pressure, while over expression of NADPH oxidase encourages the hypertensive response to Angiotensin II (AngII) (43). Recent data suggests that mtROS activates phagocytic and cardiovascular NADPH oxidases which may have an elemental role in immune cell activation and development of Ang-II-induced hypertension (62, 63). Hypertension patients have been reported with declined mitochondrial function and increased cholesterol concentration and blood pressure. PGC-1alpha gene locus, Gly482Ser polymorphisms are observed to be linked with blood pressure and hypertension among middle-aged men (64). Different mice studies have shown that the absence of mitochondrial proteins increases risk of cardiovascular disorders and potentially affects the mice survival (65). Furthermore, it has been also reported that treatment with antioxidants not only controls mtROS, but, diminishes the generated hypertension (66).

8.3 Diabetes

T2DM is a complex disorder with diminished insulin secretion and action contributing to the hyperglycemia and wide range of metabolic defects that underlie the disease (66). However, it is a matter of debate that whether the insulin resistance seen in T2DM patients is related with altered mitochondrial function (67). An inverse correlation between mtDNA content in lymphocytes and glycolated hemoglobin levels have been recently reported in early and late diagnosed patients (48). Patti and co-workers showed that NRF-1 and PGC-1 expression decreases in insulin-resistant and diabetic subjects. In addition, studies have also reported that fewer mitochondria in muscle of insulin-resistant subjects, is due to the decreased PGC-1 alpha and beta expression (68). Moreover, genome-wide association studies have identified the common variants in nuclear encoded mitochondrial genes or their transcriptional regulators that are associated with increased T2DM risk (69). Given the large number of nuclear-encoded mitochondrial genes and the largely unexplained genetic basis of T2DM, it is possible that many (tens or hundreds of) common variants in or near mitochondrial genes are associated with T2DM risk. While each gene might have a modest effect too small to be detected on its own, together they could have a more substantial collective impact. It is also possible that several nuclear regulators of mitochondrial genes could harbor common variants of modest effects on T2DM risk.

Diabetes due to mtDNA mutations is usually part of syndromatic entities with additional traits such as deafness. This type of diabetes is caused by mitochondrial genome mutations, inherited exclusively through the maternal line. These mutations are associated with OXPHOS defects resulting in tissue dysfunction and a variety of symptoms. For instance, MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) is frequently caused by an A→G transition at position 3243 of the tRNA Leu (UUR) gene. This mutation has also been associated with maternally inherited early onset diabetes and bilateral deafness (70). In addition, mutations (large rearrangements, deletions and point mutations), in genes encoding proteins for subunits of complex I, III, IV and V, rRNAs and tRNAs, have been linked to a variety of clinical disorders including T2DM (71). There is certain report which confirms that nuclear genes encoding mitochondrial proteins are also involved within sulin resistance (52). Thus, genetic factors that are inherited through nuclear or mitochondrial genes may influence the pathogenesis of the various disorders through functional impairment of mitochondria.

8.4 Atherosclerosis

Atherosclerosis is a disease broadly associated with numerous metabolic disorders including diabetes and abnormal lipid metabolism. Elevated mitochondrial ROS in response to atherosclerosis inducers, such as triglycerides, and hyperglycemia is an important factor which participates in the disease etiology leading to inflammations and endothelial cell apoptosis. Other than the ATP production, mitochondria also regulate biosynthesis of fatty acid. Atherosclerosis i.e. abnormal concentration of fatty acid in blood capillaries is a consequence of together progressive mtDNA and nDNA damage. Large numbers of mitochondrial mutations itself are sufficient to cause atherosclerosis that has been reviewed previously (72). Mice carrying mitochondrial mutation that express a proof-reading deficient copy of mtDNA polymerase shows natural phenotype at birth and in early adolescence but consequently obtain many features of premature ageing (73). Atherosclerosis may also take place without affecting ROS production in presence of mtDNA mutations that cause ageing

phenotypes. ROS exposure increases levels of mtDNA oxidative lesions and reduces mitochondrial protein and ATP production in human vascular smooth muscle cells (VSMCs). New finding showing mtDNA damage in vessel wall and circulating cells is widespread and indicates higher risk in ROS independent atherosclerosis (74). Cellular ageing as vascular cell senescence may be major cause as hypothesized. As already mentioned, clustering of metabolic risk factors, called the metabolic syndrome, occurs commonly in T2DM. The onset of hyperglycemia in patients with the metabolic syndrome appears to accelerate atherogenesis possibly by enhanced formation of glycosylated proteins and advanced glycationproducts and/or by increasing endothelial dysfunction (75). These direct consequences of hyperglycemia probably contribute to the micro-vascular disease underlying nephropathy and retinopathy,and they may promote macro-vascular disease as well.

Hypertension and dyslipidemia are threat elements for atherosclerosis. Lipid uptake into tissues is performed by lipoprotein particles, from these apolipoprotein E (ApoE) is an important component. ApoE (ApoE−/−) deficient mice build up hyperlipidaemia and succeeding to atherosclerosis. Atherogenesis has been observed in ApoE negative mice with mtDNA damage intensified by impaired antioxidant activity. It has been also reported that these ApoE−/− mice have increased nuclear and mtDNA damage with impaired DNA repair and mitochondrial complex I activity (76). Recently, Sobenin *et al*. has reported the experimental evidence of atherosclerosis associated somatic mtDNA mutations (77). A mouse model of Hutchinson-Gilford progeria syndrome is an excessive atherosclerosis premature ageing disorder that is excessive vascular calcification in vascular smooth muscle cells is caused by reduced extracellular accumulation of pyrophosphate. Progerin further accumulates in vascular tissue of individuals in age dependent manner (78). Future identification of the mechanisms linking different mitochondrial functioning defects and atherosclerosis may be further helpful in understanding the patho-physiology of the disease.

