Mitochondria-targeting particles

Mitochondria are a promising therapeutic target for the detection, prevention and treatment of various human diseases such as cancer, neurodegenerative diseases, ischemia-reperfusion injury, diabetes and obesity. To reach mitochondria, therapeutic molecules need to not only gain access to specific organs, but also to overcome multiple barriers such as the cell membrane and the outer and inner mitochondrial membranes. Cellular and mitochondrial barriers can be potentially overcome through the design of mitochondriotropic particulate carriers capable of transporting drug molecules selectively to mitochondria. These particulate carriers or vectors can be made from lipids (liposomes), biodegradable polymers, or metals, protecting the drug cargo from rapid elimination and degradation *in vivo*. Many formulations can be tailored to target mitochondria by the incorporation of mitochondriotropic agents onto the surface and can be manufactured to desired sizes and molecular charge. Here, we summarize recently reported strategies for delivering therapeutic molecules to mitochondria using various particle-based formulations.

Keywords: intracellular targeting • mitochondria • mitochondria targeting • nanotechnology • organelle specific • particles

Targeted drug delivery systems are designed to transport the drug specifically to target organs, tissues, cells or organelles at therapeutic concentrations. Specifically targeting drugs, as opposed to nontargeted systemic delivery, potentially provides a number of advantages such as improved efficacy and increased therapeutic threshold [1]. Targeted drug delivery systems not only facilitate drug delivery to the desired site of action, but often protect the active ingredient from degradation [2]. Currently, mitochondria have become a promising therapeutic target for the prevention and treatment of various illnesses such as cancer, neurodegenerative diseases, ischemia-reperfusion injury, heart failure, diabetes and obesity [3–6]. The purpose of this review is to summarize recently reported strategies for delivering therapeutic molecules to mitochondria using various particle-based formulations.

Mitochondrial dysfunction

Mitochondria are membrane-enclosed organelles found in the cytoplasm of eukaryotic cells and their crucial function is to generate energy in the form of ATP through oxidative phosphorylation via the electron transport chain. Mitochondrial dysfunction caused either by genetic mutations of mitochondrial proteins or oxidative stress can lead to a wide range of debilitating or life-threatening diseases such as Kearns–Sayre syndrome, Leber’s hereditary optic neuropathy, Alzheimer’s disease, Parkinson’s disease and diabetes [7]. Oxidative stress, or an overproduction of reactive oxygen species (ROS), can have a number of etiologies that include ischemia, hyperglycemia, ionizing radiation and anticancer drugs. Mechanistically, during ischemia-reperfusion injury, a lack of oxygen (caused during the ischemic phase) initially causes an inhibition of oxi-

Amaraporn Wongrakpanich¹,
Sean M Geary¹,
Mei-ling A Joiner²,
Mark E Anderson^{2,3}
& Aliasger K Salem^{*,1}

¹Department of Pharmaceutical Sciences
& Experimental Therapeutics, College of
Pharmacy, University of Iowa, Iowa City,
IA 52242, USA

²Department of Internal Medicine
& François M Abboud Cardiovascular
Research Center, Carver College of
Medicine, University of Iowa, Iowa City,
IA 52242, USA

³Department of Medicine, Johns
Hopkins School of Medicine, Baltimore,
MD 21205, USA

*Author for correspondence:
aliasger-salem@uiowa.edu

dative phosphorylation, cytoplasmic acidification and Ca^{2+} overload while mitochondrial Ca^{2+} levels remain low owing to a collapse of the inner mitochondrial membrane potential ($\Delta\Psi_m$), which reduces the electric driving force for mitochondrial Ca^{2+} entry. However, at reperfusion, oxidative phosphorylation and $\Delta\Psi_m$ are restored, which consequently leads to ROS and Ca^{2+} overload within the mitochondria and, combined with the restoration of a neutral cytoplasmic pH, there is an opening of the mitochondrial permeability transition pore, which triggers cell death through necrosis and/or apoptosis [8–12].

Sub-micron particles

Potential of mitochondrial-targeting particles as drug delivery systems

At present, there are a number of agents that possess the potential to ameliorate mitochondrial dysfunction that have undergone assessment in preclinical and clinical studies (Table 1) [13–16]. Since mitochondria are one of the primary sources of ROS, therapies that involve the delivery of antioxidants, such as vitamin C, vitamin E, creatine or coenzyme Q [14,17], to mitochondria could potentially counteract ROS overproduction, thereby providing benefits to patients with neurodegenerative diseases. Other than substances that can abate mitochondrial dysfunction and therefore improve cell viability, there are molecules that can act on mitochondrial function so as to promote cell death, which is desired in the case of anticancer drugs [18–20]. Examples of anticancer drugs that negatively affect mitochondrial function and cause apoptotic cell death are lonidamine, an inhibitor of aerobic glycolysis, and cisplatin, a platinum-containing drug that can promote mitochondria-dependent ROS production [19,21]. These agents, whether designed to rescue the cell or promote cell death, may prove therapeutically beneficial if targeted to mitochondria in patients with diseases caused by mitochondrial dysfunction or cancer, respectively.

In recent years, the field of intracellular drug delivery has been growing rapidly with the primary goal of increasing the therapeutic index of a certain drug moiety by specifically targeting it to relevant organelles such as lysosomes, endoplasmic reticula, mitochondria and Golgi complexes [22]. Even before considering organ-specific targeting, the transporting of drugs to mitochondria is extremely challenging in itself owing to the multiple cellular structures through which the drug must pass, such as the plasma membrane, the cytoplasm, and the outer and inner mitochondrial membranes. Some drug moieties are intrinsically capable of overcoming these barriers to access the mitochondria, while other drugs require conjuga-

tion to mitochondria-targeting molecules. Examples of the former are the small water soluble Szeto Schiller (SS) peptides that were designed to target and accumulate at the inner membrane of mitochondria. These peptides readily cross cell membranes in an energy-independent manner and target mitochondria using a sequence motif. In addition, the SS peptides themselves protect mitochondria from oxidative damage. Recently, it has been reported that one of these SS peptides, SS-31 (D-Arg-Lys-Phe-NH₂), also known as Bendavia™ (Stealth Peptides Inc., MA, USA), binds to cardiolipin at the mitochondrial inner membrane and protects mitochondria cristae during ischemia [23]. Currently, intravenously administered SS-31 is in clinical trials to assess its capacity to reduce reperfusion injury in patients with acute coronary events [24]. Many drug moieties, however, need to be conjugated to mitochondria-targeting molecules and MitoQ® (MitoQ Ltd, Auckland, New Zealand) is an example of such a modified molecule. MitoQ comprises coenzyme Q, an antioxidant, coupled to a triphenylphosphonium (TPP) cation, a widely used lipophilic cation that is taken up by and enriched in mitochondria [25,26]. Because of its mitochondrial targeting and antioxidant properties, MitoQ has the potential to be used as a protective agent in various mitochondria-related maladies such as ischemia-reperfusion injury, Parkinson's disease, Alzheimer's disease and multiple sclerosis [27,28]. *In vivo* studies showed that MitoQ can be administered safely long term to mice [29]. MitoQ, as well as alkyl-TPP *per se*, can be taken up and accumulate in the heart, brain, skeletal muscle, liver and kidney [26]. In a clinical Phase I trial, MitoQ, delivered orally, showed good pharmacokinetic behavior with 10% oral bioavailability [29]. MitoQ is currently in clinical trials for Parkinson's disease and chronic hepatitis C [27,29].

