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Review

Nanopreparations for mitochondria targeting drug delivery system: Current strategies and future prospective

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ABSTRACT

Mitochondria are a novel and promising therapeutic target for diagnosis, treatment and prevention of a lot of human diseases such as cancer, metabolic diseases and neurodegenerative disease. Owing to the mitochondrial special bilayer structure and highly negative potential nature, therapeutic molecules have multiple difficulties in reaching mitochondria. To overcome multiple barriers for targeting mitochondria, the researchers developed various pharmaceutical preparations such as liposomes, polymeric nanoparticles and inorganic nanoparticles modified by mitochondriotropic moieties like dequalinium (DQA), triphenylphosphonium (TPP), mitochondrial penetrating peptides (MPPs) and mitochondrial protein import machinery that allow specific targeting. The targeted formulations exhibited enhanced pharmacological effect and better therapeutic effect than their untargeted counterpart both *in vitro* and *in vivo*. Nanocarriers may be used for bio-therapeutic delivery into specific mitochondria that possess a great potential treatment of mitochondria related diseases.

1. Introduction

The mitochondria are an essential organelles present in most eukaryotic cells that provide the energy metabolism of the cells and play a crucial role in the regulation of programmed cell death [1,2]. Correspondingly, increasing efforts are being made toward mitochondria-targeting pharmacological intervention, leading to the emergence of 'mitochondria medicine' as a new field of biomedical research [3,4]. Mitochondria possess unusual structure that is composed of four parts (Fig. 1): the outer mitochondrial membrane (OMM), the inner mitochondrial membrane (IMM), the intermembrane space (IMS) and the matrix, that carry out specific function respectively. Therapeutic

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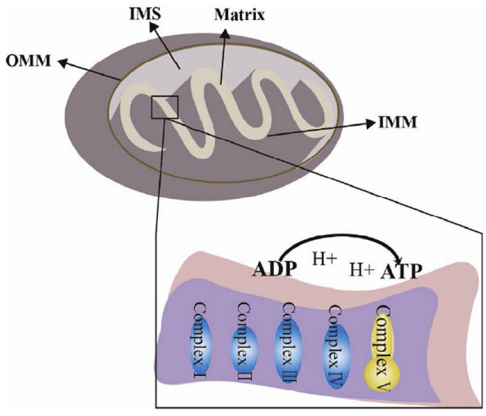

Fig. 1 – Mitochondrial four parts: the outer mitochondrial membrane (OMM), the inner mitochondrial membrane (IMM), the intermembrane space (IMS) and the matrix. And the electron transport chain contains five proteins: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc_1), complex IV (cytochrome c oxidase), and complex V (ATP synthase).

molecules are easy to cross OMM due to rather larger transition pore within the membrane where the drugs would be driven via the passive diffusion [5]. However, many therapeutic molecules suffer the difficulty in arriving at the mitochondrial matrix due to the highly folded rigid IMM with the smaller transition pores separating the intermembrane space and the mitochondrial matrix [6]. Besides, the IMM has exceptionally rich cardiolipin and maintains a strong negative internal membrane potential ($\Delta\Psi_m$) of –160 ~ –180 mV required for the electron transport chain (ETC) and adenosine triphosphate (ATP) synthesis. In addition, the IMM hosts the majority of proteins that are components of the respiratory chain. For example, complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc_1), complex IV (cytochrome c oxidase), and complex V (ATP synthase) those provide electrons transports in the tricarboxylic acid cycle which occur in the IMM and mitochondrial matrix, leading to the production of ATP (Fig. 1). During the process of ETC, the strong reduction potential between complex I and complex IV (–0.32V to +0.39V) produces superoxide anion free radicals, which might induce oxidative stress or overproduction of reactive oxygen species (ROS) [7]. Heart and lung mitochondria generate superoxide radicals mainly from complex III (cytochrome bc_1) whereas brain ROS primarily from complex I. The extensive decoupling of the ETC is detrimental to mitochondria and cells by transferring electrons to oxygen to form superoxide radicals rather than to adenosine diphosphate (ADP). ROS released from mitochondria lead to several harmful consequences including lipid oxidation, mtDNA/RNA damage, protein oxidation, Ca^{2+}-dependent mitochondrial permeability transition pore activation and the release of cytochrome c, which results in the formation of apoptosome and ultimately lead to apoptosis [8]. Both genetic mutations of mitochondrial proteins and oxidative stress bring out mitochondrial dysfunction that triggers the cell death signaling cascade and results in organ failure and disease. Recently, lots of diseases including cancer, diabetes, neurodegenerative disease (Alzheimer's disease, Parkinson's disease), heart failure and ischemia-reperfusion injury were found to be associated with mitochondrial damage [9–13]. Therapeutic intervention at the mitochondrial level could provide a solution to the treatment of related diseases. Taking the ischemia-reperfusion injury as an example, hypoxia leads to suppression of oxidative phosphorylation, cytoplasmic acidification and Ca^{2+} overload whereas the mitochondrial Ca^{2+} concentration remains low owing to a collapse of IMM potential ($\Delta\Psi_m$), which consequently reduces the electric driving force for mitochondrial Ca^{2+} entry at ischemic phase. However, during the reperfusion phase, oxidative phosphorylation and $\Delta\Psi_m$ are recovered, which results in Ca^{2+} and ROS overload within the mitochondria, together with the recovery of neutral cytoplasmic pH, those activate Ca^{2+} dependent mitochondrial permeability transition pore and trigger cell death via apoptosis due to cytochrome c leaking from mitochondria [14–19].