8.5 Cardiovascular disease

Accumulation of dysfunctional mitochondria and increased levels of oxidative damage have been observed in a broad array of cardiovascular diseases (CVD) (79, 80). mtROS has a well-known role in triggering ventricular damage and accelerating the development of cardiac failure (81, 82). Defective mitochondrial biogenesis may further lead to improper bio-energetic supply causing endothelial dysfunction and cardiovascular diseases (83). A recent study provided the first evidence that up-regulation of monoamine oxidase-A (MAO-A), a H_2O_2 producing mitochondrial enzyme causes oxidative mitochondrial damage, p53-dependent suppression of PGC-1alpha, cardiomyocyte necrosis and chronic ventricular dysfunction (84). CVD, including coronary artery disease, hypertension, heart failure, and stroke, are associated within sulin resistance and endothelial dysfunction. A large body of epidemiological and pathological data documents that diabetes is an independent risk factor for CVD in both men and women. CVD is the primary cause of death in people with either type 1 or T2DM. Major risk factors associated with CVD are cigarette hypertension and highserum cholesterol which are also related to mitochondrial disorders. Moreover, increased oxidative stress and inflammation with Ang II concoction are caused by PGC-1aplha deletion may result in to vascular dysfunction (64). Studies have also shown that PGC-1alpha attenuates migration and proliferation of vascular smooth muscle cells and diminishes neointima formation after endothelial injury (85). Additionally, PGC-1beta plays an important role in maintaining cardiac contractile function and subsequent pressure overload hypertrophy by preserving glucose metabolism and avoiding oxidative stress (86). Mitochondrial apoptosis induction and degradation, altered bioenergetics, and accumulation of lipids in the heart are the consequences of increased ROS production (87). It has been also shown that the rate of endothelial apoptosis increases in association with mitochondrial oxidative stress and vascular ageing (88). PCR analysis of cardiac patients revealed that aged individuals with higher mtDNA mutations are more prone to heart failure as compared to younger ones (89). Thus understanding the complex interplay of mitochondrial oxidative stress and dysfunction is critical to devise novel therapeutic interventions for cardiac disorders.

8.6 Neurological disorders

Neurological function depends on high energy requirement fulfilled by continual mitochondria ATP production. There are much evidence suggesting ageing-related neurodegenerative diseases happen due to mitochondrial dysfunction and oxidative stress (90, 91), suggesting that mitochondria have a central role in progression of neurological disorders. While accomplishing high energy demand, mitochondria get damaged and responsible for production of ROS and also subsequent target (92). Researches' conducted during the last decade have implicated mitochondria in a wide variety of cellular metabolic processes and molecular interactions. Therefore, it does not come as a surprise that an increasing number of human pathologies including neurodegenerative ailments have been associated with deficiencies in mitochondrial function (93, 94). Impairment of mitochondrial-nuclear cross talk related with altered redox homeostasis is widely accepted to be a perturbation in the balance of free radicals in a cell and the cell's ability to cope with this change. Techniques like RNA sequencing, microarray and proteome analysis have revealed critical regulatory machinery imbalance in cellular pathways associated with neurolonal degeneration involving cell death (95-99). Neurolonal degeneration through apoptosis alluding to

global loss of integrative brain function is closely linked to Alzheimer's disease (AD), Parkinson's disease (PD), Amyloid Lateral Sclerosis (AML), Multiple Sclerosis (MS), mild cognitive impairment (MCI), Huntington's disease, cerebral ischemia and HIV-associated neurocognitive disorder (HAND). According to the proponents of the mitochondrial cascade, progression of these diseases has been attributed to several factors, which includes reduced levels of endogenous anti-oxidants and increased demand of oxygen consumption in micro environment of the ageing-brain.

8.6.1 Alzheimer's disease

Alzheimer's is a late-onset, common in advanced age, progressive neurodegenerative disease, characterized by the gradual decline of memory, cognitive functions, and changes in behavior and personality. With increasing lifespan in humans, AD is a growing health concern in the society. Reduced cytochrome oxidase activity in postmortem cerebral tissue of AD patients confirmed confirms the disease association (100). A major vascular susceptibility factor gene apolipoprotein E gene is found to be associated with sporadic late onset of AD cases (101).

8.6.2 Parkinson's disease

Parkinson's is diagnosed by increase in muscle rigidity, frequent tremors and a slow and gradual loss of physical movements. Pathological changes in PD are the loss of dopaminergic neurons of the substantia nigra and the presence of cytoplasmic inclusions, or Lowy bodies, containing alpha-synuclein**.** PD is both chronic and progressive. Both genetic and environmental factors are involved in the development of PD (102). Complex I (NADH ubiquinone oxidoreductase) of the electron transport chain inhibition takes place in PD. Connection of PD with alpha-synuclein, Parkin, *DNAJ-1 or HSP40/HDJ-1* (*DJ1*), phosphatase and tensin homolog (PTEN) – induced kinase 1, mitochondrial serine protease *OMI*/*HTRA2*, leucine-rich repeat kinase 2 (*LRRK2*) and ubiquitin carboxy-terminal esterase L1 DNA mutations have been discussed in several recent genetic studies (103).