It is well known that doxorubicin (Dox) acts as an anticancer drug by inhibiting DNA topoisomerase II in both nuclei and mitochondria. Targeting Dox to mitochondria has been shown by Chamberlain *et al.* [30] to overcome drug resistance because most efflux pumps, such as Pgp, cannot access Dox in mitochondria. In the same study, mitochondria-targeting Dox was synthesized by coupling Dox with a succinic anhydride conjugated to the N-terminus of a mitochondria-penetrating peptide. *In vitro* studies showed that mitochondria-targeting Dox possessed increased toxicity over standard Dox in a drug-resistant human ovarian cancer cell line (A2780ADR) [30]. Should the drug molecule of interest be incapable of being conjugated to a mitochondrial-targeting molecule, then targeting particle-based carriers are required to facilitate transportation of the drug across the mitochondrial membrane. Several attempts have been made to create

Table 1. Summary of mitochondria-targeting particles (cont.).

Name	Materials/formulation	Cargo	Most advanced preclinical/clinical research progress
Biodegradable polymeric particles			
Mitochondria-targeted blended NPs	PLGA- <i>b</i> -PEG-TPP [†] blended with PLGA- <i>b</i> -PEG-OH or PLGA-COOH	Lonidamine, α -tocopheryl succinate, curcumin and 2,4-dinitrophenol [64]	<i>In vitro</i> (human)
Apoptosis-targeted HDL-mimicking NPs (TPP-HDL-apoA-1-QD NPs)	Core: PLGA, cholesteryl oleate, PLGA- <i>b</i> -PEG-QD; shell: DSPE-PEG-COOH, stearyl-TPP [†]	Contrast agent (QD in particle's core) and apoA-1 mimetic 4F peptide (on the shell) [71]	<i>In vitro</i> (human)
Mitochondria-targeting ZnPC NPs (T-ZnPC-NPs)	PLGA- <i>b</i> -PEG-TPP [†]	Zinc(II) phthalocyanine [72]	<i>In vitro</i> (human)
Metal particles			
Multilayered polypeptide-AuNP assembly (Au@CP/SA)	Core: Au NPs; shell: biotinylated CALNN-based peptide, tetrameric streptavidin and biotinylated bioactive molecules	KLA cytotoxic peptide (KLAKLAK) ₂ [76]	<i>In vitro</i> (human)
AuNP-Simdax® (Orion Corporation, Espoo, Finland) conjugate	Au NPs	Simdax (levosimendan) [79]	<i>In vivo</i>
TiO ₂ -DNA	TiO ₂ NPs	Oligo nucleotides [80]	<i>In vitro</i> (human)
CO NPs	CO NPs	Not applicable [81]	<i>In vitro</i> (human)
MoO ₃	MoO ₃ with dopamine-TPP [†] ligand	Not applicable [83]	<i>In vitro</i> (human)
[†] Substance used as a mitochondrial targeting or mitochondrial fusing agent. All <i>in vivo</i> experiments were conducted using a murine model. CALNN: Cys-Ala-Leu-Asn-Asn; CHEMS: Cholesteryl hemisuccinate (5-cholesten-3-ol 3-hemisuccinate); Chol: Cholesterol; DC-chol HCl: 3 β -[N-(N', N'-dimethylaminoethane)-carbomoyl] cholesterol hydrochloride; CP: CALNN-based peptide; DF: Dual function; DQA: Dequalinium; DOPC: Dioleoylphosphatidyl choline; DOPE: 1,2-Dioleoyl-sn-glycero-3-phosphatidyl ethanolamine; DPPG: Dipalmitoylphosphatidylglycerol; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine; EPC: Egg phosphatidylcholine; GALA: WEAALAEALAEHLAEALAEALAEALAA-NH ₂ ; GFP: Green fluorescent protein; HDL: High-density lipoprotein; KLA: Lys-Leu-Ala; MITO: Mitochondrial; NP: Nanoparticle; PE: Phosphatidylethanolamine; PLGA: Poly(lactic-co-glycolic acid); QD: Quantum dot; SA: Streptavidin; STPP: Stearyl-triphenylphosphonium; STR-S2: Stearyl-Dmt-D-Arg-FK-Dmt-D-Arg-FK-NH ₂ , STR-R8: Stearyl octaarginine; T-ZnPC-NP: Mitochondria-targeting zinc (II) phthalocyanine nanoparticle; TPGS ₁₀₀₀ : D- α -tocopheryl polyethylene glycol 1000 succinate (vitamin E polyethylene glycol succinate); TPP: Triphenylphosphonium cation; ZnPC: Zinc (II) phthalocyanine.			

submicron particle-based drug delivery systems that can penetrate cell barriers and deliver their cargo to mitochondria. Particulate-based drug delivery systems offer the flexibility to entrap a wide variety of drugs, provide protection from enzyme degradation and can be surface modified with mitochondrial-targeting moieties.

Liposomes & liposome-like vesicles

Liposomes are self-assembling colloidal structures composed primarily of phospholipids and cholesterol [31]. Liposomes have been extensively evaluated as particulate drug delivery systems for mitochondrial targeting in preclinical studies because many of the properties of liposomes, including their clearance rates subsequent to systemic injection, have undergone extensive analysis in preclinical and clinical settings [32,33]. Globally, there are approximately 12 liposome-based drugs currently on the market [32]. These include intramuscular vaccinations (for hepatitis A and influenza) and intravenously administered formulations carrying chemotherapeutic drugs such as Dox. Most liposomal formulations still in clinical trials involve systemic delivery of chemotherapeutic drugs to cancer patients. Development and therapeutic assessment of mitochondria-targeting liposomes is currently being investigated in preclinical studies.

From a formulation perspective, liposomes can be readily surface modified, and are biocompatible, biodegradable and generally nontoxic. Liposomes can entrap hydrophilic drugs inside their core and hydrophobic drugs within their lipid bilayers. A current strategy for targeting liposomes to mitochondria is to incorporate mitochondria-targeting molecules into lipid bilayers. Studies presented below have demonstrated that mitochondria-targeting liposomes are capable of delivering therapeutic moieties to mitochondria, thereby enhancing drug efficacy in both *in vitro* and *in vivo* models.

