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REVIEW ARTICLE

Mitochondrial Targeting Peptide-based Nanodelivery for Cancer Treatment

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Abstract: Mitochondria are important intracellular organelles because of their key roles in cellular metabolism, proliferation, and programmed cell death. The differences in the structure and function of the mitochondria of healthy and cancerous cells have made mitochondria an interesting target for drug delivery. Mitochondrial targeting is an emerging field as the targeted delivery of cytotoxic payloads and antioxidants to the mitochondrial DNA is capable of overcoming multidrug resistance. Mitochondrial targeting is preferred over nuclear targeting because it can take advantage of the distorted metabolism in cancer. The negative membrane potential of the inner and outer mitochondrial membranes, as well as their lipophilicity, are known to be the features that drive the entry of compatible targeting moiety, along with anticancer drug conjugates, towards mitochondria. The design of such drug nanocarrier conjugates is challenging because they need not only to target the specific tumor/cancer site but have to overcome multiple barriers as well, such as the cell membrane and mitochondrial membrane. This review focuses on the use of peptide-based nanocarriers (organic nanostructures such as liposomes, inorganic, carbon-based, and polymers) for mitochondrial targeting of the tumor/cancer. Both *in vitro* and *in vivo* key results are reported.

Keywords: Cancer treatment, nanodelivery, mitochondria, targeted peptides, liposomes, tumor.

1. INTRODUCTION

1.1. Cancer

Cancer is a broad term used to describe more than 100 distinctive diseases associated with the abnormal and uncontrolled growth of cells [1,2]. This uncontrolled growth leads to the formation of heterogeneous masses of cells called tumors. Tumors are classified as benign, premalignant, or malignant. Benign tumors are noncancerous as they stay at the primary site of origin and do not invade other tissues or organs. Premalignant tumors are characterized by the transtition from benign to malignant. In contrast to benign and premalignant tumors, malignant tumors are cancerous and spread to distant locations *via* metastasis [3]. Benign tumors can often be completely removed by surgical procedures and do not grow back, while malignant tumors have the potential to grow back. Premalignant tumors may require careful monitoring and need to be surgically removed or treated if they lead to the formation of "dysplasia" masses in which cells reproduce faster than normal and appear abnormal or "carcinoma in situ" in which cells are extremely abnormal to a point where they can potentially start invading other cells. This state of cancer growth is being referred to as "0-stage cancer" [3].

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Cancer can occur anywhere in the body and is mostly named after the region of origin. Most of the cancer types show symptoms at earlier stages, but some do not show obvious symptoms until stage 3 or 4. The lack of symptoms at earlier stages makes it harder to properly diagnose cancer, which significantly reduces the chances of survival. Such cancers are named "silent killers". Some examples include ovarian cancer, colorectal cancer, cervical cancer, lung cancer, pancreatic cancer, and breast cancer [4-8].

After heart diseases, cancer has the second highest mortality rate worldwide. According to recent statistics, there were 19.3 million new cancer cases and almost 10.0 million cancer deaths globally in the year 2020 [2]. According to the American Cancer Society statistics, about 1,898,160 new cancer cases are expected in the coming years, and 608,570 cancer deaths have already been reported in the US alone in 2022. That is approximately 4 new cases and 1 death per minute [2]. This indicates the severity of this malignant disease, which is not only impacting human life but also increasing the socioeconomic pressure worldwide. The cost of managing cancer treatments or the individual's inability to work are major outcomes that lead to poverty. It is particularly true in developing countries that lack proper health care systems and cancer care centers [9]. Thanks to cancer research, the number of survivors has increased over the last decade, giving hope to people suffering from this disease [10].