8.6.3 Amyotrophic lateral sclerosis

ALS is caused due to the degeneration of motor neurons ceasing to send messages to muscles (104). Electromyography is routinely performed to confirm the progressive signs and symptoms of upper and lower motor neuron dysfunction. The rate may be lower in some ethnic populations including American Indians as compared to Caucasians (105). Eventually, the brain loses its ability to control voluntary movement (106). Mouse studies have shown that the increased oxidative stress along with compromised mitochondria intensifies the pre-synaptic decline in the neuromuscular junctions. This initial dysfunction does lead to neuro-degeneration induced by inflammatory agents and loss of trophic support (107). Deposition of ubiquitinated TAR DNA-binding protein 43 (TDP-43) negative SOD1 protein in neurons is associated with *SOD1* mutations (108). SOD1 appears to initiate complaint in motor neurons, but astrocytes and microglia encourage disease progression, possibly through involvement of glutamate.

8.7 Mitochondrial myopathy

Mitochondrial myopathy is a large group of diseases that occurs due to dysfunctional mitochondria in muscle fibers. This dysfunction is due to mutation commonly observed in gene encoding rRNA, tRNA, and OXPHOS region of mtDNA. It results in low energy, free radical generation and lactic acidosis and may have variety of symptoms depending on the organ involved. Since, organs such as muscle, heart, brain and kidneys require high amount of energy, they are at major risk due to defective mitochondrial respiration. The group of myopathies includes Kearns-Sayre syndrome (KSS), Leigh syndrome and maternally-inherited Leigh syndrome (MILS), Mitochondrial DNA depletion syndrome (MDS), Mitochondrial encephalomyopathy, lactic acidosis and stroke like episodes (MELAS), Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), Myoclonus epilepsy with ragged red fibers (MERRF), Neuropathy, ataxia and retinitis pigmentosa (NARP), Pearson syndrome, Progressive external ophthalmoplegia and others (109). KSS is characterized by deletion of mtDNA causes dysfunction of the OXPHOS system whereas MILS is due to rare T8993C mutation in the MT-ATP6 gene that leads to OXPHOS defects linked to selective inhibition of inner membrane fusion but not outer. This mutation is also found in NARP syndrome (110). A single mtDNA point mutation in tRNA and altered complex I function is observed in MELAS that have MILS type symptoms (111). MDS has depleted mtDNA levels in nearly every tissue which causes severe ETC dysfunction, resulting in a severe depletion of ATP. MDS has been characterized with 10 gene mutations independently and more commonly found with defective nucleotide biosynthesis. Thymidine phosphorylase deficiency causes MNGIE that have a recessive mutation in the TYMP gene required for the assembly of nucleotides while MNGIE leads to the accumulation of thymidine and deoxyuridine in mitochondria that hinders with nucleotide pool and results into mtDNA instability. MERRF is accounted with point mutation 8344A>G in the mt-tRNA(Lys) gene (112). Nuclear encoded mtDNA polymerase gamma *POLG1/POLG2* mutations are major reasons for mtDNA depletion.

8.8. Ageing

It has been hypothesized that mitochondria are the major target of free radical attack that leads to human ageing in addition to well acknowledged phenomenon, shortening of telomeres. A number of mechanisms for ageing include mitochondrial dysfunction, cumulative DNA damage, telomere loss, altered gene expression, oxidative damages, Inflammation and epigenetic

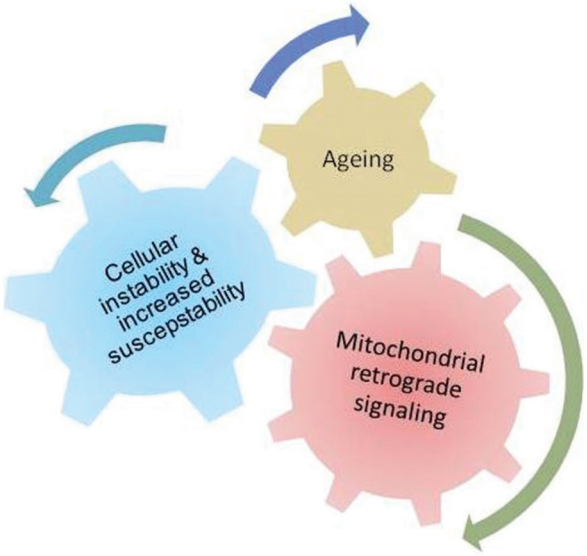

Figure 3. Figure illustrating the role of mitochondrial retrograde signaling in cellular instability and ageing.

changes (113, 114). In addition, mitochondrial dysfunction has been identified as one of the principal causes of age-related bio-energetic decline (115). Harman first proposed that ageing is consequence from accumulations of free radicals generated by mitochondria in supporting of free radical theory of ageing and further progressive loss of cellular function with ageing (116, 117). There is strong evidence that age induces alterations in the mitochondrial genome that lead to defects in mitochondrial function, especially in post-mitotic tissues with high energy requirements such as the heart, brain and skeletal muscle (118, 119). In addition, this not only insinuates the epigenetic changes, but generates inflammatory response, one of the leading causes of ageing (42, 118). Alterations in mitochondrial retrograde signaling are strongly connected with altered cellular function and ageing (Figure 3.). In the aged stage, cells have been noticed with accumulated altered proteins/molecules and these accumulations may be due to damage from elevated oxidative stress. Large number of deletions, point mutations, and tandem duplications of mtDNA has been found in various tissues of aged individuals. It has been established that many of these mtDNA mutations start to occur after the mid-thirties and they accumulate with age in post mitotic tissues of the human body.