An early mitochondria-specific particulate drug delivery system was reported by Weissig *et al.* in 1998 [34]. To facilitate gene delivery, this group incorporated pDNA into liposome-like cationic vesicles, called DQAsomes, made from dequalinium chloride, a mitochondriotropic quaternary ammonium cation. DQAsomes containing *pGL3 firefly luciferase* demonstrated transfection efficiencies comparable to those of Lipofectin® (Life Technologies, NY, USA) reagent [34]. To gain insight into the mechanism of intracellular pDNA release by these DQAsome complexes, their behavior was studied using liposomes mimicking the inner and outer mitochondrial membranes, as well as the cytoplasmic membrane. DQAsomes were shown to selectively release DNA at the inner and

outer membranes of mitochondrial-like liposomes, but not at membranes of cytoplasmic-like liposomes [35]. DQAsomes were also found to release DNA when the complexes were in contact with isolated mitochondria from mouse liver [36]. Using human breast carcinoma (BT20) cells, D'Souza *et al.* showed that DQAsomes could escape from endosomes and release DNA inside mitochondria [37]. Aside from being effective as gene carriers, DQAsomes can be used to encapsulate low-molecular-weight compounds such as paclitaxel. It was reported that, in addition to being a mitotic inhibitor, paclitaxel directly targets mitochondria upstream of caspase activation [38]. Paclitaxel was encapsulated into DQAsomes and tested for its proapoptotic activity in human colon adenocarcinoma cells (COLO205) using a nuclear morphology assay. When compared with free paclitaxel, DQAsome-encapsulated paclitaxel induced a higher percentage of apoptotic nuclei, thereby possessing a higher tumoricidal activity [39].

In an attempt to overcome intrinsic multidrug resistant (MDR) phenotypes in leukemia, Lu's group incorporated dequalinium into the lipid bilayers of liposomes (~100 nm in diameter) coloaded with the chemotherapeutic drug, epirubicin, and the calcium channel blocker, amlodipine [40]. These liposomes were shown to localize to mitochondria and were significantly more cytotoxic (compared with nontargeting formulations and soluble drugs) when incubated *in vitro* with a human chronic myelogenous leukemia cell line, K562, and the MDR subline, MDR K562/adriamycin resistant (ADR), for 24 h. These liposomes also exhibited increased antitumor activity over nontargeting liposomes when used to treat nude mice that had been previously challenged with the MDR K562/ADR cells [40]. It was proposed that the enhanced antitumor efficacy may have been due to a number of combined factors including that some of the epirubicin being released from the ruptured liposomes within the cytoplasm was free to intercalate with the nuclear DNA and the epirubicin that reached the mitochondria via the intact liposomes acted in concert with amlodipine to promote apoptosis. In a separate study, Lu's group formulated dequalinium-containing liposomes coloaded with the chemotherapeutic drug, daunorubicin, and quinacrine (a PLA2 inhibitor), with the aim of eradicating residual breast cancer stem cells, which often possess intrinsic drug resistance and are therefore resistant to standard chemotherapies such as daunorubicin alone [41]. Both drugs were incorporated with a high encapsulation efficiency (>80%) into liposomes (98 nm diameter, -1.38 mV charge) and were used to treat human breast cancer stem cells, which were derived from the breast cancer cell line, MCF-7, by selective culturing. These liposomes were ini-

tially shown, *in vitro*, to localize to mitochondria of the MCF-7 stem cells and were more cytotoxic than nontargeting liposomes or soluble daunorubicin and quinacrine. Treatment of MCF-7 stem cell-challenged female nonobese diabetic/severe combined immunodeficiency mice with daunorubicin plus quinacrine-loaded mitochondrial-targeting liposomes resulted in enhanced tumor regressions compared with nontargeting daunorubicin plus quinacrine-loaded liposomes. Mechanistically, it was demonstrated that the enhanced accumulation of daunorubicin and quinacrine within mitochondria resulted in apoptosis of cancer stem cells through the activation of the proapoptotic protein, Bax, resulting in the opening of the mitochondrial permeability transition pore, a decrease in $\Delta\Psi_m$, followed by cytochrome c-dependent caspase (-9 and -3) activation. This treatment formulation may therefore be effective at reducing relapses in cancer patients often caused by residual cancer stem cells. In another study, Lu's group, in an effort to overcome intrinsic and acquired multidrug resistance in breast cancer, used mitochondria-targeting liposomes (containing dequalinium) incorporated with D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS₁₀₀₀) and loaded with the chemotherapeutic agent, topotecan hydrochloride [42]. TPGS₁₀₀₀ can inhibit drug efflux pumps (known as ATP-binding cassette transporters) that contribute to multidrug resistance [43]. The resulting liposome compositions (65 nm) demonstrated antitumor effects when administered intravenously to female BALB/c nude mice challenged with MDR breast cancer (MCF-7/ADR) xenografts. They also significantly reduced the number of metastases of murine B16 melanoma colonies in the lungs of female BALB/c nude mice when compared with nontargeting drug-loaded liposomes.

In an attempt to enhance the efficacy of their liposomal formulations, Lu's group directly conjugated dequalinium with PEG₂₀₀₀-DSPE (DQA-PEG₂₀₀₀-DSPE) using an acylation reaction. The conjugation between dequalinium and PEG₂₀₀₀-DSPE allowed dequalinium to present at the surface of the liposomes without being sterically hindered by PEG₂₀₀₀. DQA-PEG₂₀₀₀-DSPE was used in two separate studies involving mitochondria-targeting liposomes carrying either resveratrol [44] or lonidamine [45] in an effort to overcome multidrug resistance in a lung cancer model. Resveratrol, a polyphenol found in many plant species, has anticancer effects on various cancer cells *in vitro* and *in vivo* [46]. Mitochondria-targeting resveratrol-loaded liposomes (70 nm) caused a significant reduction in tumor volumes in female nude mice challenged with MDR lung cancer A549/cisplatin (cDDP) [47] xenografts when compared with nontargeting resvera-

tol-loaded liposomes [44]. Lonidamine, an antitumor agent that has been used in clinical trials for a variety of cancers, can preferentially induce apoptosis in cancer cells by reducing cellular ATP. In this study, Lu's group prepared targeting liposomes loaded with either lonidamine or epirubicin [45]. The targeting liposomes contained DQA-PEG₂₀₀₀-DSPE for mitochondrial targeting and TPGS₁₀₀₀ for drug efflux inhibition. When lonidamine-loaded liposomes were combined with mitochondria-targeting epirubicin-loaded liposomes, the highest cytotoxic effect on A549/cDDP cells *in vitro* was observed. This combination also showed significant antitumor activity on A549/cDDP xenografts in nude mice when compared with nontargeting lonidamine or nontargeting epirubicin liposomes.

Boddapati *et al.* prepared mitochondria-targeting liposomes loaded with the proapoptotic lipid molecule, ceramide [48,49]. To facilitate the incorporation of TPP into the lipid bilayer of liposomes, they conjugated the TPP molecules to a nonpolar stearyl moiety to create stearyltriphenyl phosphonium (STPP). It was then demonstrated that fluorescently tagged STPP liposomes (54 nm diameter) could localize to mitochondria of mouse 4T1 mammary carcinoma cells after a 2-h incubation period *in vitro*. When these STPP liposomes loaded with ceramide were injected intravenously into 4T1 tumor-challenged mice, there was a significant reduction in tumor volumes [49]. In a recent *in vitro* study, STPP liposomes loaded with paclitaxel showed enhanced cytotoxicity toward a paclitaxel-resistant ovarian carcinoma cell line, Ovarcar-3, when compared with nontargeting paclitaxel-loaded liposomes [50]. In a separate study, Biswas *et al.* conjugated TPP with polyethylene glycol-phosphatidylethanolamine (TPP-PEG-PE) and incorporated this into liposomes instead of STPP to reduce the unwanted cytotoxicity caused by STPP. *In vitro* (24 h) cytotoxicity studies using a cervical cancer cell line (HeLa) demonstrated that blank mitochondria-targeting TPP-PEG-PE liposomes (i.e., carrying no cytotoxic payload, with a size and charge equal to 163 nm and 1.7 mV, respectively) had no significant effect on cell viability at the high lipid concentration of 500 $\mu\text{g}/\text{ml}$. However, the STPP liposomes were highly cytotoxic at the same concentration. Mitochondria-targeting TPP-PEG-PE liposomes loaded with paclitaxel significantly reduced cell viability of HeLa and 4T1 cells *in vitro* compared with nontargeting paclitaxel-loaded liposomes. The same targeting preparation also significantly reduced tumor volumes in mice bearing 4T1 tumors compared with mice treated with nontargeting paclitaxel-loaded liposomes or saline [51]. Patel *et al.* fabricated mitochondria-targeting liposomes (105 nm) loaded with sclareol (a diterpene alcohol that can induce

