Newly developed strategies for multifunctional mitochondria-targeted agents in cancer therapy

Erlong Zhang[1,3], Chao Zhang[1,2,3], Yongping Su[1,3], Tianmin Cheng[1] and Chunmeng Shi[1]

[1] Institute of Combined Injury, State Key Laboratory of Trauma, Burns and Combined Injury, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China
[2] Department of Orthopedics, Xinqiao Hospital, Third Military Medical University, Chongqing 400038, China

The development of multifunctional agents that could be used for simultaneous tumor targeting, imaging and treatment is a major goal in cancer research and is expected to contribute significantly to the realization of personalized oncology. Mitochondria are involved in diverse physiological activities and confer vital roles in cancer development and progression. Increasing efforts are being made to develop cancer treatment strategies based on various mitochondrial targets and novel mitochondrial drug delivery systems. Multifunctional nanostructures or multifunctional chemical compounds further broaden the current concept of tumor targeting and provide alternative solutions for mitochondrially targeted cancer therapy.

Introduction

As important cellular organelles, mitochondria exert both vital and lethal functions in diverse physiological and pathological conditions associated with cell survival and death. Cancer cell mitochondria are structurally and functionally different from their normal counterparts [1,2]. Mitochondrial dysfunctions cause many disorders and confer important roles in the entire process of cancer development and progression [3].

On the one hand, several vital functions of mitochondria are deregulated in cancer cells. Cancer cells take on different metabolic choices for energy production for their survival and these metabolic alterations affect the capacity of cancer cells to engage in catabolic processes, including apoptosis, necrosis and autophagy [4]. Increased reactive oxygen species (ROS) stress has long been observed in cancer cells, which contain more antioxidant compounds than do normal cells [5,6]. Modifications in the levels of ROS have recently been linked to multiple reactions in cancer cells, including the increase in cell proliferation, DNA damage and genetic instability, resistance to antitumor agents, promotion of carcinogenesis and metastasis [5–8]. Frequent mutations of the mitochondrial DNA (mtDNA) that affect the synthesis of respiratory chain proteins and lead to increased electron leakage and ROS overproduction have been characterized in a variety of cancer types and favor the chromosomal instability and carcinogenesis [9]. mtDNA mutations can also contribute to neoplasm metastasis [10] and tolerance against anticancer drugs [11].

On the other hand, the lethal functions of mitochondria are also deregulated in cancer cells. Mitochondria control the intrinsic pathway of apoptosis by regulating the translocation of proapoptotic proteins from the mitochondrial intermembrane space to the cytosol and also have a role in some forms of nonapoptotic cell death, such as necrosis [12]. Proapoptotic factors in cancer cells are reduced, which, combined with the overexpression of antiapoptotic proteins, enable cancer cells to be more resistant to death induction compared with normal cells [13]. Components of the permeability transition pore complex (PTPC), which controls the exchange of metabolites between the cytosol and the mitochondrial matrix and also mediates the mitochondrial permeability transition to trigger the release of cytochrome *c*, express significant alterations in malignant lesions [2,11].

According to mitochondrial dysfunctions that have been linked to multiple aspects of tumorigenesis and tumor progression, mitochondrially targeted compounds represent a promising approach to target tumors selectively [14]. Recently, the development of multifunctional agents that could be used for simultaneous tumor

Corresponding author: Shi, C. (shicm1010@yahoo.com.cn)
[3] These authors contributed equally to this article.

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.12.006

targeting, imaging and treatment has become a major goal in cancer research and is expected to contribute significantly to the realization of personalized oncology. Here, we summarize recently developed mitochondria-targeted therapeutic strategies and discuss the perspectives and future directions of developing multifunctional nanostructures and chemical compounds that can target, image and kill tumors to advance current mitochondrially targeted cancer therapy.

Mitochondria-targeted agents under preclinical and clinical evaluation for anticancer therapy

By targeting the differences between mitochondria from normal cells and from cancer cells, a variety of mitochondrially targeted compounds have been developed for anticancer therapy. Many of these compounds are currently under preclinical and clinical evaluation.

Agents targeting the transition of cell metabolism

Several chemical compounds that inhibit glycolysis, such as 2-deoxyglucose, a nonmetabolically active glucose analog under Phase I/II clinical trials [15], and 3-bromopyruvate, an analog of lactic acid under preclinical testing, have shown therapeutic effects on tumor growth [16]. Lonidamine (LND), derived from indazole-3-carboxylic acid, inhibits glycolysis and enhances the cytotoxicity of the regular chemotherapeutic agents doxorubicin and cisplatin. Multiple Phase III clinical trials of LND have been carried out in nonsmall-cell lung cancer and breast cancer [17]. In addition, phloretin, a glucose transporter inhibitor under preclinical testing, can sensitize cancer cells to daunorubicin for its anticancer activity and apoptosis to overcome drug resistance only under hypoxia [18]. Another agent, dichloroacetate, which can reverse the Warburg effect by inhibiting the key enzyme pyruvate dehydrogenase kinase in cancer cells, is currently under Phase II clinical trials in brain tumor and some solid tumors [11].