Furthermore, a decreased respiratory rate was observed in mitochondria isolated from elderly human subjects with reduced mitochondrial number and function. Gene expressions associated with mitochondrial functions are observed with ageingWallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. Annu Rev Genet 2005 Jul 19; [Epub ahead of print]. Cliquez ici pour aller à la section Réespecially muscle gene expression for subunits of ATP synthase and NADP trans-hydrogenase. At the other extremity of the ageing process (transition between adult to old animals), oxidative capacity is reduced with ageing in muscle and heart even in humans (120, 121). Because regulation of energy production isdependent on ATP needs, reduced energy expenditure with age decreases ATP needs. This leads to decreased oxidative capacity in aged skeletal muscle and heart in both animals and humans. In addition, morphology of old mitochondria changes due to increased production of ROS and decreased ATP production and respiration. In addition, mitochondrial biogenesis may be impaired by age-dependent accumulations of point mutations in human mitochondrial DNA at specific regions that control its replication. Among the known intracellular regulators of mitochondrial biogenesis, AMPK activity appears to be one of the main factors associated with deficient mitochondrial biogenesis, insulin resistance and impaired lipid metabolism observed in aged cells. AMPK seems to play a critical role in several important processes such as the control of insulin resistance, obesity, fatty acid oxidation and mitochondrial biogenesis. Therefore, its dysfunction during ageing seems to be one of the key factors involved in the deficiency in mitochondrial activity and metabolism regulation during ageing. Furthermore,age-dependent reduction in PGC-1alpha expression may account for reduced OXPHOS in elderly humans (54). Thus, genetic factors, altered mitochondrial biogenesis, increased ROS production and ageing all contribute to mitochondrial dysfunction.

9. PREVENTIVE STRATEGIES AND TRANSLATIONAL PERSPECTIVE

Mito-prevention is the protection of semi-autonomous organelle either using mito-protectors or armament from misshapenness in mitochondria. It mainly emphasizes on the methods of restoring the mitochondrial function which is an important aspect of mitochondria related diseases (122). Since mitochondria are one of the primary sources of ROS, therapies that involve the delivery of anti-oxidants, such as vitamin C, vitamin E, creatinine or coenzyme Q, to mitochondria that could potentially counteract ROS overproduction (123). Whereas, molecules that can abate mitochondrial dysfunction are helpful for various degenerative diseases, molecules that promote cell death are highly desired for cancer control. Optimized diet that contains higher amount of anti-oxidants like flavonoids and polyunsaturated omega-3 fatty acids helps to maintain mitochondria dynamics. Supplementation of lipoic acid prevents lipid peroxidation thereby reducing oxidative stress (6). Exercise could be a major abiotic factor to lower the risk of age associated mitochondrial dysfunctions as it increases PGC-1alpha, a major biosynthetic regulator (124).

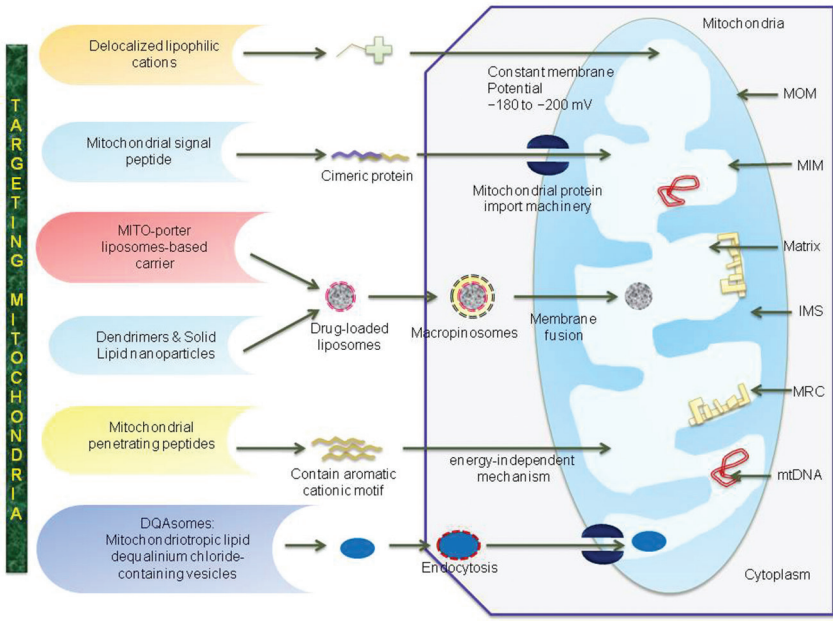

Figure 4. Figure summarizing different nano-particulate strategies for effective mitochondrial targeting.