death of cancer cells via the mitochondrial apoptosis pathway) and demonstrated their improved cytotoxicity toward the colon carcinoma cell line, COLO205, when compared with sclareol-loaded nontargeting liposomes *in vitro* [52]. Lu's group conjugated TPP with TPGS₁₀₀₀ (TPP-TPGS₁₀₀₀) and incorporated this into paclitaxel-loaded liposomes. Mitochondrial targeting of paclitaxel-loaded liposomes showed greater antitumor activity compared with the commercial paclitaxel liposome, Taxol® (Bristol-Myers Squibb, NY, USA), in a murine model of drug-resistant human lung cancer (A549/cDDP cells) [53].

Wagle *et al.* designed mitochondrial-targeting liposomes (~150 nm diameter) with negative charge using mitochondrial extracts as a mitochondriotropic agent. It was found that liposomes made from the enriched lipid mitochondrial fraction exhibited higher mitochondrial targeting. The authors suggested that such formulations may be more biocompatible than synthetic liposomes [54].

The Harashima group designed liposomes called MITO-Porter (200–300 nm in diameter) that were capable of fusing with, and delivering macromolecules to, mitochondria [55]. MITO-Porter liposomes in the cytosol localized to mitochondria through electrostatic interactions (the zeta potential value of MITO-Porter liposomes was approximately +50 mV) and the fusion between mitochondrial membranes and MITO-Porter liposomes was induced by fusogenic lipid components such as 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl ethanolamine and sphingomyelin, or 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl ethanolamine and phosphatidic acid. Propidium iodide, a membrane impermeable fluorescent dye that can stain nucleic acid and mitochondrial DNA, was loaded into MITO-Porter liposomes. It was subsequently shown (as proof of principle for a drug moiety) that MITO-Porter liposomes are capable of delivering propidium iodide into the mitochondrial matrix of *in vitro* cocultured HeLa cells [56]. In a subsequent study by the same group, dual function (DF)-MITO-Porter multilayer liposomes (150 nm; +30 mV) encapsulating macromolecules targeting to mitochondrial DNA were developed [57,58]. The surface of these DF-MITO-Porter liposomes contained the stearyl octaarginine peptide, STR-R8, which promotes macropinocytosis-mediated uptake of the liposomes and aids liposomal escape from macropinosomes into the cytosol [59]. As proof of principle that these liposomes could specifically target bioactive molecules to mitochondria, it was shown in HeLa cells *in vitro* that DNase I could be delivered to the mitochondria, resulting in digestion of mitochondrial DNA while nuclear DNA remained intact [60]. Recently, STR-R8 in DF-MITO-Porter liposomes was replaced

with a small sequence peptide (STR-S2; stearyl-Dmt-D-Arg-FK-Dmt-D-Arg-FK-NH₂) that selectively targets to the inner membrane of mitochondria [11]. STR-S2-modified DF-MITO-Porter liposomes showed lower cytotoxicity in HeLa cells while demonstrating similar mitochondrial-targeting activities [57].

Biodegradable polymeric particles

Polymeric particles are attractive as mitochondria-targeting drug delivery systems owing to their ease of manufacture, flexibility with respect to surface modification and tunable drug release profiles [61]. Moreover, polymers offer the ability to protect drug moieties from harsh physiological environments, possibly even more than liposomes, thereby increasing drug stability [62]. Poly(lactic-*co*-glycolic acid) (PLGA), a copolymer of glycolic acid and lactic acid, is able to entrap hydrophilic or hydrophobic compounds [63]. Because of its biodegradable and biocompatible traits, US FDA-approved PLGA is a good candidate for polymer-based drug delivery systems that target mitochondria [61].

Dhar's group studied the relationship between size and surface charge of PLGA nanoparticles (NPs) and their mitochondrial uptake efficacy in HeLa cells *in vitro* [64]. A mitochondria-targeting polymer was synthesized by conjugating TPP to PLGA-block-PEG (PLGA-*b*-PEG-TPP). All NPs were created using a nanoprecipitation method that generated particles with a size range of 80–140 nm in diameter and with a surface charge ranging from -30 to +30 mV. Optimal mitochondrial targeting was observed with positively charged NPs in the size range of 80–100 nm. Negatively charged NPs could not be detected inside mitochondria. These findings might stem from the fact that the mitochondrial inner membrane is highly negatively charged relative to the matrix, thus facilitating the uptake of positively charged NPs. Mitochondria-targeting PLGA NPs localized to mitochondria of HeLa cells after exposure to NPs for 4 h. These PLGA-based NPs can carry hydrophobic drugs, such as curcumin for Alzheimer's disease and 2, 4-dinitrophenol (DNP) for obesity, and showed superior therapeutic effects compared with nontargeted NPs and free drugs. Curcumin has the potential to prevent and treat Alzheimer's disease owing to its antioxidant and anti-inflammatory properties, and its ability to prevent β -amyloid formation, which accumulates in the brains of patients with Alzheimer's disease [65,66]. Mitochondria-targeting curcumin-loaded PLGA NPs exhibited significantly higher protective effects against β -amyloid peptides in human neuroblastoma (IMR-32) cells when compared with nontargeted particles or free curcumin. DNP has the ability to reduce the ATP concentration in cells and reduce fatty acid synthesis, but is also reported to have