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A well-known cause of cancer is gene mutations. There are two types of these mutations: acquired mutations and germline mutations. Acquired mutations occur due to damage to the genes of a particular cell at any point during a person's life. Factors that can trigger an acquired gene mutation include ultraviolet radiation, use of tobacco, viral/bacterial infections, and age. Germline mutations occur from a mutation in the egg cell or the sperm cell and can pass from one generation to the next generation. Such mutations lead to cancers that can be transferred from parent to offspring and are named "inherited cancers" [11]. Mutations may also occur in genes that control cell proliferation mechanisms by either over-activating growth-promoting genes or suppressing tumor-suppressor genes [12]. Depending on the type and stage of cancer, a patient may undergo different treatments. Some of the most common cancer treatments include surgical removal of tumors and cancers, chemotherapy, and radiation therapy. With recent advances in cancer research, some new technologies have emerged as means of more effective cancer treatment. These include hormone therapy that uses hormones to control cellular behavior, immunotherapy that uses a person's self-defense system to target and kill cancerous cells, bone marrow (or stem cell) transplants in which defective cells are replaced by bone marrow/stem cells to late grow to normal cells and targeted therapy in which tumor or cancer cells are targeted with minimal or no impact on the normal cells. Drug-based treatments (such as chemotherapy, immunotherapy, and hormone therapy) are often named systematic treatments as they usually affect the whole body [13]. Radiation-based therapies are useful for small and localized tumors but may affect the growth of normal cells or sometimes induce them to generate secondary tumors. Surgical procedures for cancer removal and bone marrow transplant are invasive and expensive procedures with common side effects of developing infections and loss of blood resulting in a weakened immune system. Owing to these side effects, a patient undergoing cancer treatment often suffers from hair loss, fatigue, nausea, diarrhea, infertility, low blood count, and skin infections [13,14]. Targeted therapies are getting more attention from scientists and extensive research is ongoing in this field to develop a platform for targeted delivery that will have the potential to effectively target and treat cancer with minimal side effects to normal non cancerous cells.

2. MITOCHONDRIA AS A TARGET FOR CANCER TREATMENT

The mitochondrion is a double membrane bound organelle of the cell (Fig. **1**). It facilitates vital functions such as cellular respiration and homeostasis (calcium). It also initiates programmed cell death processes (apoptosis) that are pivotal to the survival of an organism. Mitochondria are considered the 'powerhouse' of the cell as they provide adenosine 5'-triphosphate (ATP) that is considered the 'energy currency' of the cell. Due to the involvement in cell cycle regulation and energy metabolism, mitochondria are essential in cancer physiology [15].

Studies have found a difference in the energy production process between normal cells and tumor cells. In normal cells, mitochondria generate energy for the cell in three steps: glycolysis, tricarboxylic acid cycle (TCA cycle), and oxidative phosphorylation [16]. Glycolysis breaks down glucose into pyruvate that is used to fuel the TCA cycle. The conversion of glucose to pyruvic acid releases energy that is stored as ATP. In the mitochondrial matrix, pyruvate is converted to Acetyl Coenzyme A, which then starts the TCA cycle where ATP, NADH, and $FADH_2$ are produced while carbon dioxide is released as the waste product. The high energy electrons from NADH and $FADH_2$ are used as fuel in the third cycle and passed through the electron-transport chain, the energy released is used to pump the protons from the mitochondrial matrix to the intermembrane space. The transfer of protons across the membrane creates an electrochemical gradient that drives the ATP production process. The energy is released when the protons move back into the matrix and are used by ATP synthase to generate ATP from ADP [17]. On the other hand, tumor cells perform a less efficient ATP production pathway through glycolysis followed by lactic acid fermentation. This specific type of respiration is called the "Warburg effect" or "aerobic glycolysis" because it occurs even in the presence of oxygen (although many tumors are hypoxic) [15]. This effect is opposite to the Pasteur effect that describes increased fermentation in hypoxic conditions. The conflict between the two effects is often associated with prolyl hydroxylase and the expression of hypoxia-inducible factor (HIF) that converts normoxia to hypoxia [18]. Studies have found that the mitochondria of cancer cells have the ability to switch between aerobic glycolysis and oxidative phosphorylation depending on the cellular needs [18]. Although most of cancer cells have functional mitochondria, some studies have found mutated enzymes in TCA such as isocitrate dehydrogenase (IDH), aconitate hydratase (AH), succinate dehydrogenase (SDH), fumarate hydratase (FH), and KGDHC, *etc*. In such instances, an increased rate of glycolysis is the sole way to fulfill ATP needs [16]. Cancer cells usually demand higher energy due to faster and uncontrolled proliferation of cells resulting in higher energy demands causing cellular stress, which in response to that, the nuclear genome increases the biogenesis of mitochondria in cancer cells [18,19].