Nanopreparations may be able to address the current limitations and offer a sustained and targeted drug delivery system to mitochondria [20–22]. Moreover, nanopreparations could significantly improve the pharmacokinetic properties and biodistribution profiles of therapeutic molecules. In order to deliver the payload within the mitochondrial matrix and to have spatio-temporal control for the release of the payload in different mitochondrial compartments, the delivery vector based on the nanoparticles (NPs) needs to be rigorously designed to have a precise size, lipophilic surface, appropriate charge and specific density of targeting moieties. In this review, the information on mechanisms of mitochondrial dysfunctions-related prevalent diseases would firstly be provided that allows the development of targeting strategies to mitochondria. We focus on the discussion of several different targeting mechanisms and summarized the current mitochondria targeted nanopreparations.

2. Representative mitochondrial diseases

2.1. *Cancer*

There were 8.8 million people worldwide who died from cancer in 2015. That was nearly 1 in 6 of all global deaths from World Health Organization (WHO) data [http://www.who.int/cancer/en/ (accessed on 23 March 2017)]. A lot of cell death-related signal transduction pathways are activated by mitochondria. Mitochondria have the ability to control the activation of programmed cell death through regulating the translocation of pro-apoptotic proteins from the mitochondrial intermediate space to the cytosol. Apoptosis is triggered by a series of mitochondrial events, including the collapse of intimal potential, the swelling of mitochondria, and the opening of permeability transition pore [23]. Besides, mitochondrial dysfunction is associated with many characteristics of cancer cells such as enhanced anabolism, limitless proliferative potential, insensitivity to anti-growth signals, impaired apoptosis and decreased autophagy [24,25]. Since the local blood vessels do not provide enough oxygen, the microenvironment of rapidly proliferating cancer cells becomes hypoxia [26]. Once mitochondria fail to supply adequate adenosine triphosphate (ATP) for cell survival under this hypoxic condition, the tumor cells up-regulate the glycolysis

pathway to increase energy production by inducing the hypoxia-inducible factor-1 (HIF-1) [27]. In the malignant cells, down-regulation of the aerobic respiration pathway leads to impairment of normal cellular functions. Tricarboxylic acid cycle-related substrates like succinate accumulate as a result of the activation of HIF-1α, which leads to the inhibition or mutation of succinate dehydrogenase and the production of signal stimulation to glycolysis. Cancer cells would succeed in avoiding hypoxia-mediated cell death through low-expressed or mutated p53 [28], which is a cancer suppressor protein regulated cellular stress response. Mutations of p53 in tumor cells lead to down-regulation of mitochondrial respiration and change the main cellular energy metabolism to glycolysis. ROS is another important factor in mitochondrial dysfunction. Dysfunctional mitochondrial oxidation of the respiratory chain to produce excess ROS leads to oxidative stress that makes the imbalance of the oxidation and anti-oxidation state of the cells *in vivo*, leading to the peroxidation of protein and lipid. Furthermore, mutated mtDNA was found to be related to the oxidative stress as oxidative 8-hydroxyguanosine base is 10–20 folds higher in mtDNA than that in nuclear DNA in cancer [26]. Mitochondrial-mediated intrinsic apoptosis in cancer cells is also inhibited due to the overexpression of anti-apoptotic proteins, which protect mitochondrial membrane from permeabilization, which is a key step in activating cell death through the apoptotic pathway.

2.2. *Diabetes*

Diabetes directly killed 1.6 million people worldwide in 2015, up from less than 1 million in 2000, making it the 6th leading cause of global deaths in 2015 from WHO data [http://www.who.int/mediacentre/factsheets/fs310/en/ (accessed on 23 March 2017)]. Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin (type 1 diabetes) or when the body cannot effectively use the insulin produced (type 2 diabetes), the latter comprises the majority of people with diabetes around the world [http://www.who.int/mediacentre/factsheets/fs312/en/ (accessed on 23 March 2017)]. Recent studies suggest that mitochondrial dysfunction is the cause of type 2 diabetes, which increases intracellular glucose concentration and reduces insulin production [29]. Briefly, lipid oxidation and increased acetyl coenzyme A (CoA) in the citrate of mitochondria inhibit some enzymes using glucose, such as phosphofructokinase
and pyruvate dehydrogenase. Thereby it reduces the consumption of glucose that leads to increased intracellular glucose levels and decreased production of insulin as well. Recent studies have shown that the patients who suffered from the type 2 diabetes have lower mitochondrial oxidase activity, smaller mitochondria and reduced biological energy compared to normal subjects, which directly related to low insulin-mediated glucose metabolism [30]. Decreased mitochondrial function is significantly associated with a decrease in electron transport activity (approximately 4 times) and a reduction in mitochondrial protein yield (approximately 2 times). Further, the reduction in electron transport activity is highly correlated with reduced insulin-induced glucose metabolism. Reduced mitochondrial content and function in type 2 diabetic patients can be attributed to a reduction in mtDNA content (approximately 1850 vs. 2514 copy number). Oxidative stress also plays an important role in the process of diabetes, and even hypotheses to consider that oxidative stress is the culprit of diabetes [31]. In order to analyze the mechanism of mitochondrial dysfunction in diabetes, a research team specifically knocked out the mouse mitochondrial transcription factor A (mtTFA) to prevent the expression of mitochondrial DNA. Seven weeks after birth, these mice severely lost mtDNA in the islet tissue with the mitochondrial deletion of complex IV and abnormal tubular cristae morphology, leading to deficient respiratory chain activity and eventually reduced blood insulin levels.