significant adverse effects and a narrow therapeutic window [67]. Mitochondria-targeting DNP-loaded PLGA NPs were incubated with mouse 3T3-L1 preadipocytes and found to reduce intracellular lipid accumulation at lower doses compared with the nontargeted DNP NPs and free DNP [64]. In a separate study, PLGA-based NPs were also used as a core for high-density lipoprotein (HDL)-mimicking NPs (TPP-HDL-apoA-1-QD NPs) as a proof of concept for detection (imaging) and prevention of atherosclerosis. The core of these NPs was composed of PLGA, cholesterol oleate and quantum dots (QD) in the form of PLGA-*b*-PEG-QD, while the outer surface comprised apoA-1, the major protein in HDL that removes fat by reverse cholesterol transport mediated by macrophages [68]. Cationic stearyl-TPP was also synthesized and incorporated onto the surface of the NPs to differentiate between cells that had a healthy versus collapsed $\Delta\Psi_m$, as an indicator of atherosclerotic plaque development [69,70]. The size and charge of the TPP-HDL-apoA-1-QD NPs were 123 nm and +39 mV, respectively. In a mouse macrophage cell line, RAW 264.7, and in human adipose-derived mesenchymal stem cells, the TPP-HDL-apoA-1-QD NPs showed no cytotoxicity even at concentrations of 50 mg/ml after 72 h of exposure. In healthy cells, the TPP on the surface of TPP-HDL-apoA-1-QD NPs facilitated the transport and localization of the NPs into the mitochondrial matrix. Apoptotic cells experienced the loss of $\Delta\Psi_m$, and the TPP-HDL-apoA-1-QD NPs could not accumulate in the mitochondrial matrix, and therefore accumulated in the cytoplasm instead. This differential accumulation profile is a proof of concept for an imaging technique designed for the detection of apoptosis. The TPP-HDL-apoA-1-QD NPs also demonstrated the ability to reduce triglyceride and cholesterol levels in rats suggesting the potential for these particles to be a preventive therapy for atherosclerosis [71]. Dhar's group also investigated PLGA-*b*-PEG-TPP encapsulating zinc (II) phthalocyanine (ZnPc), a catalyst for oxygen reduction, for vaccine applications in cancer immunotherapy. Positively charged mitochondria-targeting ZnPc NPs (T-ZnPc-NPs) were manufactured with a size range of 65 to 75 nm. Human breast adenocarcinoma cells (MCF-7) were incubated with T-ZnPc-NPs and exposed to a laser light (660 nm light, 1 min) to create cancer cell supernatants from mixtures of apoptotic and necrotic cells. These supernatants showed significantly higher stimulatory effects on murine bone marrow-derived dendritic cells compared with nontargeted ZnPc-NPs [72].

Metal particles

The use of gold (Au), silver and titanium dioxide (TiO₂) NPs as diagnostic and therapeutic tools has

grown rapidly in recent years [73,74]. Metallic particles can be synthesized to obtain often ultra-small sized (3–30 nm) and homogeneous NPs. They can also be functionalized and conjugated with various drugs and targeting compounds.

AuNPs (or colloidal gold) have been thoroughly investigated for pharmaceutical applications owing to their low cytotoxicity, ease of synthesis and surface functionalization [75]. One of the problems with surface-modified AuNPs is their tendency to aggregate. In order to overcome this, Fei *et al.* designed a multilayered polypeptide-AuNP assembly to create a prototype of AuNPs for functionalization with various drugs/peptides [76]. The 20 nm AuNP core was stabilized with a Cys-Ala-Leu-Asn-Asn (CALNN)-based peptide (CP; biotin-NNLACCALNN-COOH) as the first layer by directly mixing. For the second layer, the biotin in the CALNN-based peptide was conjugated to streptavidin (SA), which then allows for the attachment of biotinylated drugs of interest at the outermost layer. These multilayered AuNPs (Au@CP/SA) had hydrodynamic diameters equal to 27 nm and were stable in phosphate-buffered saline. Rhodamine-labeled Au@CP/SA localized to the mitochondria of three different human cancer cell lines (HeLa [cervical], A549 [alveolar] and MCF-7 [breast]) after 1-h incubation periods *in vitro*. In studies to delineate the mechanism by which these NPs localize to mitochondria, it was found that Au@CP/SA were taken up by cells using caveolae-mediated endocytosis. To study the mitochondrial-targeting ability of these NPs, different NP formulations (SA, CP/SA, Au@CP and Au@CP/SA) were labeled with rhodamine and tested separately in HeLa cells. It was found that the Au core was necessary for localization in mitochondria since SA and CP/SA alone did not localize to mitochondria. Only the Au@CP/SA group displayed a mitochondrial localization effect. The authors suggested that the mitochondrial-targeting ability of Au@CP/SA might come from the assembly of three substances in the right order. To test the ability of AU@CP/SA as a potential nanomedicine, mitochondrial membrane disrupting (KLAKLAK)₂ peptides (biotin-KLA) [77] were conjugated to NPs (Au@CP/SA/KLA). Au@CP/SA/KLA significantly reduced cell viability in all three cell lines when compared with free KLA and AU@CP/SA [76]. AuNPs were also investigated in the treatment of heart failure in a Dox-induced advanced heart failure model in rats. AuNPs (30 nm in diameter) were conjugated to Simdax® (Orion Corporation, Espoo, Finland; AuNPs-Simdax), an inotropic agent and a mitochondrial K_(ATP) channel opener that plays a role in cardioprotection [78]. Different treatments (AuNPs, AuNPs-Simdax and Simdax only) were administered

via two different routes, intrapleural (local delivery) and intravenous (systemic), after intravenous administration of Dox to stimulate heart failure. The mean life continuity of the rats was found to be longest in AuNPs-Simmax, AuNPs and Simmax, respectively. AuNPs-Simmax had a significantly higher cardioprotective effect than Simmax alone. The NPs were found to localize to mitochondria of heart cells. Sonoporation (using ultrasound to increase cell membrane permeation) was also introduced after NP administration and was found to increase mitochondrial localization of AuNPs [79].

Other mitochondria-targeting metal particles to have been studied include TiO_2 NPs (3–5 nm in diameter) conjugated to one of two DNA-targeting oligonucleotides. One DNA-targeting oligonucleotide targets genes encoding ribosomal RNA located in the nucleus, and the other targets mitochondrial genes. TiO_2 NPs conjugated to a mitochondrial gene (*NADH dehydrogenase subunit 2*) targeting DNA oligonucleotide were transfected into the rat pheochromocytoma (PC12: a neuroendocrine tumor) cell line by electroporation. Although electroporation allowed for infiltration of NPs into all cell compartments equally, the targeting NPs were retained at the targeted organelle (the mitochondrion) after 24 h, as determined by transmission electron microscopy. Using x-ray fluorescence microscopy, TiO_2 -DNA NPs were also found in isolated mitochondria of PC12 cells [80]. In a separate study, cuprous oxide NPs (CONPs; 40–110 nm in diameter) were tested for cytotoxicity in two tumor cell lines (HeLa and human melanoma [YUMAC]) and two normal cell lines (human embryonic kidney [293T] and mouse embryonic fibroblast) at concentrations ranging from 1.25 to 40.00 $\mu\text{g}/\text{ml}$. Tumor cell lines were more sensitive to toxicity from CONPs when compared with normal cell lines, especially at low concentrations. Using transmission electron microscopy, CONPs were observed to bind to the outer membrane of mitochondria and some were found inside mitochondria within vesicles. The CONPs caused structural changes and damage to the mitochondrial membrane and may have been the cause for the CONP-induced apoptosis [81]. The antitumor effect of CONPs was also tested in B16-F10 tumor-bearing mice. Intratumoral injections of CONPs (400 μg of NPs per day for 16 days) resulted in a reduced tumor mass and significantly prolonged survival of mice when compared with the control group. *In vitro* results using B16-F10 and HeLa cells suggested that CONPs could cause a reduction in $\Delta\Psi_m$ and cytochrome *c* release leading to apoptosis [82].