Under certain clinical situations such as cardiac failure, the presence of insulin resistance may adversely affect the disease progression. The factors responsible for developing such resistance include lipid and glucose toxicity, neuro-humoral deregulation, cytokine imbalance and oxidative stress. In addition, insulin receptor substrate-1 (IRS-1) mediated signaling mechanism is also considered to be responsible for developing insulin resistance. Studies have shown that direct targeting of mitochondrial signaling and metabolic pathways may prove beneficial for positive clinical outcome. In recent past, the use of both angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) have proved beneficial in minimizing the diabetes incidence in cardiac failure patients (125, 126). Thus for improved management of insulin resistance in cardiac patients identifying novel mechanism-based therapeutic targets holds good promise. Similarly, studies have shown that restriction of calories also stimulates mitochondrial function and increases longevity. It is supposed that sirtuin-1 activates PGC-1beta followed by the activation of genes involved in mitochondrial biogenesis. The biogenesis related genes are also stimulated by mTOR inhibition and activation of the NRF-2 which improves the antioxidant gene expression thereby reducing oxidative stress and improving mitochondrial respiration (127).

Mitochondrial targeting using programmed bio-degradable nanoparticles is an emerging strategy for treatment of mitochondrial dysfunction associated diseases (128) (Figure 4). However these nanoparticles should be optimized for their charge and size to check the versatility of this platform for different therapeutics applications those including for cancer, AD and obesity (129). Importantly, technology development has improved the perception of complex mitochondrial function, which is a field of burgeoning research. Understanding the mitochondrial genome regulatory mechanisms like mtDNA damage neutralization and RNA import by mitochondria may be beneficial. In addition, the most important is the clinical translation of the *in-vitro*/*in-vivo* assessed therapeutic modalities. Targeted drug delivery systems are designed to transport the drug specifically to mitochondria of target organs, tissues, cells or organelles at therapeutic concentrations. Specifically targeting drugs, as opposed to non-targeted systemic delivery, potentially provides a number of advantages such as improved efficacy and increased therapeutic threshold. Targeted drug delivery systems not only facilitate drug delivery to the desired site of action, but often protect the active ingredient from degradation.

9.1 Utilization of mitochondrial properties for targeting

Mitochondria exhibit some specific features that differ from other cellular compartments and also among normal and diseased mitochondria. These include: high membrane potential across the inner mitochondrial membrane; the organelle's protein import machinery; and the mitochondrial fusion process. In particular, these special features are being exploited for developing targeted strategies for transportation of biologically active molecules to and from mitochondria. A trans-membrane electrochemical gradient and pH difference maintains a high membrane potential, which is negative inside and acidic outside. Therefore, cation molecules

are attracted to mitochondria and selectively accumulate in the mitochondrial matrix because of membrane potential (130).

Mitochondrial membrane protein import pores with specific targeting sequences present for both outer and inner membrane of mitochondria could potentially be utilized for transporting drug or DNA molecules to and/or into the matrix of mitochondria. The voltage-dependent anion channels-class of porin ion channel (VDAC) situated only in the outer mitochondrial membrane can be targeted specifically to direct drug to the inter membrane space. Moreover, mitochondrial membranes enclosing any mitochondria-specific binding sites and unique protein receptor could be also exploited for drug targeting purposes. Drug carriers that are too large to pass the mitochondrial protein import pores may be targeted using mitochondrial fusion such as drug carrier which is decked with moieties, peptide or non-peptide ones. But mitochondrial targeting by mitochondrial fusion process is area that is unexplored. Although some of the nanotechnology formulations follow passive-targeting to probe mitochondria but most of them use active-targeting mechanism. Active modes of targeting include the intrinsic mitochondriotropic design of DQA-somes, the surface alteration of liposomes and solid lipid nanoparticles (SLP) with mitochondriotropic residues and the immuno- and peptide-conjugation to SLP. The mitochondriotropic molecules partially enhance mitochondrial targeting through accumulation at the site of mitochondria or inside mitochondria of living cells. The use of antibodies or mitochondrial leader sequences conjugates are general mechanisms for targeting.

9.2 Impediments in mito-targeting

Various extracellular and intracellular barriers including cell membrane and the mitochondrial membrane create challenges to the drug delivery to mitochondria. Plasma membrane, cytosol and mitochondrial membrane, are key trafficking obstacles. Mitochondrial matrix targeting is most difficult where almost all potential targets are located, because drug or drug carriers should cross two mitochondrial membranes after exceeding the cellular membrane and cytoplasm. It is essential to overcome both mitochondrial outer membrane which have only small pores and highly lipophilic mitochondrial inner membrane. The molecular size and hydrophobicity are important determinants that affect the diffusion rate through mitochondrial membrane.

Controlled drug release and protection of encapsulated macromolecules from proteolytic degradation are the major advantages of utilizing nanoparticles for mito-targeting. Nanoparticles enter to the cellular system by general mechanisms like clathrin-mediated endocytosis, caveolae-mediated endocytosis, phagocytosis and macropinocytosis (131). Initial binding of nanoparticles to the membrane receptor is followed by formation of clathrin coated vesicular pit and pinching off the vesicle from the cell membrane into the cytosol. While clathrin independent mechanism is mediated by receptor endocytosis, most commonly known as caveolae-mediated endocytosis. Receptor targeting ensures the selective uptake and internalization of drugs where nanoparticles are firstly entrapped in endosomes which upon maturation form late endosome and fuse with lysosomes in order to exert its therapeutic effects. However, the nanoparticles entering via the endosomal route faces drug and carrier degradation issues due to the stability problems at endosomal/lysosomal pH. In this regard, using a novel classes of peptides molecules known as cell penetrating peptides (CPPs) (10–30 amino acid polycationic sequences) may be beneficial as they possess the ability to transfer molecules straight to the cytosol by detouring endocytic pathway (131).