However, with regard to the role of glycolysis and use of glycolytic inhibitors to treat cancers, the dependence of cancer cells on glycolysis has been disputed. Some unsuccessful clinical trials have shown that the application of glycolytic inhibitors is limited to atypical cancers with low aerobic capacity, and is not applicable to most tumors. Recent work by Moreno-Sanchez *et al.* has shown that some tumors, such as those located in lung, mammary gland, skin and uterine cervix, depend on oxidative phosphorylation (OXPHOS) instead of glycolysis, suggesting that these tumors do not depend on glycolysis, but instead have a dependency on mitochondrial function [4].

Agents targeting cellular damage caused by abnormal ROS production

Several agents have been developed to increase ROS generation for cancer therapy. Arsenic trioxide, a clinically used drug, can cause an increase in electron leakage by interfering with the OXPHOS, therefore promoting ROS generation, leading to cancer cell apoptosis [19]. Alpha-tocopheryl succinate, a redox-inactive vitamin E analog under Phase II study in melanoma, prostate cancer, colorectal cancer, mesothelioma and breast cancer, also exhibits strong proapoptotic and anticancer activities. It causes rapid production of ROS through its role as a competitive inhibitor of the ubiquinone binding sites in Complex II to inhibit succinate dehydrogenase activity in the mitochondrial electron transport chain [20].

Other agents have also been observed to exert anticancer activity through the inhibition of antioxidant enzymes or the reduction of cellular oxidant-buffering capacity. 2-Methoxyoestradiol, an estrogen derivative, selectively kills human leukemia cells (but not normal lymphocytes) by inhibiting superoxide dismutase [21]. The Phase I/II studies have demonstrated that 2-methoxyoestradiol is well tolerated and causes disease stabilization in patients with solid malignancies or with multiple myeloma [22,23]. Additionally, buthionine sulphoximine, which elevates ROS levels by inhibiting the synthesis of reduced glutathione (GSH) [24], and imexon, which depletes the GSH pool owing to its thiol-binding activity [25], are both in Phase I clinical trials [25,26].

However, the 'double-face' of ROS might also need some consideration. Given that ROS can be an important factor in cancer initiation and progression, some researchers have proposed that the reduction of ROS by chemical compounds might represent a different strategy for cancer treatment [5].

Agents targeting the disabled apoptosis pathway

Correcting the altered apoptosis pathway and promoting the normal apoptotic pathway could also be used to treat tumors. Cancer cell apoptosis can be induced directly through the enhancement of proapoptotic processes or the attenuation of the antiapoptotic pathway [27,28]. Antiapoptotic protein Bcl-2 is a promising target molecule in cancer therapy. Antisense oligonucleotides specific for Bcl-2 RNA sequences (such as G3139) suppress specifically the proliferation of cancer cells or to enhance their sensitivity to chemotherapeutic drugs. G3139 has now entered Phase III clinical trails in combination with chemotherapeutic agents in a variety of tumors, such as leukemia, small-cell lung cancer, multiple myeloma and prostate cancer [29]. The mimetics of Bcl-2 homology domain 3 (BH3) can act as Bcl-2 inhibitors and some BH3 mimetics are currently being evaluated as anticancer agents in clinical settings [30]. Gossypol, a BH3 mimetic, has been introduced into Phase III clinical trials in chronic lymphatic leukemia, hormone refractory prostate cancer and advanced breast cancer [29]. Furthermore, the peripheral benzodiazepine receptor (PBR), which interacts with the PTPC through voltage-dependent anion channels (a component of PTPC), correlates with the aggressive phenotype in breast, colorectal and prostate cancer. PBR ligands, such as PK11195, RO5-4864 and diazepam, have demonstrated antitumor effects both *in vitro* and *in vivo*, either as single agents or combined with chemotherapeutic agents, such as etoposide. PK11195 and RO5-4864 have entered clinical trials and promising results have been obtained in patients with recurrent glioblastoma treated with diazepam plus lonidamine [31].

Agents targeting mutated mtDNA

Many compounds that target mtDNA or enzymes related to its replication hold promise for potential clinical applications [17]. Among them, cisplatin, a classic anticancer drug in clinical use, is found to bind preferentially to mtDNA more than to nuclear DNA and shows higher cisplatin–mtDNA adduction levels, resulting in the inhibition of NADH-ubiquinone reductase and the decrease of ATP generation [32]. Ditercalinium, a bis-intercalating agent that

accumulates mainly in the mitochondria, can cause specific elimination of mtDNA and inhibit its replication [33]. This compound exhibits *in vivo* antitumor activity in animal models and ultrastructural studies have shown a complete loss of mitochondrial cristae and depletion of mtDNA after ditercalinium treatment [34]. Vitamin K3 exhibits specific inhibitory affect on DNA polymerase γ, the mitochondrial enzyme responsible for mtDNA replication. *In vitro* studies have shown that vitamin K3 exhibits anticancer activity in breast and pancreatic cancer cells, but its use as a potential anticancer agent remains to be evaluated [17].

Current strategies for the selective delivery of anticancer agents to the mitochondria of tumor cells

Although mitochondria are emerging as promising cancer therapeutic targets, there are potential concerns and challenges related to the clinical use of current mitochondrially targeted drugs owing to their low cancer selectivity and specificity. It is known that certain normal tissues, including brain, retina and testis, also use glucose as their main energy source; therefore, inhibition of glycolysis could be toxic to these tissues. Some mitochondrially targeted agents might bind specifically to normal cellular components, which is expected to be a problem for *in vivo* application. A further concern is that possible intrinsic drug-resistant mechanisms, such as the intervention of multidrug resistance pumps, might exclude these potential drugs from cancer cells [35]. Although promising, their use might face difficulties posed by the known systemic toxicity. A challenge to improving the therapeutic efficacy of mitochondrially targeted drugs is to deliver these agents selectively and in high enough concentrations at the tumor sites to increase their efficacy and decrease their side effects. Efficient mitochondria-specific delivery systems are urgently needed and several transport pathways have been described (Table 1).