Recently, it was shown *in vitro* that molybdenum trioxide NPs, when functionalized with TPP, could confer sulfite oxidase activity to a cultured liver cell

line (HepG2) with knocked down sulfite oxidase [83]. It was suggested that these mitochondrial-targeting particles may be used as a therapy for infants with molybdenum cofactor deficiency or sulfite oxidase deficiency.

Conclusion

Mitochondria are involved in many human disorders and are therefore attractive organelles for drug targeting. Liposomes have been the most intensely investigated pharmaceutical formulations for use as mitochondria-targeting systems since the 1990s. This is primarily owing to the ground work performed with liposomes in various clinical settings and because they are nontoxic, amenable to surface modification and capable of hosting a wide range of drug molecules. The practice of using polymeric particles, such as PLGA, is still relatively new; however, they have many of the advantages of liposomes without some of the limiting drawbacks (e.g., liposomes not being amenable to long-term storage and being costly to manufacture), making them potential candidates for diagnostic and therapeutic mitochondrial targeting. Metallic particles are appealing because of their small size and because they are readily functionalized with targeting molecules. Although no pharmaceutical formulation designed to deliver a drug moiety specifically to mitochondria is currently available on the market, many promising preparations are showing promise as therapeutic and/or diagnostic agents.

Future perspective

Using mitochondria-targeting formulations as a therapy is a relatively new concept. Despite promising results with formulations in *in vitro* and *in vivo* preclinical studies, to the best of our knowledge, none have reached clinical trials. In the future, we expect to see an introduction of these formulations into clinical settings. The major challenge will be to design organ-specific mitochondrial-targeting particles since the cargo should preferentially accumulate at the desired site of action, such as the brain in Alzheimer's disease and the heart or brain in ischemia-reperfusion injury. In this regard, the fact that TPP can preferentially localize to those tissues mostly affected by mitochondrial dysfunction is promising; however, further surface modifications of particles are required to generate greater specificity.

Financial & competing interests disclosure

The authors gratefully acknowledge support from the American Cancer Society (RSG-09-015-01-CDD), the National Cancer Institute at the NIH (1R21CA13345-01/1R21CA128414-01A2/UI Mayo Clinic Lymphoma SPORE), and the Lyle and

Sharon Bighley Professorship. A Wongrakpanich acknowledges support from the Royal Thai Government Scholarship. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial inter-

est in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Potential of mitochondria-targeting particles as drug delivery systems

- A number of drug moieties are capable of targeting mitochondria. However, transporting drugs to mitochondria necessitates their passage through tissue and cellular barriers that include the plasma membrane and mitochondrial membranes. Many drugs cannot overcome these barriers without assistance. Thus, particle-based carriers are needed to help transport drugs to mitochondria.

Liposomes & liposome-like vesicles

- Amongst the particulate delivery systems with the potential for targeting mitochondria, liposomes are the most studied. The mitochondrial-targeting properties of liposomes were established by incorporating mitochondriotropic agents into the lipid bilayer during liposome formation.
- Although there are many liposome formulations in clinical trials and on the market to date, there are no mitochondria-targeting liposome formulations in clinical trials.

Biodegradable polymeric particles

- Considering that many types of biodegradable polymers are used in the pharmaceutical industry, it is surprising that so far only poly(lactic-co-glycolic acid) has been used in mitochondria-targeting particulate formulations with success. Poly(lactic-co-glycolic acid) particles were prepared using a nanoprecipitation method with triphenylphosphonium as the mitochondria-targeting molecule.

Metal particles

- Various metal particles were investigated as mitochondria-targeting carriers partly owing to their small and uniform size. Among all metal particles investigated to date, gold nanoparticles have been the most intensely studied.
- Further research is needed to understand the mechanism of mitochondrial targeting and the cytotoxicity caused by metal particles.

References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest.

- Wang B, Galliford CV, Low PS. Guiding principles in the design of ligand-targeted nanomedicines. *Nanomedicine (Lond.)* 9(2), 313–330 (2014).
- Martinho N, Damge C, Reis C. Recent advances in drug delivery systems. *J. Biomater. Nanobiotechnol.* 2(5A), 510–526 (2011).
- Weissig V, Boddapati SV, Jabr L, D'Souza GG. Mitochondria-specific nanotechnology. *Nanomedicine (Lond.)* 2(3), 275–285 (2007).
- Murphy MP, Smith RA. Drug delivery to mitochondria: the key to mitochondrial medicine. *Adv. Drug Deliv. Rev.* 41(2), 235–250 (2000).
- Bayeva M, Gheorghiade M, Ardehali H. Mitochondria as a therapeutic target in heart failure. *J. Am. Coll. Cardiol.* 61(6), 599–610 (2013).
- Moreira PI, Zhu X, Wang X *et al.* Mitochondria: atherapeutic target in neurodegeneration. *Biochim. Biophys. Acta* 1802(1), 212–220 (2010).
- Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. *Nat. Rev. Genet.* 6(5), 389–402 (2005).
- Walters AM, Porter GA Jr, Brookes PS. Mitochondria as a drug target in ischemic heart disease and cardiomyopathy. *Circ. Res.* 111(9), 1222–1236 (2012).
- Joiner ML, Koval OM, Li J *et al.* CaMKII determines mitochondrial stress responses in heart. *Nature* 491(7423), 269–273 (2012).
- Pradelli LA, Beneteau M, Ricci JE. Mitochondrial control of caspase-dependent and -independent cell death. *Cell Mol. Life Sci.* 67(10), 1589–1597 (2010).
- Szeto HH, Schiller PW. Novel therapies targeting inner mitochondrial membrane – from discovery to clinical development. *Pharm. Res.* 28(11), 2669–2679 (2011).
- Kinnally KW, Peixoto PM, Ryu S-Y, Dejean LM. Is mPTP the gatekeeper for necrosis, apoptosis, or both? *Biochim. Biophys. Acta* 1813(4), 616–622 (2011).
- Dumont M, Lin MT, Beal MF. Mitochondria and antioxidant targeted therapeutic strategies for Alzheimer's disease. *J. Alzheimers Dis.* 20(Suppl. 2), S633–S643 (2010).
- Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. Mitochondria-targeted antioxidants for treatment of Parkinson's disease: preclinical and clinical outcomes. *Biochim. Biophys. Acta* 1842(8), 1282–1294 (2013).
- Reddy PH. Mitochondrial medicine for aging and neurodegenerative diseases. *Neuromolecular Med.* 10(4), 291–315 (2008).
- Moreira PI, Zhu X, Wang X *et al.* Mitochondria: a therapeutic target in neurodegeneration. *Biochim. Biophys. Acta* 1802(1), 212–220 (2010).