9.3. Mitochondria targeting approaches

Several approaches have been designed to selectively direct the drugs or molecules into the mitochondria. The strategies include ligands like lipophilic cations (e.g. mitoQ and sk compounds), mitochondria-penetrating peptides, membrane fusion, mitochondrial protein import machinery, liposomes, polymeric nanoparticles, bolasomes and metal nanoparticles etc. (130-132). In addition, several mitochondrial-specific probes have also been fabricated capable of selective delivering biologically active molecules to mitochondria, thereby enhancing the bioavailability of drugs.

9.3.1. Chimeric proteins

Molecules having a mitochondrial signal peptide can easily enter into the mitochondria through the mitochondrial protein import machinery. An N-terminal specific amino acid sequence or mitochondrial signal peptide attached to the proteins or on the surface of targeting moieties can be exploited for direct drug delivery into the mitochondrial matrix. Moreover, proteins sited in the mitochondrial inner or outer membrane can be targeted for mitochondrial delivery of therapeutics (133). By covalent linking of peptide or proteins having signal for mitochondrial to the oligonucleotide or DNA at the C-terminus for delivery to the mitochondria has also been achieved (134,135). Using this method an efficient nano-particle based delivery molecule is developed, which enters into the cytosol via polycations or transient membrane channels. These nano-vectors have N-terminal mitochondrial targeting peptide linked to a polyamide nucleic acid (PNA), molecule with an achiral polyamide backbone which is annealed to an oligonucleotide with a selected sequence (136). Although significant effort has been commenced to create the mitochondria-targeted nano-preparations, but the full knowledge of the process of intracellular trafficking, pay-load delivery and time-dependent localization of nano-preparation have yet to be attained.

9.3.2. Colloidal dequalinium vesicles

DQAsomes/Bolasomes are dequalinium liposomes that are di-cationic amphiphilic vesicle-like aggregates (137). The higher positive charge of DQAsomes attract towards the negatively charged mitochondrial transmembrane (138). They enter the cell by endocytosis followed by fusion with the outer memberane and exhibits higher mitochondrial accumulation and retention. The mitochondriotropic nature of DQAsomes has been evaluated to determine their potential for non-viral transfection vector for shipping DNA to the mitochondria (139). A more effective delivery of plasmid DNA (pDNA) to the mitochondria have been made easy by the conjugation of mtDNA with mitochondrial leader sequence peptide (MLS) followed by tagging on the DQAsomes. With this method, DQAsomes accumulate on the mitochondrial membrane and get in to the mitochondrial matrix by the mitochondrial protein import machinery (138). The advantage of this process is that DQAsomes are able to escape from endosomes without losing their enclosed load. Studies have also accounted DQAsomes for anti-cancer activities. Paclitaxel encapsulated DQAsomes displayed better apoptosis activity and accumulation in the mitochondria as compared to the un-encapsulated paclitaxel in COLO25 cells (139). Surface specification with folic acid of these molecules has been reported to improve their anti-tumor efficiency (140) and can be utilized for human tumor therapeutics. The internalization of the folate conjugated nano-carriers in a tumor cell-specific manner occurs through folate receptor-mediated endocytosis and results in an increased toxicity of the encapsulated drug.

9.3.3. Liposomes

Due to the biocompatibility and non-toxic properties, liposomes are one of the oldest in drug delivery systems with greatest translational potential for mitochondrial delivery and most successful in developing FDA approved therapeutics. The first liposomes used for mitochondrial targeting was proteo-liposomes that have a crude mitochondrial membrane fraction in the liposomes (141, 142). The fusion properties of mitochondria can be utilized to make liposomes that fuse with mitochondrial membranes to release their drug load into the mitochondrial outer and inner membranes (143). Besides this, liposomes can be conjugated to the cationic and lipophilic ligand for same purpose. A class of liposomes i.e. lipoplexes formed of nucleic acid mixed with cationic liposome are small and partially stable particles for delivery of nucleic acid. Lipoplexes protects nucleic acid from nuclease degradation and have increased cellular transfection efficiency (144). A group developed with cationic and lipophilic properties, stearyl triphenyl phosphonium (STPP) functionalized liposomes to deliver an anti-tumor drug molecule i.e. ceramide specifically in the mitochondria (145). Ceramide targets cytochrome c release, ROS production, and apoptosis by their anti-cancer property. About 250 nm fusogenic liposomes can deliver enclosed molecules to the mitochondria. While the exact non-fusogenic liposome's delivery mechanism is unknown, it is assumed that it may involve through the surface binding to the mitochondria to deliver drug in close proximity to their target.