Delocalized lipophilic cations as mitochondrial transporters

Delocalized lipophilic cations (DLCs) are small membrane-permeable cationic molecules with delocalized positive charge. Driven by higher mitochondrial membrane potential ($\Delta\Psi_m$) and plasma membrane potential ($\Delta\Psi_p$) in cancer cells [36,37], DLCs, such as rhodamine 123 (Rh123), can selectively accumulate in the mitochondria of cancer cells with a longer retention time compared with normal epithelial cells [38–40]. The $\Delta\Psi_p$ of -60 mV and $\Delta\Psi_m$ of −180 mV can result in tenfold and 10,000-fold concentrations of the DLCs in the cytoplasm and mitochondria, respectively [41]. With their selective localization, they can act as mitochondrial transporters [42]. A complex called platinum–Rh123, comprising Rh123 and platinum, has been reported to display some degree of selectivity toward transformed cells [43]. Recently, a series of antioxidants has also been conjugated to DLC molecules and further support the potential of DLCs as mitochondrial transporters. However, because some normal cells, such as myocardial cells, also have a similar mechanism for drug retention, these DLCs could cause cardiac toxicity [40]. As small-molecule compounds, DLC-mediated delivery of large molecules could influence their targeting activity [44].

Mitochondrial targeting sequences as mitochondrial transporters

Most mitochondrial proteins are encoded by nuclear DNA and then synthesized as precursors in the cytoplasm; they are then transported to the mitochondria post-translationally with modified mitochondrial targeting signal peptide sequences via the protein import machinery. Typically, mitochondrial matrix proteins, as well as several proteins of the inner membrane and intermembrane space, carry amino-terminal mitochondrial targeting sequences (N-MTSs) that contain approximately 20–40 amino acids without sequence identity. By contrast, outer and polytypic inner membrane proteins are mostly synthesized without an N-MTS, but contain an internal MTS (INT-MTS) [45]. The mitochondrial membranes contain specific machineries (translocases) for the recognition, translocation and membrane insertion of precursor proteins. According to this unique transporting process, the MTS has the potential to become a useful device for the selective delivery of therapeutic agents to the mitochondria of cancer cells.

TABLE 1

Mitochondria-based drug delivery systems in cancer cells[a].

Strategies	Targeting mechanisms	Agents	Advantages	Limitations	Refs
DLCs	Higher $\Delta\Psi_m$ and $\Delta\Psi_p$, relatively lipid soluble	Rh123, MKT-077, EDKC, DECA and TPP	Biocompatible, high efficiency, straightforward conjugation to various types of small molecules	Potential toxicity; size and polarity limitations, and ineffective transport for large polar molecules	[36–44]
MTSs	Structural motifs comprising approximately 20–40 amino acids and recognized by the mitochondrial protein import machinery	Bacterial signal peptides, N-MTS, INT-MTS and mitochondrial leader sequence peptides	Endogenous system, biocompatible; able to transport various biomolecular species	Limited by the size and structural properties of biomolecules, poor aqueous solubility and cellular permeability	[45–49]
MPPs	Higher $\Delta\Psi_m$ and Ψ_p (different from DLCs with point charges), PTDs for improving transmembrane transportation	Engineered CPPs, constructs with guanidinium groups	Biocompatibility, versatility and generality in the delivery of molecules with diverse physicochemical properties	Requires necessary balance of positive charge and crucial lipophilicity	[49–53]
Vesicles	Macropinocytosis, endosomal escape and membrane fusion	DQAsome and MITO-Porter	Sufficient for transport of macromolecules, negatively charged and impermeable agents	Low efficiency of cellular internalization	[54,55]

[a] *Abbreviations*: CPP, cell-penetrating peptide; MPP, mitochondria-penetrating peptide.

It has been reported that a bacterial signal peptide from Toho-1 and the N-MTS of human 3-oxoacyl-CoA thiolase can carry proteins into cell mitochondria [46,47]. However, mitochondrial delivery using MTS is limited by the size of the agents [48]. Macromolecules cannot be transported by this pathway and their aqueous solubility and cellular permeability is another limiting factor for exogenous delivery [49].

Mitochondria-penetrating peptides as mitochondrial transporters

Recent studies have described a variety of amino acid- and peptide-based mitochondrial transporters that are designed to exploit charge-driven uptake into the organelle. These peptides appear to enter cells via a direct mode of uptake, bypassing endocytic uptake and thereby avoiding endosomal and/or lysosomal sequestration that would prohibit their ability to accumulate in mitochondria. It has been reported that peptide transduction domains (PTDs), such as the human immunodeficiency virus-1 TAT protein, can improve the delivery of various biologically active molecules [49,50]. TAT has been fused to a peptide derived from the VHL tumor suppressor and intraperitoneal administration of the TAT–VHL peptide slows the growth of subcutaneous renal cell carcinoma tumors in nude mice [51]. Ellerby *et al.* developed synthesized targeted proapoptotic peptides composed of two functional domains. The targeting domain is designed to guide the 'homing' proapoptotic peptides to targeted cell mitochondria and enable their internalization. The proapoptotic domain is designed to be nontoxic outside of cells, but toxic when internalized into targeted cells by the disruption of mitochondrial membranes [52]. On the basis of this work, a variety of amino acid- and peptide-based mitochondrial transporters are under investigation [49,53]. Although the use of these fused peptides as transporters is only now being investigated, they hold great promise as changeable and versatile mitochondrial delivery agents owing to their advantages of straightforward synthesis, facile derivitization and biocompatibility [53].