3. Mitochondrial targeting strategies

Since the mitochondria were recognized as an emerging pharmacological target, a number of strategies for targeting mitochondria have been provided for the effective therapies of mitochondrial dysfunction based diseases (Fig. 2). Many mitochondrial targeting strategies take advantage of the highly hydrophobic and strong negative membrane potential of IMM. For example, triphenylphosphonium ion (TPP) derivatives as the most widely studied mitochondrial targeting moieties utilize their strong lipophilic and delocalized cationic nature to target IMM. The delocalized cation provides the crucial effect for this action, which is suggested to reduce the free energy change involved in transitioning from an aqueous to a hydrophobic environment in response to the electrochemical gradient potential of IMM. The mitochondrial accumulation of the delocalized cation in the mitochondrial matrix is 1000 times higher than that in the cytosol based on the Nernst equation when $\Delta\Psi_m$ is ~180 mV. Similarly, dequalinium (DQA) with two delocalized cation centers in a single-chain bola-amphiphile molecule which self-assembled into liposome-like cationic vesicles also demonstrated mitochondria-targeting profiles (Fig. 3). Besides delocalized charge, the hydrophobic nature of mitochondria targeting inducer facilitated to penetrate through a phospholipid membrane as compared with hydrophilic moiety [32]. Actually, these hydrophobic delocalized protonophores act as mitochondria uncouplers that could increase proton leak across the IMM to induce toxic effects [33–35]. Murphy et al. revealed that TPP might lead to negative impact on mitochondrial damage at concentration above 10 μM due to proton leak and then mitochondrial membrane depolarization.

An alternative strategy for mitochondrial targeting uses a carrier of short peptide sequences (mitochondria penetrating peptides) with specific physicochemical properties. Due to structural similarity between mitochondria and bacteria, a series of gramicidin mimics were developed to serve as a template for mitochondrial targeting since gramicidin S (GS) shows the ability to disrupt the bacterial membrane. The hemi-GS sequence conjugated with the ROS scavenger offered preferable protective benefits against oxidative cellular damage. The structure activity based on the Monte Carlo simulations indicated that the optimized localization of scavenger moiety inside the polar region of mitochondrial membranes was essential to allow efficient effects.

Szeto–Schiller (SS) tetra-peptide represents the first of a class of new chemical entities that selectively target mitochondrial