In addition, cellular entry properties of a virus can be utilized for development of an efficient lipid envelope equipped with different useful devices to mimic envelope-type viruses (143). Such kind of device is a multifunctional envelope-type nano-device (MEND) and facilitates efficient and simple loading of plasmid DNA, proteins or other macromolecules for gene delivery. These can be further modified to create a unique drug delivery system with specific receptors or ligands and peptides for endosomal escape to locate nanoparticles selectively in the mitochondria. However, cytotoxicity effect of targeted liposomes has yet to be examined. In the most cases liposomes used for the cytotoxic drugs delivery for the anti-cancer therapy but their potential use for other therapeutic applications is unknown and must be explore.

Similarly, MITO-Porter is another liposome based system to deliver both large and small bioactive molecules into mitochondria by the mechanism of membrane fusion (146). These molecules have advantage over others that their lipid composition promotes both fusion to the mitochondrial membrane and the intra-mitochondrial release of enclosed cargo. Nano-preparation formulated of DOPE/sphingmyelin/Strearyl-R8 (9:2:1) carries its macromolecular cargo (146, 147). Cytosolic delivery of this can be increased by the liposomal surface-modification with high-density R8 that have dual benefit of cytoplasmic and mitochondrial macromolecule delivery. This mimics the trans-activating transcriptional activator (TAT), a useful moiety for cellular uptake as well as mitochondrial targeting. The modification also increases the chance of cellular uptake by macropinocytosis. When these macropinosomes releases their contents into the intracellular spaces, the electrostatic interaction between the MITO-Porters and mitochondria cause fusion which enables MITO-Porters entry into the mitochondria (146). A study shows that MITO-Porters encapsulating the Bongkrekic acid (BKA) which is an adenine nucleotide translocator block the apoptosis via mitochondrial permeability transition. MITO-Porter encapsulated BKA have been reported to possess a strong anti-apoptotic effect compared to naked BKA in HeLa cells (148).

9.3.4. Polymeric Nanoparticles

Use of polymeric nanoparticles encapsulated drugs now becoming the principal preference due to their bio-degradable nature, bio-compatibility, easy chemical modifications and conjugations to the targeting ligands and/or drugs. Polymeric nanoparticles stuffed with anti-oxidant molecules are able to protect cells

from oxidative stress induced cell death. In a recent study, formulated polylactic-co-glycolic acid (PLGA) nanoparticles encapsulating SOD have more effective prevention against H_2O_2 induced neuronal cell death in contrast to SOD alone and SOD conjugated to poly-ethylene glycol (149). Zinc phthalocyanine (ZnPc) (photosensitizer) containing polymeric nanoparticles are biodegradable and successfully induced immune response through mitochondria-targeting (150). Poly (amidoamine) (PAMAM) dendrimer surface conjugated with mitochondriotropic ligand tri-phenyl-phosphonium (TPP) have been shown to hold good cellular capture and mitochondrial targeting ability (151-153).

9.3.5. Metal nanoparticles

Metal elements including gold, platinum and titanium dioxide containing nanoparticles are unique, size < 10 nm, benefited with anti-oxidant capabilities and simple for attachment with targeting ligands for mitochondrial targeting. However, the safety and efficacy of metal nanoparticles is controversial among scientists. Metals like gold can be easily functionalized with targeting ligands by their thiol reactivity. According to a study, gold nanoparticles cause tumor cell death by disrupting the integrity of the mitochondrial outer memberane following release of cytochrome c (154) This study found gold nanoparticles of 3 nm could permeate the mitochondrial outer memberane but not of 6 nm (155). This practice may be of high therapeutic value for induction of cellular apoptosis for inhibition of tumor growth. Titanium dioxide nanoparticles have also been investigated as mitochondrial targeting nano-therapeutic molecules for the control of gene regulation (156). Surface modification of metal nanoparticles with mitochondrial specific oligo-nucleotide molecule also increases the specificity of these nanoparticles but the mechanism of shuttling or trafficking to the mitochondria is unknown. Similarly, platinum nanoparticles also exhibit unique antioxidant properties as they are capable of quenching superoxide anion radical (O_2^-) and H_2O_2 (157). Bimetallic nanoparticles exhibit antioxidant capacity with dual property of both the delivery vehicle and therapeutic agent though their cellular toxicity has yet to be tested.

9.3.6. Mitochondrial-targeted ligands

Delivery systems for mitochondrial-targeting drugs are emerging as a prime pharmacological target in the treatment of metabolic disorders like cancer. Significant efforts are being carried out to discover mitochondria targeting molecules. Various molecules with such specificity involve delocalized lipophilic cations like small molecule ligands, cationic bolalipid based vesicles, mitochondria penetrating peptides (MPP), protein import machineries of mitochondrial.