- 17 Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 7(1), 65–74 (2009).
- 18 Rohlena J, Dong LF, Ralph SJ, Neuzil J. Anticancer drugs targeting the mitochondrial electron transport chain. *Antioxid. Redox Signal.* 15(12), 2951–2974 (2011).
- 19 Ferrin G, Linares CI, Muntane J. Mitochondrial drug targets in cell death and cancer. *Curr. Pharm. Des.* 17(20), 2002–2016 (2011).
- 20 Wang F, Ogasawara MA, Huang P. Small mitochondria-targeting molecules as anti-cancer agents. *Mol. Aspects Med.* 31(1), 75–92 (2010).
- 21 Marullo R, Werner E, Degtyareva N *et al.* Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. *PLoS One* 8(11), e81162 (2013).
- 22 Sakhrani NM, Padh H. Organelle targeting: third level of drug targeting. *Drug Des. Devel. Ther.* 7, 585–599 (2013).
- 23 Birk AV, Liu S, Soong Y *et al.* The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. *J. Am. Soc. Nephrol.* 24(8), 1250–1261 (2013).
- 24 Chakrabarti AK, Feeney K, Abueg C *et al.* Rationale and design of the EMBRACE STEMI study: a Phase 2a, randomized, double-blind, placebo-controlled trial to evaluate the safety, tolerability and efficacy of intravenous Bendavia on reperfusion injury in patients treated with standard therapy including primary percutaneous coronary intervention and stenting for ST-segment elevation myocardial infarction. *Am. Heart J.* 165(4), 509–514.e7 (2013).
- 25 Smith RA, Murphy MP. Mitochondria-targeted antioxidants as therapies. *Discov. Med.* 11(57), 106–114 (2011).
- 26 Smith RA, Porteous CM, Gane AM, Murphy MP. Delivery of bioactive molecules to mitochondria *in vivo*. *Proc. Natl Acad. Sci. USA* 100(9), 5407–5412 (2003).
- 27 Mao P, Manczak M, Shirendeb UP, Reddy PH. MitoQ, a mitochondria-targeted antioxidant, delays disease progression and alleviates pathogenesis in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. *Biochim. Biophys. Acta* 1832(12), 2322–2331 (2013).
- 28 Miquel E, Cassina A, Martinez-Palma L *et al.* Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* 70, 204–213 (2014).
- 29 Smith RA, Murphy MP. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Ann. NY Acad. Sci.* 1201, 96–103 (2010).
- 30 Chamberlain GR, Tulumello DV, Kelley SO. Targeted delivery of doxorubicin to mitochondria. *ACS Chem. Biol.* 8(7), 1389–1395 (2013).
- 31 Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* 13(1), 238–252 (1965).
- 32 Chang HI, Yeh MK. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. *Int. J. Nanomedicine* 7, 49–60 (2012).
- 33 Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.* 65(1), 36–48 (2013).
- 34 Weissig V, Lasch J, Erdos G, Meyer HW, Rowe TC, Hughes J. DQAsomes: a novel potential drug and gene delivery system made from Dequalinium. *Pharm. Res.* 15(2), 334–337 (1998).
- **Early report on a mitochondria-specific particulate drug delivery system.**
- 35 Weissig V, Lizano C, Torchilin VP. Selective DNA release from DQAsome/DNA complexes at mitochondria-like membranes. *Drug Deliv.* 7(1), 1–5 (2000).
- 36 Weissig V, D'souza GG, Torchilin VP. DQAsome/DNA complexes release DNA upon contact with isolated mouse liver mitochondria. *J. Control. Release* 75(3), 401–408 (2001).
- 37 D'souza GG, Rammohan R, Cheng SM, Torchilin VP, Weissig V. DQAsome-mediated delivery of plasmid DNA toward mitochondria in living cells. *J. Control. Release* 92(1–2), 189–197 (2003).
- 38 Andre N, Carre M, Brasseur G *et al.* Paclitaxel targets mitochondria upstream of caspase activation in intact human neuroblastoma cells. *FEBS Lett.* 532(1–2), 256–260 (2002).
- 39 D'souza GG, Cheng SM, Boddapati SV, Horobin RW, Weissig V. Nanocarrier-assisted sub-cellular targeting to the site of mitochondria improves the pro-apoptotic activity of paclitaxel. *J. Drug Target.* 16(7), 578–585 (2008).
- 40 Men Y, Wang XX, Li RJ *et al.* The efficacy of mitochondrial targeting antiresistant epirubicin liposomes in treating resistant leukemia in animals. *Int. J. Nanomedicine* 6, 3125–3137 (2011).
- 41 Zhang L, Yao HJ, Yu Y *et al.* Mitochondrial targeting liposomes incorporating daunorubicin and quinacrine for treatment of relapsed breast cancer arising from cancer stem cells. *Biomaterials* 33(2), 565–582 (2012).
- **Demonstrated both *in vitro* and *in vivo* cytotoxicity of mitochondrial-targeting liposomes toward breast cancer stem cells and provided a mechanistic explanation.**
- 42 Yu Y, Wang ZH, Zhang L *et al.* Mitochondrial targeting topotecan-loaded liposomes for treating drug-resistant breast cancer and inhibiting invasive metastases of melanoma. *Biomaterials* 33(6), 1808–1820 (2012).
- 43 Collnot EM, Baldes C, Schaefer UF, Edgar KJ, Wempe MF, Lehr CM. Vitamin E TPGS P-glycoprotein inhibition mechanism: influence on conformational flexibility, intracellular ATP levels, and role of time and site of access. *Mol. Pharm.* 7(3), 642–651 (2010).
- 44 Wang XX, Li YB, Yao HJ *et al.* The use of mitochondrial targeting resveratrol liposomes modified with a dequalinium polyethylene glycol-distearoylphosphatidyl ethanolamine conjugate to induce apoptosis in resistant lung cancer cells. *Biomaterials* 32(24), 5673–5687 (2011).
- 45 Li N, Zhang CX, Wang XX *et al.* Development of targeting lonidamine liposomes that circumvent drug-resistant cancer by acting on mitochondrial signaling pathways. *Biomaterials* 34(13), 3366–3380 (2013).
- 46 Carter LG, D'orazio JA, Pearson KJ. Resveratrol and cancer: a focus on *in vivo* evidence. *Endocr. Relat. Cancer* 21(3), R209–R225 (2014).