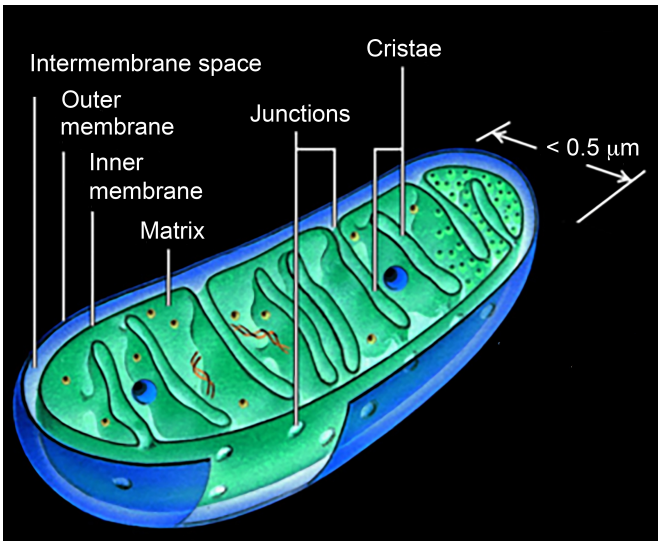


Fig. (1). Mitochondria; structure and compartments. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Sometimes, mitochondrial dysfunction may also trigger the generation of tumors in the body because of the generation of reactive oxygen species (ROS) byproducts during respiration cycles that may promote genetic instability, DNA damage, and disturbance to metabolic signaling pathways that ultimately lead to the formation of tumors. These reactive species are produced when electrons leak from the respiratory complexes and react with molecular oxygen forming superoxide anions. Higher accumulation of ROS can also trigger the release of cytochrome c into the cytoplasm and initiate cell death mechanisms. Hence, cancer cells find a balance between other metabolic processes and ROS accumulation to maintain cell survival [19].

A major challenge to current cancer treatment is multidrug resistance (MDR), which is known to be associated with increased overexpression of ATP-driven transmembrane efflux pump in cancer cells. Cancer cells showing MDR often contain higher polarized mitochondrial mass than non-MDR cancerous cells. As the ATP required for MDR comes from the cancer cells' mitochondria, cancer cell death can be induced by targeting these mitochondria. Hence, mitochondria are an emerging target to overcome multidrug resistance (MDR) resulting from chemotherapeutics [20].

A relevant study conducted by Han and coworkers used a chimeric peptide-based drug delivery system to target mitochondria (Fig. **2**). It consists of a photosensitizer (protoporphyrin IX), a targeting peptide D(KLAKLAK)2-GRGD, and the drug doxorubicin. The drug release from mitochondria, followed by photodynamic therapy, induces the production of ROS in HeLa and human breast carcinoma cells. Increased ROS levels result in mitochondrial disruption, ultimately leading to decreased ATP synthesis. This reduction in ATP inhibits ATP-dependent doxorubicin efflux, hence overcoming drug resistance [21].

With a specific delivery of anticancer drugs to mitochondria, not only MDR but side effects associated with nuclear DNA targeting of drugs can also be avoided. An example is doxorubicin, which is a potent anticancer agent but is associated with cardiotoxicity that causes congestive heart failure in almost 20% of cancer patients. If the mitochondria of cardiac cells get damaged under stress, such as oxidative damage or heavy exercise, cardiac cells can regenerate functional mitochondria through mitochondrial biogenesis. However, when doxorubicin is used for therapeutic purposes, it damages nuclear DNA by inhibiting topoisomerase activity, resulting in decreased nuclear-encoded members of the mitochondrial transcriptome that lead to inhibition of mitochondrial biogenesis and cardiomyocytes die. In this regard, mitochondrial targeting of doxorubicin can save the nucleus of cardiac cells without disturbing their regeneration ability [22]. Different strategies can be employed to trigger a specific cellular response by mitochondria, such as targeting mitochondrial metabolism by using inhibitors of glycolysis, oxidative phosphorylation, TCA cycle, or directly inducing cell death mechanisms by targeting apoptotic factors such as Bcl-2 or Bcl-xL proteins or regulating ROS production to induce cell death [18]. To achieve that goal, several chemical agents and drug molecules are currently in clinical trials or in use that can effectively act on mitochondria to trigger a specific cellular response such as paclitaxel, 2-DG, WZB117, DCA, metformin, ABT-737, TW-37, VP-16 (etoposide), vinorelbine, ceramide, MKT077, CD437, lonidamine, and betulinic acid [17, 23].

Considering the dynamics in cancer physiology and the potential benefits of mitochondrial targeting of anticancer drugs, there is no doubt that mitochondria are an interesting target for cancer therapy. As mitochondrial targeting is an emerging area, there is still a need of developing better targeting agents that may lead anticancer drugs, imaging probes, and other cytotoxic payloads towards the mitochondria of cancer cells with increased accumulation and overall enhanced efficacy with minimal side effects.