9.3.6.1. Lipophilic cations

Lipophilic moieties combined with delocalized positive charge are mitochondriotropic molecules capable to enter mitochondria. Various lipophilic cations such as triphenylphosphonium (TPP), Flupirtine, rhodamine 123, MKT-077 and anthracyclins have been demonstrated for intracellular delivery and selectively accumulated in the mitochondria (158). Due to presence of high negative membrane potential across of about −180 to −200 mV their lipid bilayer of mitochondrial membrane gives unique advantage for selective accumulation of lipophilic cations targeted to the mitochondria (159). Because of cationic property of TPP, it can be attached to the surface of drug-loaded liposomes for effective delivery of therapeutic cargo to the mitochondria (160). For example surface conjugation using TPP to a macromolecule like dendrimer led to the efficient mitochondrial targeting (161). Another TPP group and a photosensitizer mesochlorine e6 (Mce6) conjugate attached on the polymer backbone, the N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer was used in mitochondriotropic drug delivery system (162). MitoQ has been used extensively to rectify oxidative damage in diseases associated with oxidative stress it is a TPP-conjugated ubiquinone derivative based mitochondria targeted antioxidant (158-163). MitoQ has high accumulation efficiency up to 100-fold within mitochondria and notably more effective than the non-targeted CoQ10 analog cecycloubiquinone (163). One more lipophilic cation Rhodamine 123 is also utilized for drug delivery to the mitochondria. The complex forms of tetrachloroplatinate and rhodamine- 123 were used to produce cytotoxic and antitumor effects.

9.3.6.2. “Sk” compounds

A group developed an alternative string of mitochondria-targeted antioxidant molecules based on the hydrophobic cation targeting approach. A quinone in the electron transfer chain of chloroplasts i.e. plastoquinone used in place of ubiquinone antioxidant moiety of MitoQ because of better antioxidant activity (164, 165). SkQ1 (plastoquinonyl-decyl-tri-phenyl-phosphonium) molecules are able to promote survival in infections, age-dependent pathology and decreases functional decline (160). SkQ1 and MitoQ, both molecules increase survival and health span in some situations but *in vivo* validation of efficacy and explanation of the mechanism of action needs to be explored.

9.3.6.3. Mitochondria-penetrating peptides

A class known as Szeto–Schiller (SS) peptides contains aromatic cationic motif in which the aromatic group is either tyrosine or a dimethyltyrosine, separated by a basic amino acid in polypeptide. The mode of mitochondrial targeting followed by these molecules is energy-independent mechanism after the rapid accumulation in the cell (166-169). They get accumulated in the inner mitochondrial memebrane and serve as scavenger H_2O_2, peroxynitrile and reduce lipid peroxidation (170). Another group of mitochondrial-specific peptides used are the XJB peptides that belong to a series of hemigramicidin-TEMPO compounds. These

targeting moieties have ROS scavenging properties. The convergence of nanoscience, nanotechnology and mitochondrial medicine will lead to the development of novel tools and methodologies for observing, quantitatively characterizing and manipulating molecular and supra-molecular components of mitochondria.

10. CONCLUSIONS

Emerging evidence supports the notion that imbalance of mitochondrial-nuclear cross talk plays a critical role in origin, development and patho-physiology of several human diseases. Mitochondria are most likely the dominant site for initiation and propagation metabolic perturbations because of their role in substrate oxidation and ATP generation. Although age-related abnormalities in key signature processes culminate in decrease in mitochondrial biogenesis, reduced mitochondrial content, mtDNA mutations, increased oxidative stress, energy dysfunction, decrease in anti oxidant defence and aberrant homeostasis of cytosolic calcium, there exists several missing links. Further, mitochondria also provoke process such as epigenetic changes and inflammatory responses that additively drive to the path of degenerative ailments. For closing these gaps in knowledge, innovative approaches will be essential that may help ramify the extent to which mitochondrial anomalies contributes to the development of these diseased conditions. Given the key role of mitochondrial dysfunction, novel treatments and therapies are developed for many of these disorders. Selective targeting using engineered nano-vectors is an interesting strategy that might provide a new tool for mitochondria engineering and pave way for treatment of these fatal mitochondrial diseases in humans.

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Abbreviations: ATP: adenosine triphosphate; ADP: adenine diphosphate; NADH: nicotinamide adenine dinucleotide; FADH: flavin adenine dinucleotide; ETC: electron transport chain; ROS: reactive oxygen species; mtDNA: mitochondrial DNA; SAM: S-adenosyl methionine; DNMT: DNA methyltransferase; NO: nitric oxide; Mfn: metofusins; mtROS: mitochondrial ros; GSH: glutathione; NRF: nuclear respiratory factor; ERR-ALPHAlpha: estrogen-related receptor-ALPHAlpha; PGC-1: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; T2DM: diabetes mellitus type 2; SOD: superoxide dismutases; CVD: cardiovascular diseases; Apo E: apolipoprotein E; MAO-A: monoamine oxidase-A; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; MELAS: mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes; VSMC: vascular smooth muscle cell; AD: alzheimer's disease; PD: parkinson's disease; ALS: amyotrophic lateral sclerosis; AMPK: 5' AMP-activated protein kinase; IRS-1: insulin receptor substrate-1; mTOR: mammalian target of rapamycin; MIM: mitochondrial inner membrane; MOM: mitochondrial outer membrane; VDAC: voltage-dependent anion channels; SLP: solid lipid nanoparticles; DQA-Somes: dequalinium-based liposome-like vesicles; CPPs: cell penetrating peptides; PNA: polyamide nucleic acid; MLS: mitochondrial leader sequence peptide; STPP: stearyl triphenyl phosphonium; MEND: multifunctional envelope-type nano-device; BKA: bongkrekic acid; TPP: triphenylphosphonium; MPP: mitochondria penetrating peptides; SS: Szeto–Schiller; FDA: food and drug administration.

Key Words: Mitochondrial Dysfunction, Oxidative Stress, Oxidative Damage, Redox Signaling, Review, Mitochondria Targeting, Review

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