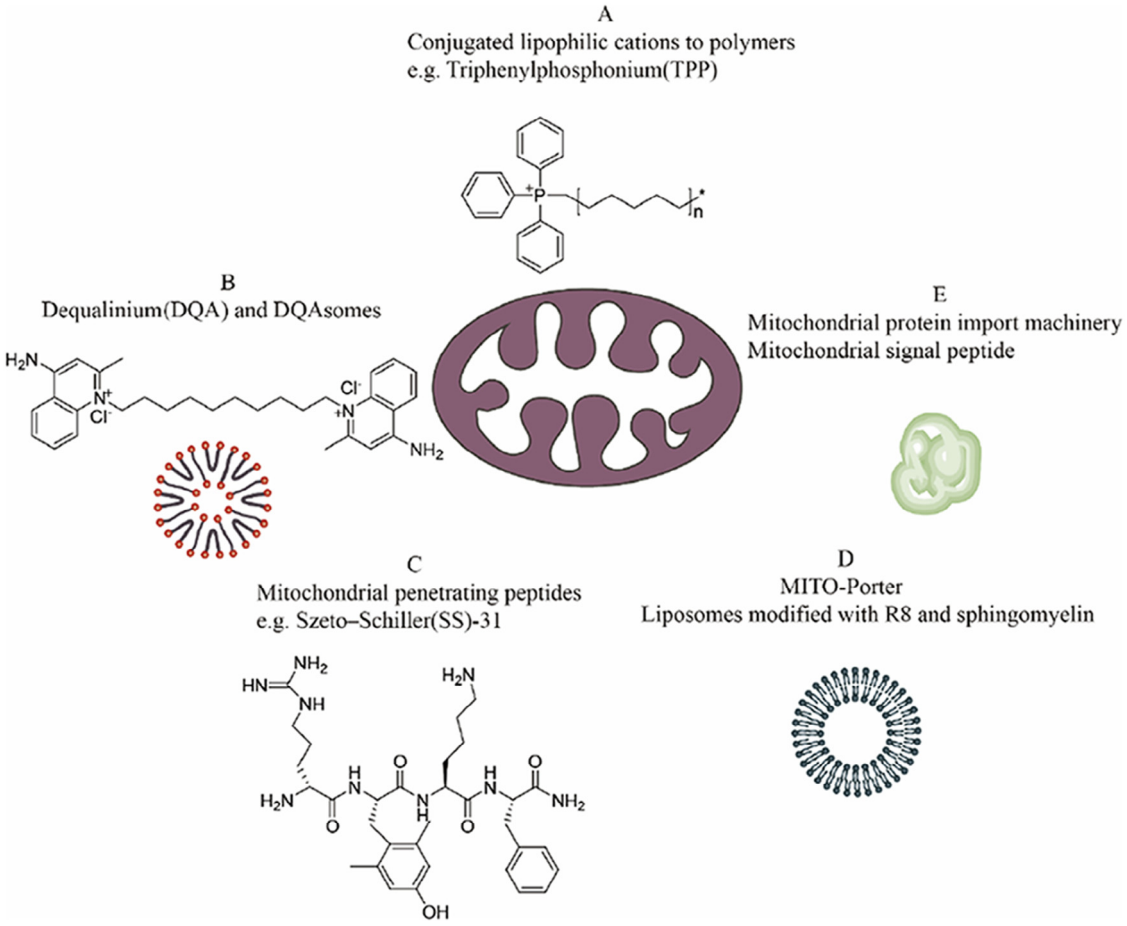

Fig. 2 – The five main mitochondria targeting strategies: lipophilic cations such as TPP (A), DQA and DQAsomes (B), mitochondrial penetrating peptides like SS-31 (C), a special liposomes named MITO-Porter (D), and mitochondrial protein import machinery (E).

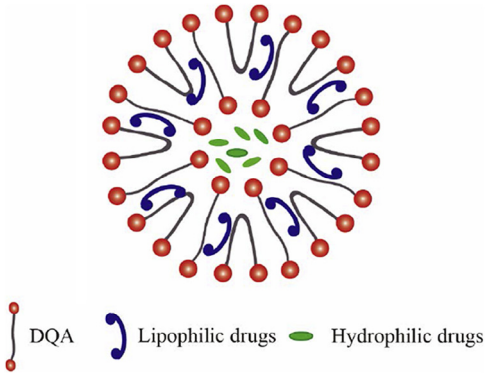

Fig. 3 – The schematic of DQAsomes. Lipophilic drugs are encapsulated between two hydrophobic chains while the hydrophilic drugs are wrapped in the core of DQAsomes.

cardiolipin to improve mitochondrial plasticity and restore optimal bioenergetics (Fig. 4). Positive charge and lipophilic character were found to be the 2 main properties used to design SS peptides, which are known to be important for the passage across both the plasma and mitochondrial membranes. More specifically, the alternating aromatic residues and basic amino acids is the key point to target the mitochondria. Finally, SS-31 (D-Arg-Dmt-Lys-Phe-NH_2) was found to be the best effective in scavenging ROS and inhibiting lipid oxidation. The free radical scavenging abilities of the peptide are likely to originate from the Dmt residue. On the contrary to TPP which causes toxicity at >10 μM, the uptake of these peptides is not self-limiting and they do not cause mitochondrial depolarization even at 1 mM. On account of the efficacy and safety, SS-31 have been on the way in clinical phase II trial that focused in ischemia-reperfusion injury and microvascular injuries in patients experiencing acute ST-segment elevation myocardial infarction. Another second phase II trial involved SS-31 is for the treatment of acute kidney injury and renal microvascular dysfunction in hypertension. In addition, it has been scheduled to potential treatment of heart failure and diabetic macular edema in clinic [16,36].

Another example of peptide or mimics for targeting IMM is cationic amphipathic α-helical D-$(KLAKLAK)_2$, which has been attempted to improve the potencies of anticancer peptides [37,38]. The alternative cationic and hydrophobic residues make the attractive point to design the similar MPPs. P11LRR is also such an arginine modified amphiphilic peptide that consists of pre-organized poly-proline scaffolds to allow helical structure. Li et al. suggested that the accumulation of P11LRR in

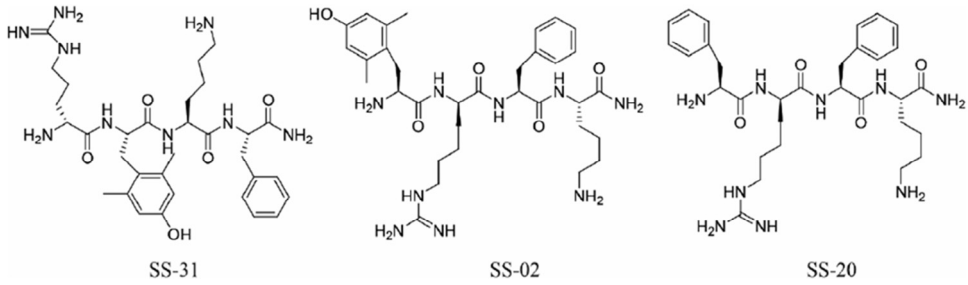

Fig. 4 – The chemical structure of Szeto–Schiller (SS) peptide.

mitochondria was driven by the mitochondrial transmembrane potential judging from the inhibition of the localization of the molecule after elimination of potential. In addition, amphipathic α-helical structure might have impacts on the mitochondrial targeting efficiency since it has been revealed to be crucial for the import of peptide sequence to mitochondria [39].

Recent work has shown that the new liposomes composed by 1,2-dioleoyl-sn-glycero-3-phosphatidyl ethanolamine(DOPE), sphingomyelin or phosphatidic acid named MITO-Porter whose surface-modified with octaarginine(R8) and pH sensitive membrane fusogenic peptide GALA could introduce cargos into mitochondria via a membrane fusion mechanism. A high density of R8 enables the system to be efficiently internalized by cells via micropinocytosis and further bind to mitochondria. Moreover, GALA permits the carrier to escape from the endosome into the cytosol. MITO-Porter system could effectively fuse with both the OMM and IMM by fusion lipid and released the encapsulated antisense RNA, which might also be imported into the mitochondrial matrix via import machinery [40,41].