2.1. Peptides for Mitochondrial Targeting

The importance of mitochondria in cancer physiology makes them a universal pharmacological target for almost all cancer types. Conventional cancer therapies for treatment suffer from non-specific targeting and off-target toxicity. To date, there are certain chemical moieties developed for mitochondrial targeting, for example, lipophilic cations (triphenylphosphine or TPP, triethylammonium, phenylsulfonylfuroxan, guanidine, dequalinium, indolinium) and fluorescent dyes (rhodamine, cyanine, pyridinium, and borondipyrromethane). However, these chemical compounds have some drawbacks that include fetal toxicity in physiological environments and complex synthetic requirements. Furthermore, linkers are usually required for ligation with other components of drug conjugates. Cellular uptake is charge dependent, which may result in the significant uptake of drug conjugates by normal cells [24]. The concept of targeting intracellular organelle mitochondria with peptides is a promising approach and it started with the use of modified cell-penetrating TAT peptides by adding arginine and cyclohexyl groups to make it more lipophilic for targeting the mitochondria [25]. Recent studies have shown three types of peptides for mitochondrial targeting that include mitochondria penetrating peptides (MPP), Szeto-Schiller peptides (a class of short cell-permeable peptides having antioxidative activity), and the mitochondrial targeting sequence peptide (MTS) [26].

MPPs are synthetic peptides (usually 4-16 amino acids in length) that have the ability to translocate through the cell membranes and localize specifically in the mitochondrial matrix. The uptake of these peptides is driven by an electrochemical gradient. MPPs are designed to carry cationic charges and hydrophobic amino acid residues [27].

SS peptides are short amino acid sequences consisting of four alternating aromatic and basic amino acid residues (tyrosine, dimethyltyrosine (Dmt), phenylalanine (Phe), arginine, and lysine). These positively charged peptides have the ability to permeate the cell membrane and selectively localize in the inner membrane of mitochondria. SS peptides are known to have antioxidant activity against ROS owing to the presence of aromatic amino acids (dimethyltyrosine and tyrosine) that can capture free radicals. The mechanism of uptake is known to be the energy-independent uptake, so SS peptides can freely penetrate at physiological pH. [28, 29]. Among other reported SS peptides, SS-31 (*D*-Arginine-Dmt-Lysine-Phe-NH_2) has progressed to clinical trials for use in

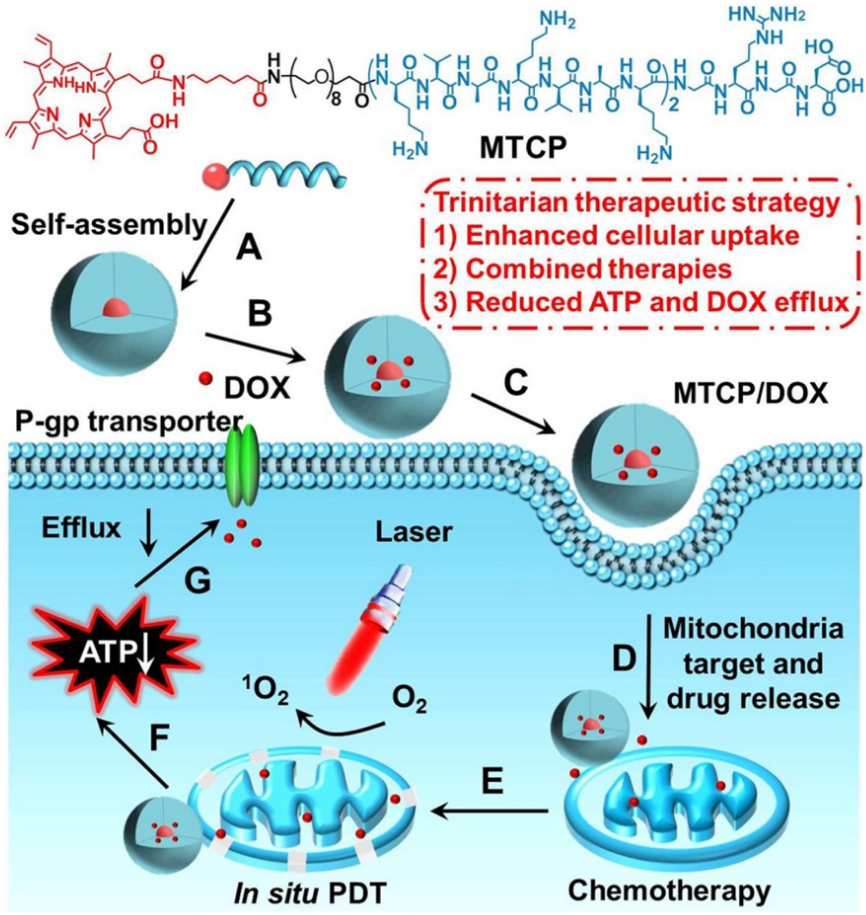


Fig. (2). "Self-assembly of mitochondrial targeting chimeric peptide-doxorubicin and trinitarian inhibition of drug resistance at tumor site**).** (**A-C**) Peptide self-assembly, encapsulation of doxorubicin and internalization of peptide-doxorubicin complex (**D**) Peptide-mediated mitochondria-targeted accumulation of peptide-doxorubicin followed by controlled release of drug (**E**) in situ photodynamic therapy leading mitochondrial disruption (**F-G**) Decreased intracellular ATP generation and suppressed efflux of doxorubicin." Reprinted with permission from reference [21]. Copyright 2016, American Chemical Society, Washington, DC. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