Vesicle-based mitochondrial transporters

Active molecules or agents can also be delivered using mitochondrially targeted liposomes as potential mitochondrial transporters. Mitochondriotropic liposomes made of positively charged amphiphilic molecules have been developed. The most intensively investigated examples are the 'DQAsomes' (dequalinium-based liposome vesicles), dicationic mitochondriotropic compounds that self-assemble and form vesicle-like aggregates that can be internalized actively by cells for drug delivery [54]. A novel liposome-based carrier called MITO-Porter, which introduces agents into mitochondria via a membrane fusion mechanism, is also currently receiving more research attention. With the octa-arginine moieties at high density, this transporter is internalized by macropinocytosis and escapes from endosomal phagocytosis. The MITO-Porter then binds to the mitochondria membrane via electrostatic interactions and fuses with the outer mitochondrial membrane, releasing the drugs [55].

Multifunctional agents for mitochondria-based cancer therapy

Currently, multifunctional agents for simultaneous tumor targeting, imaging and treatment are under intensive development for personalized oncology. The most common and general tumor-targeting approach is to conjugate the imaging or therapeutic agents to specific drug delivery systems to develop multifunctional agents. As described above, the growth of mitochondria-based targeting strategies through direct chemical conjugation of therapeutic agents and targeting transporters has demonstrated some promising efficacy, but there is still debate about their selectivity and specificity. Thus, there is an urgent need to study these problems further and to develop innovative or alternative strategies for overcoming them. The relatively new field of cancer nanomedicine investigates the application of such multifunctional agents in cancer detection and therapy. Significant advances have been made in the delivery of targeting, imaging and therapeutic components on nanometer-sized particles (such as quantum dots, colloidal gold and polymeric nanomicelles) to create multiple modalities that are unavailable from individual components [56]. Recently, multifunctional mitochondria-targeted compounds have also been identified and could shed light on cancer-targeted imaging and therapy. Current strategies for the development of multifunctional mitochondria-targeted agents are summarized in Fig. 1.

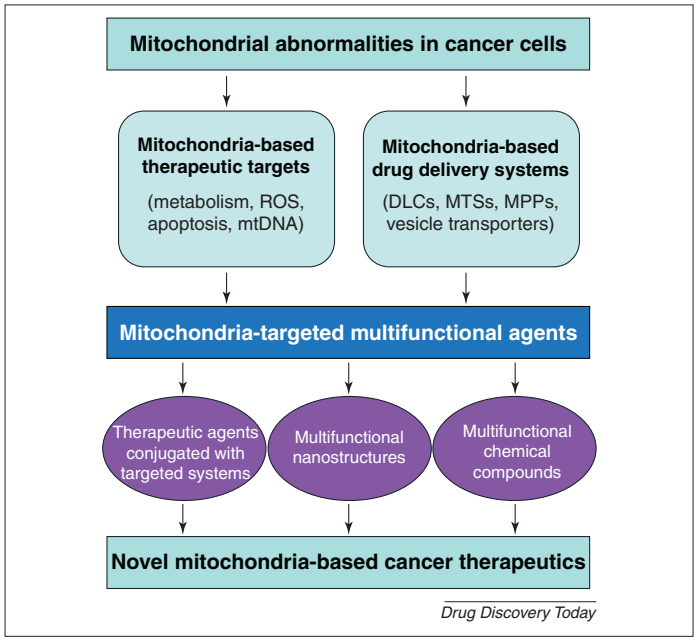

FIG. 1

Strategies for the development of multifunctional agents in mitochondria-based cancer therapy.

Multifunctional nanoparticles

Nanomaterials have been demonstrated to be excellent tools for molecular imaging, diagnosis and targeted therapy. Nanotechnology has been applied to target cancer cells, especially the mitochondria of cancer cells, through various strategies. It has been reported that zinc oxide (ZnO) nanoparticles induce mitochondrial cytotoxicity in human colon carcinoma LoVo cells through alteration of the electron transport chain, triggering the apoptotic pathways of the cell [57]. Currently, multiple functionalities, including targeting, imaging and therapeutics, can be integrated into one nanoparticle. Through mitochondrial targeting of cancer cells, nucleic acids, proteins, antioxidants and anticancer agents