The mitochondrial protein import machinery would facilitate the internalization of oligonucleotide, DNA or protein through the transporters in the membrane of mitochondria [42]. Typically, the mitochondrial signal peptide with an N-terminal specific amino acid sequence covalently conjugated with therapeutics is designed to improve the recognition of transporters for mitochondrial delivery [43,44].

4. Nanosystems

4.1. *DQAsomes*

DQAsomes are the earliest reported mitochondrial targeting vectors as gene delivery system (1998) [45]. DQAsome–DNA complexes (DQAplexes) take the advantages of simple preparation by mixing DNA with the requisite amount of DQAsomes [46]. The plasmid DNA ligated to the mitochondrial homing sequence is specifically targeted to mammalian cell mitochondria delivery by the DQAsomes [47].

One study by Weissin et al. demonstrated that the DQAsomes could selectively release DNA at the membranes of mitochondrial-like liposomes whereas not at membranes of cytoplasmic-like liposomes due to the higher electrochemical gradient across the mitochondrial-like liposomes [48]. In another *in vitro* investigation, it was also shown that DNA was selectively released from the DQAplexes after contact with the mitochondria harvested from mouse liver. D'Souza et al. further accessed the mechanism that allowed DQAplexes to escape from endosomes and mitochondrial targeting delivery. They found that the cationic DQAsomes exerted a destabilizing effect on endosomal membranes leading to the release of DQAsomes and then DNA was uptake by mitochondrial protein import machinery [46].

Interestingly, a recent study by Hegarty et al. demonstrated that *in vitro* antisense treatment was delivered by cationic DQAsomes to inhibit the growth of the gram-positive pathogen. By increasing its hydrophobicity via cyclohexyl modification, the DQAplexes could deliver plasmid DNA across bacterial membranes due to the similar cardiolipin-rich structure in bacterial membranes and IMM [49]. In addition to be used as effective gene carrier, DQAsomes can be also used to encapsulate anti-cancer drugs that involved paclitaxel and curcumin [50,51]. These studies concluded that the encapsulated paclitaxel or curcumin in DQAsomes enhanced the tumoricidal or antioxidant activity as compared to free paclitaxel or curcumin *in vitro* and *in vivo*.

4.2. *Liposomes*

Encouraged by success in DQAsomes, many liposomes systems have been developed for the mitochondrial targeting delivery. Guo et al. found TPP modified liposomes co-loaded with both chlorin e6 (Ce6, photosensitizer) and IR780 iodide (IR780, photothermal and near infrared imaging agent) showed higher toxicity to the HeLa cells and tumor vessels *in vitro* than non-targeted ones, leading to enhanced photodynamic therapy efficacy [52].

Boddapati et al. conjugated a stearyl residue to TPP and incorporated stearyltriphenylphosphonium (STPP) into lipid bilayers [53]. The authors revealed that ceramide loaded STPP-modified liposomes treated group showed significant decreased tumor volumes in BALB/c mice. Similarly, Patel et al. encapsulated sclareol in STPP-modified liposomes and improved the cytotoxic and apoptotic action of therapeutic molecules due to the stimulation of caspase-8 and caspase-9 activities in COLO205 cells [54]. To overcome the non-specific cytotoxicity of STPP-modified liposomes, Biswas et al. synthesized polyethylene glycol-phosphatidylethanolamine (PEG-PE) conjugated with TPP group and incorporated this moiety into the liposomal lipid bilayer to form TPP-PEG-PE-modified liposomes. The carrier exhibited low toxicity both in human cervical cancer cell line (HeLa) and in mouse mammary carcinoma cells (4T1). Fortunately, TPP-PEG-PE modified liposomes loaded with paclitaxel remained its ability to mitochondria-targeting and

demonstrated enhanced antitumor effects *in vitro* and *in vivo* as compared to non-targeted counterparts [55]. In order to overcome the instability and aggregation of liposome-based drug formulations when administrated in blood, the hybrid cerasomes (CER) based on Si–O–Si framework and liposomes were introduced by Katagiri in 1999 [56]. Cerasomes and TPP were conjugated by the linker (3-aminopropyl triethoxysilane, APS). TPP modified doxorubicin-loaded cerasomes (TPP–CER–DOX) were prepared via self-assembly process and then the formation of phospholipid bilayer vesicles to cover cerasomes. The prepared TPP–CER–DOX exhibited excellent biocompatibility, stability, sustained release ability and great drug accumulation in the mitochondria [57].

Besides TPP, another study done by Tuo et al. looked at a different targeting approach to mitochondria. They delivered PEGylated liposomes conjugated with 9-C_{16} berberine and folic acid into MCF-7/adr cell mouse xenografts' tumor. They found that 9-C_{16} berberine binded specifically to mitochondria owing to the delocalized cation and 16-carbon aliphatic alkylated chain of berberine derivative. The delivery systems exhibited greater cytotoxicity and apoptosis, enhanced 15-fold cellular uptake of doxorubicin, increased the drug distribution in tumor and stopped resistant MCF-7/adr cell mouse xenografts' tumor growth as compared to free doxorubicin and regular liposomal doxorubicin [58].