- 47 Pan Y, Zhao W, Wang J, Liu Q, Yang G, Li J. [Establishment of a multidrug resistance cell line A549/cDDP of human lung adenocarcinoma and expression analysis of multidrug resistance-associated genes.]. *Zhongguo Fei Ai Za Zhi* 12(3), 187–192 (2009).
- 48 Mullen TD, Obeid LM. Ceramide and apoptosis: exploring the enigmatic connections between sphingolipid metabolism and programmed cell death. *Anticancer Agents Med. Chem.* 12(4), 340–363 (2012).
- 49 Boddapati SV, D'souza GG, Erdogan S, Torchilin VP, Weissig V. Organelle-targeted nanocarriers: specific delivery of liposomal ceramide to mitochondria enhances its cytotoxicity *in vitro* and *in vivo*. *Nano Lett.* 8(8), 2559–2563 (2008).
- 50 Solomon MA, Shah AA, D'Souza GG. *In vitro* assessment of the utility of stearyl triphenyl phosphonium modified liposomes in overcoming the resistance of ovarian carcinoma Ovar-3 cells to paclitaxel. *Mitochondrion* 13(5), 464–472 (2013).
- 51 Biswas S, Dodwadkar NS, Deshpande PP, Torchilin VP. Liposomes loaded with paclitaxel and modified with novel triphenylphosphonium–PEG–PE conjugate possess low toxicity, target mitochondria and demonstrate enhanced antitumor effects *in vitro* and *in vivo*. *J. Control. Release* 159(3), 393–402 (2012).
- 52 Patel NR, Hatziantoniou S, Georgopoulos A *et al.* Mitochondria-targeted liposomes improve the apoptotic and cytotoxic action of sclareol. *J. Liposome Res.* 20(3), 244–249 (2010).
- 53 Zhou J, Zhao WY, Ma X *et al.* The anticancer efficacy of paclitaxel liposomes modified with mitochondrial targeting conjugate in resistant lung cancer. *Biomaterials* 34(14), 3626–3638 (2013).
- 54 Wagle MA, Martinville LE, D'Souza GG. The utility of an isolated mitochondrial fraction in the preparation of liposomes for the specific delivery of bioactives to mitochondria in live mammalian cells. *Pharm. Res.* 28(11), 2790–2796 (2011).
- 55 Yamada Y, Akita H, Kamiya H *et al.* MITO-Porter: aliposome-based carrier system for delivery of macromolecules into mitochondria via membrane fusion. *Biochim. Biophys. Acta* 1778(2), 423–432 (2008).
- 56 Yasuzaki Y, Yamada Y, Harashima H. Mitochondrial matrix delivery using MITO-Porter, a liposome-based carrier that specifies fusion with mitochondrial membranes. *Biochem. Biophys. Res. Commun.* 397(2), 181–186 (2010).
- 57 Kawamura E, Yamada Y, Harashima H. Mitochondrial targeting functional peptides as potential devices for the mitochondrial delivery of a DF-MITO-Porter. *Mitochondrion* 13(6), 610–614 (2013).
- 58 Yamada Y, Furukawa R, Yasuzaki Y, Harashima H. Dual function MITO-Porter, a nano carrier integrating both efficient cytoplasmic delivery and mitochondrial macromolecule delivery. *Mol. Ther.* 19(8), 1449–1456 (2011).
- 59 Khalil IA, Kogure K, Futaki S, Harashima H. High density of octaarginine stimulates macropinocytosis leading to efficient intracellular trafficking for gene expression. *J. Biol. Chem.* 281(6), 3544–3551 (2006).
- 60 Yamada Y, Harashima H. Delivery of bioactive molecules to the mitochondrial genome using a membrane-fusing, liposome-based carrier, DF-MITO-Porter. *Biomaterials* 33(5), 1589–1595 (2012).
- 61 Lu JM, Wang X, Marin-Muller C *et al.* Current advances in research and clinical applications of PLGA-based nanotechnology. *Expert Rev. Mol. Diagn.* 9(4), 325–341 (2009).
- 62 Vilar G, Tulla-Puche J, Albericio F. Polymers and drug delivery systems. *Curr. Drug Deliv.* 9(4), 367–394 (2012).
- 63 Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. *J. Biomater. Sci. Polym. Ed.* 17(3), 247–289 (2006).
- 64 Marrache S, Dhar S. Engineering of blended nanoparticle platform for delivery of mitochondria-acting therapeutics. *Proc. Natl Acad. Sci. USA* 109(40), 16288–16293 (2012).
- **Well characterized mitochondrial-targeting biodegradable polymeric delivery system.**
- 65 Mishra S, Palanivelu K. The effect of curcumin (turmeric) on Alzheimer's disease: an overview. *Ann. Indian Acad. Neurol.* 11(1), 13–19 (2008).
- 66 Potter PE. Curcumin: a natural substance with potential efficacy in Alzheimer's disease. *J. Exp. Pharmacol.* 5, 9 (2013).
- 67 Grundlingh J, Dargan PI, El-Zanfaly M, Wood DM. 2,4-dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J. Med. Toxicol.* 7(3), 205–212 (2011).
- 68 Rothblat GH, Phillips MC. High-density lipoprotein heterogeneity and function in reverse cholesterol transport. *Curr. Opin. Lipidol.* 21(3), 229–238 (2010).
- 69 Seimon T, Tabas I. Mechanisms and consequences of macrophage apoptosis in atherosclerosis. *J. Lipid Res.* 50(Suppl.), S382–S387 (2009).
- 70 Karafllou M, Lambrinoudaki I, Christodoulakos G. Apoptosis in atherosclerosis: a mini-review. *Mini Rev. Med. Chem.* 8(9), 912–918 (2008).
- 71 Marrache S, Dhar S. Biodegradable synthetic high-density lipoprotein nanoparticles for atherosclerosis. *Proc. Natl Acad. Sci. USA* 110(23), 9445–9450 (2013).
- 72 Marrache S, Tundup S, Harn DA, Dhar S. *Ex vivo* programming of dendritic cells by mitochondria-targeted nanoparticles to produce interferon-gamma for cancer immunotherapy. *ACS Nano* 7(8), 7392–7402 (2013).
- 73 Dykman LA, Khlebtsov NG. Gold nanoparticles in biology and medicine: recent advances and prospects. *Acta Naturae* 3(2), 34–55 (2011).
- 74 Ong C, Lim JZ, Ng CT, Li JJ, Yung LY, Bay BH. Silver nanoparticles in cancer: therapeutic efficacy and toxicity. *Curr. Med. Chem.* 20(6), 772–781 (2013).
- 75 Dreaden EC, Austin LA, Mackey MA, El-Sayed MA. Size matters: gold nanoparticles in targeted cancer drug delivery. *Ther. Deliv.* 3(4), 457–478 (2012).
- 76 Ma X, Wang X, Zhou M, Fei H. A mitochondria-targeting gold-peptide nanoassembly for enhanced cancer-cell killing. *Adv. Healthc. Mater.* 2(12), 1638–1643 (2013).

- 77 Law B, Quinti L, Choi Y, Weissleder R, Tung CH. A mitochondrial targeted fusion peptide exhibits remarkable cytotoxicity. *Mol. Cancer Ther.* 5(8), 1944–1949 (2006).
- 78 McCully JD, Levitsky S. The mitochondrial K(ATP) channel and cardioprotection. *Ann. Thorac. Surg.* 75(2), S667–S673 (2003).
- 79 Spivak MY, Bubnov RV, Yemets IM, Lazarenko LM, Tymoshok NO, Ulberg ZR. Development and testing of gold nanoparticles for drug delivery and treatment of heart failure: a theranostic potential for PPP cardiology. *EPMA J.* 4(1), 20 (2013).
- 80 Paunesku T, Vogt S, Lai B *et al.* Intracellular distribution of TiO₂-DNA oligonucleotide nanoconjugates directed to nucleolus and mitochondria indicates sequence specificity. *Nano Lett.* 7(3), 596–601 (2007).
- 81 Wang Y, Zi XY, Su J *et al.* Cuprous oxide nanoparticles selectively induce apoptosis of tumor cells. *Int. J. Nanomedicine* 7, 2641–2652 (2012).
- 82 Wang Y, Yang F, Zhang HX *et al.* Cuprous oxide nanoparticles inhibit the growth and metastasis of melanoma by targeting mitochondria. *Cell Death Dis.* 4, e783 (2013).
- 83 Ragg R, Natalio F, Tahir MN *et al.* Molybdenum trioxide nanoparticles with intrinsic sulfite oxidase activity. *ACS Nano* 8(5), 5182–5189 (2014).