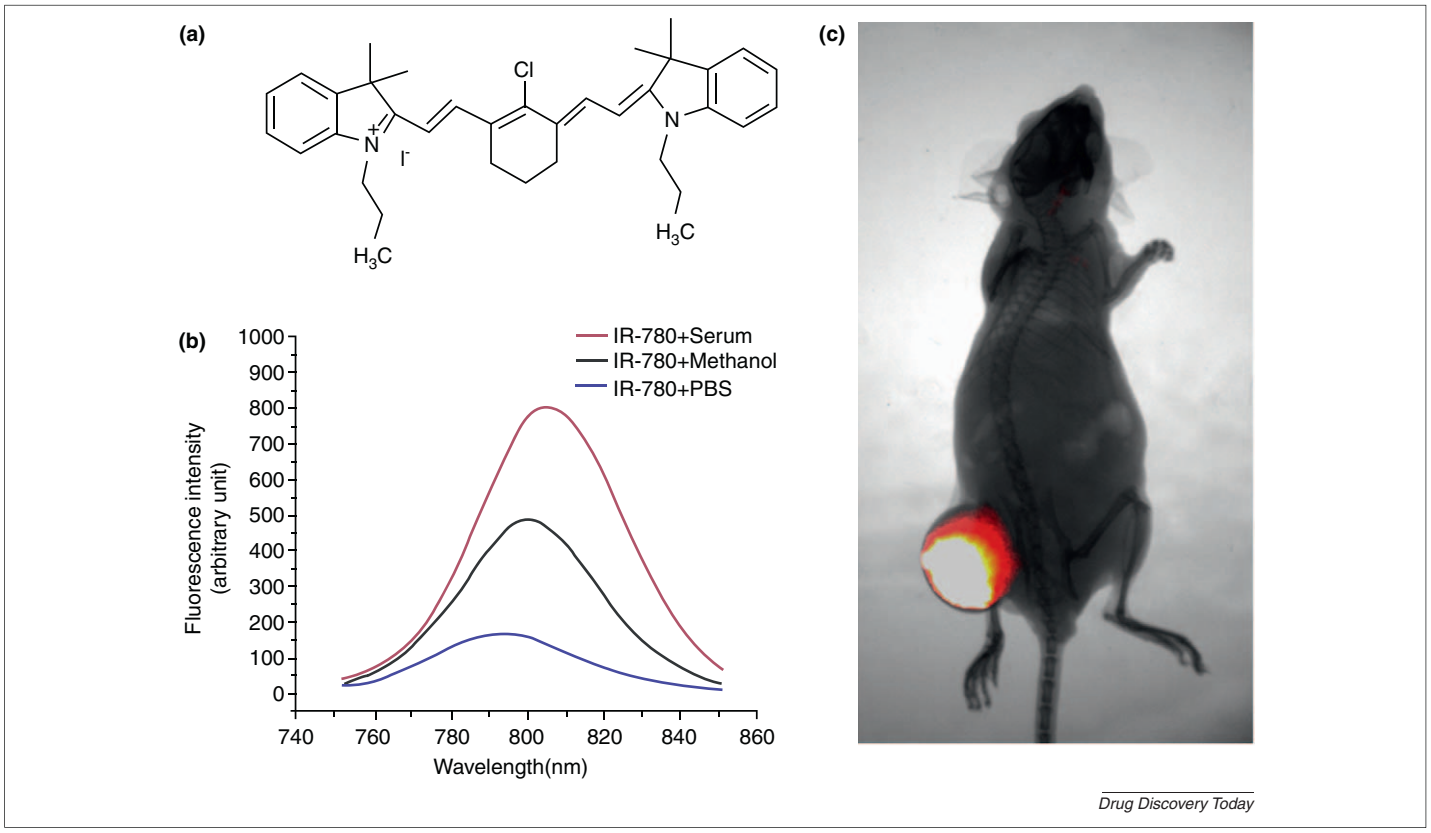

FIG. 2

Chemical, optical properties and preferential tumor retention of an active NIR heptamethine dye, IR-780 iodide. **(a)** Chemical structure of IR 780 iodide. **(b)** Emission fluorescence wavelength scan of 1 μm IR-780 iodide in methanol (black), phosphate-buffered saline (blue) and 100% fetal bovine serum (red). **(c)** NIR imaging of human HeLa tumor xenografts in an athymic nude mouse. The animal was subjected to NIR fluorescence and X-ray imaging with the Kodak In-Vivo Imaging System FX Pro after 48 hours administration of IR-780 iodide at a single dose of 0.2 mg kg^{-2}. The images are merged to show tumor localization.

can be delivered in nanostructures and act as targeted therapeutic agents.

In addition to many nanoparticles with single functions, some multifunctional nanoparticles are now under investigation to provide a promising method for mitochondria-targeted cancer treatment. The multifunctional envelope-type nano-device (MEND), which is based on the new packaging concept 'programmed packaging', is one such example [45]. MEND, which is similar to envelope-type viruses, comprises a condensed core, such as plasmid DNA, and a lipid envelope equipped with various functional devices, which keep their original properties and exist as separate structures in the compound. Condensation of these therapeutic molecules has many advantages, including protection from enzymes, size control, improved packaging efficiency and release over a short period of time. To locate these nanoparticle selectively in the mitochondria of cancer cells, different functional devices, which contain ligands for specific receptors, peptides for endosomal escape and mitochondrial targeting drugs, could be easily incorporated into the core and onto the surface of particles. These unique characteristics make this design a promising agent for drug delivery [58,59]. Several multifunctional mitochondrial nanoparticles have been successfully used for cancer therapy. For example, dendrimer phthalocyanine-encapsulated polymeric micelles have the ability to photodamage the mitochondria upon photoirradation and doxorubicin (DOX)-conjugated micelles can increase DOX mitochondrial accumulation through an αvβ3 integrin-targeting ligand on the micellar surface [60,61].