In order for mitochondrial delivery of genome targeting nucleic acid, Yamada et al. developed a new liposome named MITO-Porter which contained mitochondrial fusogenic lipid envelopes for targeting mitochondria via membrane fusion mechanism. Sphingomyelin or phosphatidic acid in the formulations facilitated mitochondrial membrane fusion and then the cargos were released to intra-mitochondrial compartment (Fig. 5). Normally, MITO-Porter was prepared by hydration method, which is conventional method for preparing liposomes. The authors clarified that the density of R8 was crucial for the intracellular trafficking of MITO-Porter: high-density R8-modified MITO-Porter was internalized by micropinocytosis but low density R8-modified MITO-Porter was taken up via clathrin-mediated endocytosis and then degraded by lysosomal enzymes [40,59]. Yasuzaki et al. later used MITO-Porter to encapsulate a fluorescent dye for staining nucleic acids (propidium iodide) that could detect mitochondrial genes and achieve the mtDNA visualization [60]. In another study, this group succeeded to develop a dual function MITO-Porter (DF-MITO-Porter) that could penetrate the endosomal and mitochondrial membranes via step-wise membrane fusion [61]. Yamada et al. compared the effective dose 50(ED_{50}) for two nanocarriers, and the results manifested that the conventional MITO-Porter (ED_{50} = 5.4 μg) was 15-fold less efficient than DF-MITO-Porter (ED_{50} = 0.33 μg) in mitochondrial delivery [62]. To reach more efficient mitochondrial delivery, they used mitochondrial targeting signal peptide (MTS, NH_2-MVSGSSGLAAARLLSRTFLLQQNGIRHGSYC) instead of R8 to form MTS-MITO-Porter. However, MTS-MITO-Porter was found to be liable to aggregate although it indeed provided more efficiently delivery to mitochondria than R8-MITO-Porter [63]. Therefore, Kawamura et al. shifted to use a stearyl modified S2 peptide (stearyl-Dmt-D-Arg-FK-Dmt-D-Arg-FK-NH_2, STR-S2) to decorate DF-MITO-Porter. It showed that DF-S2-MITO-Porter induced lower cytotoxicity than DF-R8-MITO-Porter despite co-localized with mitochondria

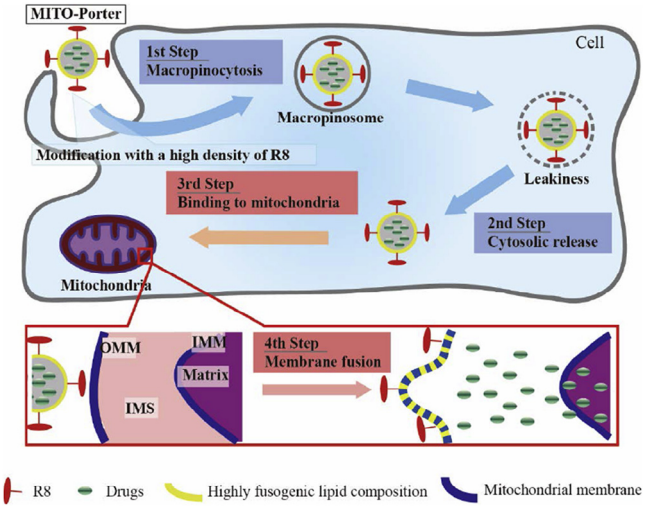

Fig. 5 – The mitochondrial targeting mechanism of MITO-Porter: cells internalized MITO-Porter through micropinocytosis, then it escaped from macropinosomes and bound to mitochondria via electrostatic interactions with R8. The membrane fusion was helped by sphingomyelin or phosphatidic acid which is fusogenic lipids with mitochondrial membrane and the cargos released to intra-mitochondrial compartment. Redrawn based on reference [33].

found in both treatments via the nanocarriers [64]. R8/GALA-modified MITO-Porter was a nanocarrier with surface-modified by both high density of R8 and pH-sensitive membrane fusogenic peptide (GALA). It has been successfully used for delivering the D-arm modified antisense RNA oligonucleotide to mitochondria, which led to knockdown of the mitochondrial mRNA and protein, hence down regulate the respiratory chain [65]. Besides nucleic acids, Yamada et al. recently succeeded in the delivery of coenzyme Q10 (CoQ10) to liver mitochondria in the hepatic ischemia/reperfusion injury mice via the systemic injection of a CoQ10-MITO-Porter [66].

4.3. *Polymer nanoparticles*

Biocompatible and biodegradable polymer such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone(PCL) are most promising drug delivery materials [21,22,67,68]. Through emulsification-solvent evaporation or nanoprecipitation, these polymer can be prepared into nanoparticles which are able to encapsulate both water-soluble and water-insoluble medicine via engineering hydrophilic block like FDA approved polyethylene glycol(PEG) to hydrophobic blocks [21,69–71]. Hydrophobic blocks generally increase the stability of the formulation, and PEG augments the residence time *in vivo* or usually used to conjugate the targeting moieties [6]. Sharma et al. used PEG and TPP modified PCL by ring-opening polymerization via click chemistry. PCL-PEG-TPP was used to be self-assembled into the micelles with the diameter of 38–60 nm and the loading efficiency of CoQ10 is 8.3–9.3% (see in Table 1). The high loaded micelles were proven to be accumulated in the mitochondria under the observation

Table 1 – Summary of mitochondria-targeting nanopreparations.