Multifunctional chemical compounds

To achieve simultaneous tumor targeting, imaging and therapy, an ideal strategy is to develop agents that are natively multifunctional. Recently, a class of multifunctional heptamethine indocyanine dyes was developed for tumor targeting, imaging and even killing without the need for chemical conjugation to target ligands [62]. These multifunctional dyes are lipophilic cationic molecules that preferentially accumulate in the mitochondria of tumor cells with unique near-infrared (NIR) imaging properties. For instance, a prototypical dye, IR-780 iodide (Fig. 2a), can reach a NIR region at 780 nm and can be detected conveniently by a NIR fluorescent detection system without significant autofluorescence interference [63]. Furthermore, this dye has a fluorescence enhancement effect in serum (Fig. 2b). More interestingly, this multifunctional dye preferentially accumulates in the mitochondria of a variety of human tumor xenografts to achieve good signal:noise ratios with minimal background autofluorescence (Fig. 2c). The signal:noise ratio can reach up to 20, whereas in other studies, a ratio of more than 2.5 is considered as substantial accumulation [64]. Additionally, the fluorescence signal is relatively stable and is able to persist in tumors for at least 20 days, enabling repeated imaging. These multifunctional compounds raise the possibility of developing

new and sensitive approaches for tumor growth detection and the evaluation of the effectiveness of anticancer drug treatments. Furthermore, these dyes can also be used to detect small lesions, such as metastatic tumors or even tumor cells in circulation. For instance, after mixing human prostate cancer cells with human blood cells, cancer cells could be visualized by NIR dye imaging; the dye was sufficiently sensitive to detect as few as ten cancer cells per milliliter in whole blood [65]. These results demonstrate the high cancer specificity of these dyes and make *in vivo* tumor targeting by NIR dyes more attractive as an imaging tool.

In addition to cancer targeting and imaging properties, these dyes also have potential tumoricidal activities. Similar to most DLCs, these dyes are toxic to cell mitochondria at high concentrations [42]. This cytotoxicity might be related to the direct or indirect inhibition of mitochondrial ATP synthesis. Some cyanine dyes and DLCs have also been studied extensively as potential photosensitizers, suggesting that some of these multifunctional dyes could also be used for cancer photodynamic therapy [66]. For clinical use, the agents must be biocompatible and safe. These multifunctional dyes have shown superior pharmacokinetic and safety properties without any apparent acute toxicity at the imaging dose. The metabolic pathway of IR-780 was similar to that of most cyanine dyes, such as indocyanine green, excreted by liver and exclusively into the bile. However, although they can be cleared from the host at the imaging dose in a couple of days, their long-term toxicity needs to be carefully assessed. Taken together, for future cancer treatment purpose, these dyes can be used in at least three ways: (i) they can be native anticancer drugs after accumulation in cancer cells owing to their intrinsic antitumor activities; (ii) they can be converted to potent inhibitors of oxidative phosphorylation by photothermal therapy; and (iii) they can be cancer-specific delivery platforms that preferentially target and deliver anticancer drugs to the cytoplasm of cancer cells, which could open a desirable area for cancer therapeutic design.

Concluding remarks

The success in using synthesized compounds to gain access to mitochondria of cancer cells has inspired the development of a variety of systems that use artificial structures and sequences to target and heal cancers. A variety of DLCs, amino acids and peptides with mitochondrial-specific properties have been described and primary results show significant promise for their use as effective mitochondrial delivery agents. With rapid advances in chemical synthesis and modification, these agents provide a robust framework in which to incorporate two or more components to give multifunctional capabilities. As described above, many compounds have been used to transport exogenous cargo into the mitochondria of cancer cells. The ability to target, image and deal with cancers simultaneously by incorporating multiple components to develop multifunctional agents could prove advantageous over conventional chemotherapy. However, there are still challenges to improving the transfer of these therapeutic principles into clinical practice, such as structural changes that occur during the conjugation procedure.

To improve the specificity and selectivity of cancer mitochondria, a better understanding of the key pathophysiological differences between mitochondria in cancer cells and normal cells will help to improve the selectivity of mitochondrially targeted anticancer agents. Nanostructures have shown new hope for drug delivery, although the development of these multifunctional nanoparticles for clinical applications has proven to be challenging and they are still at an early or proof-of-concept stage. However, significant efforts must be made to overcome several fundamental problems and technical barriers, such as surface opsonization, uptake and retention in reticuloendothelial organs, difficulties in targeting and penetrating tumors, and long-term fate and toxicity concerns. The newly identified cancer mitochondria-targeted multifunctional compounds broaden the current concept of actively targeting tumors and could provide an alternative strategy for the development of novel solutions for cancer diagnosis and therapy.

Acknowledgements

This work was supported by following grants from China, including Chongqing Municipal Natural Science Foundation Program (CSTC2009BA5043); State Key Basic Research Development Program (2010CB529403); the National Natural Science Foundation Program (NSFC81072523) and the State Education Ministry Program (FANEDD200777).