Vehicle	Induce by	Cargo	Size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Drug loading (%)	Ref.
DQAsomes	DQA	Curcumin	170–200	+50	90	61	[51]
DQAsomes	DQA	Antisense oligonucleotides	165–192	+50	/	/	[49]
Liposomes	Rh123	Paclitaxel	188	-16.23	/	92	[72]
Liposomes	STPP	Sclareol	105	+9.85	/	/	[54]
Liposomes	STPP	Ceramide	54	+30	/	/	[53]
Liposomes	Berberine	Doxorubicin	99	–18.40	96	/	[58]
Liposomes	TPP	Paclitaxel	163	+1.66	/	/	[55]
Liposomes	DQA	Topotecan	65	–0.52	96	/	[73]
Liposomes	DQA	Resveratrol	69	–10.14	97	/	[74]
Liposomes	MITO-Porter	Coenzyme Q10	82	+23	86.3	/	[66]
Liposomes	TPP	Paclitaxel	84	+1.93	86	/	[75]
Cerasomes	TPP	Doxorubicin	220	+15.37	3.34	53.4	[57]
Micelles	TPP	Coenzyme Q10	~40	/	83–93	8.3–9.3	[76]
PLGA NPs	TPP	Curcumin, 2,4-dinitrophenol, lonidamine, α-tocopheryl succinate	80–400	–30~+30	64–95	3–21	[69]
PLGA NPs	TPP	Zinc phthalocyanine	65–75	+24~+34	22–35	3–11	[70]
Composite NPs	TPP	Zinc phthalocyanine	80–150	+18~+45	60–87	3–4.5	[77]
Chitosan NPs	TPP	Doxorubicin	100	15.6	91.3	13.2	[78]
mPEG NPs	TPP	Doxorubicin	161	/	76	4.5	[79]
Mesoporous Silica NPs	TPP	α-tocopheryl succinate	115	+5~+20	7.3%	3.65%	[80]

of confocal microscopy method and they eventually significantly reduced the level of reactive oxygen species and inflammation [76]. Dhar's group blended TPP modified PLGA-PEG(PLGA-b-PEG-TPP) and PLGA-b-PEG-OH or PLGA-COOH to optimize the size, zeta potential and stability of nanoparticles. They revealed that the nanoparticles with the diameter of less than 100 nm and positive zeta potential greater than 22 mV could be efficiently uptaken by mitochondria. The delocalized positive charge and large steric hindrance from triphenyl groups allow the nanoparticles less aggregation and protein adsorption. To demonstrate the wide application of their engineered nanocarriers, they used four drugs as payloads: curcumin (an inhibitor of amyloid-β protein for Alzheimer's disease), 2,4-dinitrophenol (a mitochondrial decoupler as antiobesity drug), lonidamine (mitochondrial glycolysis inhibitor) and α-tocopheryl succinate (a tumor-selective drug for cancer) respectively [81–85]. The results indicated that the targeted NPs improved therapeutic window of 2,4-dinitrophenol, decreased the cytotoxicity induced by amyloid-β protein (loading curcumin) and reduced the IC_{50} values in HeLa cells (loading lonidamine and α-tocopheryl succinate) [69]. Yue et al. also used PEGylated TPP to conjugate thioketal linker-modified camptothecin (TL-CPT) to form TL-CPT-PEG_{1K}-TPP, which is blended with DSPE-PEG-NH_2 to prepare chemo- and photodynamic dual functional NPs through loading photosensitizer zinc phthalocyanine (ZnPc). When irradiated with 633 nm laser to TL-CPT-PEG_{1K}-TPP, the ZnPc loaded NPs produce ROS, which induced the rupture of the thioketal linker and then camptothecin can be released. As a result of it, the anticancer efficacy against lung cancer testing demonstrated that TL-CPT-PEG_{1K}-TPP led to develop a mitochondria-targeting ROS-activated chemo- and photodynamic therapy with a single light source for lung cancer and exhibited approximately 6 times malignant cells killing ability than free ZnPc and camptothecin in NCI-H460 cells [77]. Hou et al. developed TPP functionalized chitosan NPs loaded with doxorubicin and the nanoparticles were capable to improve antitumor efficiency in HeLa and A549 cells [78].

To address the limitation of toxicity associated with TPP, Khatun et al. synthesized polyethylene glycol (mPEG)-TPP via the linker of disulfide bond (mPEG-(ss-TPP)$_2$) and the amphiphilic polymer self-assembled in aqueous media to form a hydrophilic PEG shell and a hydrophobic TPP core. After DOX-loaded NPs endocytosed into cells, the disulfide bonds between mPEG and TPP would be cleaved due to the presence of intracellular glutathione, thereby removed the mPEG shell, exposing TPP to the outside, and driven the drug target to mitochondria. The results demonstrated that mPEG-(ss-TPP)$_2$ NPs encapsulated DOX had an improved mitochondria-targeting efficiency and better therapeutic effect than nonbioreducible NPs since the delocalized cationic TPP became the shell of bioreducible NPs increasing lipophilicity [79].

4.4. *Inorganic nanoparticles*

Inorganic nanoparticles have advantages in smaller and more uniform particle size over organic NPs. Correspondingly, a lot of inorganic materials have also been used to prepare mitochondrial targeting NPs. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) is the main inorganic constituent of the hard tissue of humans, which exhibited excellent biocompatibility and drug-loading capacity. More importantly, HAP NPs may enter the mitochondria of tumor cells as well and then induce apoptosis via changing mitochondrial membrane potential, leading the leakage of cytochrome C [86–88]. Sun et al. synthesized rod-shaped HAP NPs (~10 nm in width and ~50 nm in length) which was an intracellular uptake via caveolae-mediated endocytosis in both lung cancer cells (A549) and normal bronchial epithelial cells (16HBE). But curiously more NPs were taken up into A549 cells and caused sustained rise of intracellular calcium concentration in contrast to transitory increase in 16HBE cells. Increased cellular uptake and mitochondria targeting combined with the prolonged elevation of intracellular calcium concentration resulted in approximately 40% tumor growth inhibition in a nude mouse lung cancer model even if without

loading drugs [89]. To further improve the anti-tumor efficiency, Xiong et al. designed DOX-loaded HAP NPs and coated hyaluronic acid (HA) shell outside that could both target CD44-overexpressing tumor cells and overcome burst drug release. The DOX/HAP-HA exhibited nearly 4 and 7-fold higher cytochrome c release than free DOX and the control group respectively on account of its mitochondria-targeting profiles [90].

Similar to the organic NPs, there are many groups that focus on TPP modified inorganic NPs to target mitochondria. For example, Kwon et al. prepared DSPE-PEG-TPP-coated ceria nanoparticles with the diameter of 22 nm and zeta potential of +45 mV. The ceria NPs could reversibly bind oxygen atoms and shuttle between the Ce^{3+} (reduced) and Ce^{4+} (oxidized) states on their surface, in that way that showed great ROS-scavenging ability in cells especially in mitochondria. The TPP-ceria NPs showed nearly 4-fold higher targeting mitochondria than ceria NPs through calculating co-localization coefficient between mitochondria and NPs. TPP-ceria NPs showed suppression of the pathogenesis of Alzheimer's disease in the 5XFAD mouse model *in vivo* with enhanced ability of reduction of Aβ-induced neuronal cell loss than non-targeted one [91]. In another example, Qu et al. used TPP modified mesoporous silica NPs loaded α-tocopheryl succinate (α-TOS) to achieve mitochondrial delivery. The targeted NPs' overlapping coefficient index of confocal images was 20% higher than non-targeted counterpart between the NPs and mitochondria. The anticancer efficacy of α-TOS can be significantly enhanced by mitochondria targeted NPs via activating higher activity of caspase 9 and caspase 3 thereby inducing apoptosis, what is more, α-TOS loaded mitochondria-targeting NPs showed no obvious cytotoxicity to normal cells [80].

Besides, several studies demonstrated that internalization of AuNPs could accumulate in the mitochondria to induce apoptosis [92–94]. Mkandawire et al. conjugated AuNPs with a green fluorescent protein (GFP) harboring the mitochondrial localization sequence of the inner membrane protein COX8 at its amino terminus (mitoTGFP). To surmount the aggregate of AuNPs, this group applied cationic maltotriose-modified poly(propylene imine) dendrimers to coat with mitoTGFP-AuNPs. The designed mitoTGFP-AuNPs, however, required a transfection reagent like cationic glycodendrimer PPI-Mal-III G3 to transverse the cellular membrane. Afterwards, the NPs successfully escaped from early endosomes and then they could rupture the OMM, finally localized in the IMM as observed by transmission electron microscope. As a result of it, cytochrome *c* was released to cytosol to trigger the apoptosis [95]. In another example, Ma et al. coated multilayered polypeptide to surround the AuNPs: the first layer is CALNN-based peptide (CP: biotin-NNLACCALNN-COOH) to avoid AuNPs aggregation. The second layer is tetrameric streptavidin which is the linker to connect biotinylated biomolecules via its multimeric binding sites; the outside layer is biotinylated peptide (KLA: $(KLAKLAK)_2$) that is both mitochondriotropic moiety and cytotoxic peptide to kill cancer cells. The KLA-anchored AuNPs showed dramatically enhanced anticancer activity with thousands of times stronger than that of the free KLA peptide, which is attributed to its improved cell-entry efficiency, mitochondria-specific delivery and the polyvalent effect of the nanoassembly [96].

5. Conclusion and future direction

Efficient targeted mitochondria delivery is the key for successful treatment of many human diseases such as cancer, metabolic diseases and neurodegenerative disease. To date, there is no mitochondria-targeting pharmaceutical formulation on the market, mainly owing to the lack of an efficient delivery system that can both stabilize therapeutic biomaterials and target them to specific accumulation within the mitochondria.

Nanotechnology offers a promising way for efficient mitochondrial delivery. Nano-formulation systems such as liposomes, polymeric NPs and inorganic NPs have been shown to carry a variety of drugs to mitochondria *in vitro* models, although with more limited success in *in vivo* animal models. As discussed above, the NPs allow mitochondrial delivery on account of the strategies that include delocalized lipophilic cations, mitochondria-penetrating peptides and mitochondrial protein import machinery. The nanoparticles however produced a positive zeta-potential on the surface, leading to non-specific interaction with blood components and then rapid clearance from the blood circulation. One possible way to resolve the issue is to develop a charge reversal delivery system, as the system provides better stabilization *in vivo*. There is still much to learn about nanoparticles beyond their use as effective targeting agents. Recent studies of mitochondrial homing moieties such as TPP revealed the potential toxicities. Future researches may focus on safer mitochondria-specific nanopreparations and explain the mechanism of mitochondria-targeting toxicity clearly.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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