References

1 Fulda, S. *et al.* (2010) Targeting mitochondria for cancer therapy. *Nat. Rev. Drug Discov.* 9, 447–464
2 Galluzzi, L. *et al.* (2010) Mitochondrial gateways to cancer. *Mol. Aspects Med.* 31, 1–20
3 Duchen, M.R. (2004) Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol. Aspects Med.* 25, 365–451
4 Moreno-Sanchez, R. *et al.* (2007) Energy metabolism in tumor cells. *FEBS J.* 274, 1393–1418
5 Storz, P. (2005) Reactive oxygen species in tumor progression. *Front. Biosci.* 10, 1881–1896
6 Pelicano, H. *et al.* (2004) ROS stress in cancer cells and therapeutic implications. *Drug Resist. Updat.* 7, 97–110
7 Pelicano, H. *et al.* (2006) Glycolysis inhibition for anticancer treatment. *Oncogene* 25, 4633–4646
8 Radisky, D.C. *et al.* (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 436, 123–127
9 Chatterjee, A. *et al.* (2006) Mitochondrial DNA mutations in human cancer. *Oncogene* 25, 4663–4674
10 Ishikawa, K. *et al.* (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 320, 661–664
11 Ohta, S. (2006) Contribution of somatic mutations in the mitochondrial genome to the development of cancer and tolerance against anticancer drugs. *Oncogene* 25, 4768–4776
12 Kroemer, G. *et al.* (2007) Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* 87, 99–163
13 Sakuragi, N. *et al.* (2002) Bax Bcl-2, and p53 expression in endometrial cancer. *Gynecol. Oncol.* 86, 288–296
14 Gogvadze, V. *et al.* (2009) Mitochondria as targets for chemotherapy. *Apoptosis* 14, 624–640
15 Simons, A.L. *et al.* (2007) 2-Deoxy-D-glucose combined with cisplatin enhances cytotoxicity via metabolic oxidative stress in human head and neck cancer cells. *Cancer Res.* 67, 3364–3370
16 Xu, R.H. *et al.* (2005) Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. *Cancer Res.* 65, 613–621
17 Wang, F. *et al.* (2010) Small mitochondria-targeting molecules as anti-cancer agents. *Mol. Aspects Med.* 31, 75–92
18 Cao, X. *et al.* (2007) Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother. Pharmacol.* 59, 495–505

19 Pelicano, H. *et al.* (2003) Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. *J. Biol. Chem.* 278, 37832–37839

20 Dong, L.F. *et al.* (2008) Alpha-tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. *Oncogene* 27, 4324–4335

21 Huang, P. *et al.* (2000) Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* 407, 390–395

22 Matei, D. *et al.* (2009) Activity of 2 methoxyestradiol (Panzem NCD) in advanced, platinum-resistant ovarian cancer and primary peritoneal carcinomatosis: a Hoosier Oncology Group trial. *Gynecol. Oncol.* 115, 90–96

23 Tevaarwerk, A.J. *et al.* (2009) Phase I trial of 2-methoxyestradiol NanoCrystal dispersion in advanced solid malignancies. *Clin. Cancer Res.* 15, 1460–1465

24 Maeda, H. *et al.* (2004) Effective treatment of advanced solid tumors by the combination of arsenic trioxide and L-buthionine-sulfoximine. *Cell Death Differ.* 11, 737–746

25 Dragovich, T. *et al.* (2007) Phase I trial of imexon in patients with advanced malignancy. *J. Clin. Oncol.* 25, 1779–1784

26 Moulder, S. *et al.* (2010) A phase I trial of imexon, a pro-oxidant, in combination with docetaxel for the treatment of patients with advanced breast, non-small cell lung and prostate cancer. *Invest. New Drugs* 28, 634–640

27 Kang, M.H. and Reynolds, C.P. (2009) Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin. Cancer Res.* 15, 1126–1132

28 Vogler, M. *et al.* (2008) A novel paradigm for rapid ABT-737-induced apoptosis involving outer mitochondrial membrane rupture in primary leukemia and lymphoma cells. *Cell Death Differ.* 15, 820–830

29 O'Brien, S.M. *et al.* (2005) Phase I to II multicenter study of oblimersen sodium, a Bcl-2 antisense oligonucleotide, in patients with advanced chronic lymphocytic leukemia. *J. Clin. Oncol.* 23, 7697–7702

30 Lessene, G. *et al.* (2008) BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discov.* 7, 989–1000

31 Oudard, S. *et al.* (2003) Phase II study of lonidamine and diazepam in the treatment of recurrent glioblastoma multiforme. *J. Neurooncol.* 63, 81–86

32 Giurgiovich, A.J. *et al.* (1997) Elevated mitochondrial cisplatin–DNA adduct levels in rat tissues after transplacental cisplatin exposure. *Carcinogenesis* 18, 93–96

33 Okamaoto, M. *et al.* (2003) Ditercalinium chloride, a pro-anticancer drug, intimately associates with mammalian mitochondrial DNA and inhibits its replication. *Curr. Genet.* 43, 364–370

34 Segal-Bendirdjian, E. *et al.* (1988) Selective loss of mitochondrial DNA after treatment of cells with ditercalinium (NSC 335153), an antitumor bis-intercalating agent. *Cancer Res.* 48, 4982–4992

35 Biasutto, L. *et al.* (2010) Mitochondrially targeted anti-cancer agents. *Mitochondrion* 10, 670–681

36 Chen, L.B. (1988) Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol.* 4, 155–181

37 Davis, S. *et al.* (1985) Mitochondrial and plasma membrane potentials cause unusual accumulation and retention of rhodamine 123 by human breast adenocarcinoma-derived MCF-7 cells. *J. Biol. Chem.* 260, 13844–13850

38 Nadakavukaren, K.K. *et al.* (1985) Increased rhodamine 123 uptake by carcinoma cells. *Cancer Res.* 45, 6093–6099

39 Modica-Napolitano, J.S. and Aprille, J.R. (2001) Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. *Adv. Drug Deliv. Rev.* 49, 63–70

40 Summerhayes, I.C. *et al.* (1982) Unusual retention of rhodamine 123 by mitochondria in muscle and carcinoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 79, 5292–5296

41 Yousif, L.F. *et al.* (2009) Targeting mitochondria with organelle-specific compounds: strategies and applications. *ChemBioChem* 10, 1939–1950

42 Lampidis, T.J. *et al.* (1984) Effects of the mitochondrial probe rhodamine 123 and related analogs on the function and viability of pulsating myocardial cells in culture. *Agents Actions* 14, 751–757

43 Teicher, B.A. *et al.* (1986) Intracellular distribution of a platinum–rhodamine 123 complex in *cis*-platinum sensitive and resistant human squamous carcinoma cell lines. *Biochem. Pharmacol.* 35, 3365–3369

44 Ross, M.F. *et al.* (2004) Cell-penetrating peptides do not cross mitochondrial membranes even when conjugated to a lipophilic cation: evidence against direct passage through phospholipid bilayers. *Biochem. J.* 383, 457–468

45 Yamada, Y. and Harashima, H. (2008) Mitochondrial drug delivery systems for macromolecule and their therapeutic application to mitochondrial diseases. *Adv. Drug Deliv. Rev.* 60, 1439–1462

46 Mukhopadhyay, A. *et al.* (2005) Bacterial signal peptide recognizes HeLa cell mitochondrial import receptors and functions as a mitochondrial leader sequence. *Cell. Mol. Life Sci.* 62, 1890–1899

47 Zhang, C. *et al.* (1998) Photosensitisation properties of mitochondrially localised green fluorescent protein. *Biochem. Biophys. Res. Commun.* 242, 390–395

48 Esaki, M. *et al.* (1999) Two distinct mechanisms drive protein translocation across the mitochondrial outer membrane in the late step of the Cytochrome b(2) import pathway. *Proc. Natl. Acad. Sci. U. S. A.* 96, 11770–11775

49 Yousif, L.F. *et al.* (2009) Mitochondria-penetrating peptides: sequence effects and model cargo transport. *ChemBioChem* 10, 2081–2088

50 Del Gaizo, V. *et al.* (2003) Targeting proteins to mitochondria using TAT. *Mol. Genet. Metab.* 80, 170–180

51 Datta, K. *et al.* (2001) The 104–123 amino acid sequence of the β-domain of von Hippel–Lindau gene product is sufficient to inhibit renal tumor growth and invasion. *Cancer Res.* 61, 1768–1775

52 Ellerby, H.M. *et al.* (1999) Anti-cancer activity of targeted pro-apoptotic peptides. *Nat. Med.* 5, 1032–1038

53 Horton, K.L. *et al.* (2008) Mitochondria-penetrating peptides. *Chem. Biol.* 15, 375–382

54 Weissig, V. *et al.* (2004) Mitochondrial pharmaceutics. *Mitochondrion* 3, 229–244

55 Yamada, Y. *et al.* (2008) MITO-Porter: a liposome-based carrier system for delivery of macromolecules into mitochondria via membrane fusion. *Biochim. Biophys. Acta* 1778, 423–432

56 Wang, X. *et al.* (2008) Application of nanotechnology in cancer therapy and imaging. *CA Cancer J. Clin.* 58, 97–110

57 De Berardis, B. *et al.* (2010) Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. *Toxicol. Appl. Pharmacol.* 246, 116–127

58 Yamada, Y. *et al.* (2007) Mitochondrial drug delivery and mitochondrial disease therapy – an approach to liposome-based delivery targeted to mitochondria. *Mitochondrion* 7, 63–71

59 Kogure, K. *et al.* (2004) Development of a non-viral multifunctional envelope-type nano device by a novel lipid film hydration method. *J. Control. Release* 98, 317–323

60 Nishiyama, N. *et al.* (2009) Enhanced photodynamic cancer treatment by supramolecular nanocarriers charged with dendrimer phthalocyanine. *J. Control. Release* 133, 245–251

61 Xiong, X.-B. *et al.* (2010) The therapeutic response to multifunctional polymeric nano-conjugates in the targeted cellular and subcellular delivery of doxorubicin. *Biomaterials* 31, 757–768

62 Zhang, C. *et al.* (2010) A near-infrared fluorescent heptamethine indocyanine dye with preferential tumor accumulation for in vivo imaging. *Biomaterials* 31, 6612–6617

63 Zhang, C. *et al.* (2010) Sentinel lymph node mapping by a near-infrared fluorescent heptamethine dye. *Biomaterials* 31, 1911–1917

64 Andreev, O.A. *et al.* (2007) Mechanism and uses of a membrane peptide that targets tumors and other acidic tissues in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 104, 7893–7898

65 Yang, X. *et al.* (2010) Near IR heptamethine cyanine dye-mediated cancer imaging. *Clin. Cancer Res.* 16, 2833–2844

66 Mojzisova, H. *et al.* (2007) Structural and physico-chemical determinants of the interactions of macrocyclic photosensitizers with cells. *Eur. Biophys. J.* 36, 943–953