ischemia reperfusion and microvascular injury in patients [29]. MTS peptides usually consist of 20-40 amino acid residues. These peptides are recognized by receptors present on the surface of mitochondria [29,30].

The use of peptides as targeting entities in cancer therapy is considered a safer, biocompatible, and highly specific approach. The use of peptides to target the mitochondria also gives the advantage of better protein-protein or protein-lipid interactions that are not possible with small drug molecules or nanoparticles alone [31].

Mitochondria have their own DNA (mtDNA) that encodes for 13 OXPHOS (mitochondrial oxidative phosphorylation) proteins, while the other cellular proteins are encoded by the nuclear DNA and are passed to the mitochondria with the help of a N-terminal mitochondrial targeting signal sequence. The mitochondrial targeting signal sequences are recognized by the TIM/TOM protein complexes that let the translocation of proteins through the outer and inner membranes of the mitochondria [31]. Scientists have used a similar approach of developing mitochondrial targeting sequences to target the mitochondria for the delivery of cytotoxic payloads to the mitochondria. To design a peptide for mitochondrial targeting, the nature of both mitochondrial membranes should be considered as the outer membrane of mitochondria (OMM) allows the movement of metabolites, and the inner mitochondrial membrane (IMM) is highly dense and nonpolar. The peptides should be designed in a way that they can easily translocate through the mitochondrial membrane barriers *via* TIM/TOM transporter channels [23]. The overall charge (Z) of the peptide and its lipophilicity (log P that is defined as the logarithm of the octanol−water partition coefficient) are two important features to be considered while designing a peptide. This is important because of the highly nonpolar nature of mitochondrial membranes and to overcome their negative potential [31]. Studies have shown that peptides designed with an overall charge greater than 0 and log P values greater than −1.7 give a high mitochondrial localization [23,32]. Horton *et al.* developed a series of peptides for mitochondrial localization by combining positively charged amino acids (lysine and arginine) and the hydrophobic amino acid phenylalanine, as well as the chemically modified not natural amino acid cyclohexylalanine. This study also revealed that peptides with the cationic charge of +3 or above and log P values of approximately −2 localize well inside the mitochondria [32].

2.2. Nanomedicine: History and Importance

Nanomedicine has been practiced since ancient times, but the pioneering work in the history of modern nanomedicine was performed by Metchnikov and Ehrlich who were awarded the Nobel Prize for Medicine in 1908 for the work on phagocytosis and cell-specific diagnostic and therapy, respectively [33]. The major drawback of using peptides for targeted delivery is their low bioavailability. Peptides are prone to proteolytic digestion by a plethora of active proteases in biological serum that hence results in shorter half-lives [34,35]. On the other hand, drugs used for chemotherapy often show low bioavailability and poor selectivity. In this regard, nanoparticles as drug delivery vehicles have received great attention owing to their nanosize and high surface area. The encapsulation of such therapeutic peptides and anticancer drugs within nanocarriers may enhance their bioavailability. Moreover, specificity surface modification of nanocarriers with mitochondria targeting peptides can be performed easily, specifically targeting a certain tumor site. The controlled release of the encapsulated drug in response to a specific stimulus is another attractive feature of the nanoplatforms [36]. Depending on the nature of the material, nanocarriers can be classified as polymer-based [37], organic [37], inorganic [38], and carbon-based [39]. Organic nanomaterials are considered biocompatible due to the biodegradable nature and are less toxic compared to other nanomaterials [37]. Some of the examples include liposomes, micelles, dendrimers, and peptide nanosponges [40]. Inorganic nanomaterials consist of inorganic metal and metal oxide nanoparticles [38]. Some examples include gold (Au) [41], iron (Fe) [8], and iron oxide (Fe_2O_3), aluminum (Al) and aluminum oxide (Al_2O_3), zinc (Zn) and zinc oxide (ZnO) nanoparticles. Carbon-based nanomaterials are made up of only carbon. Some of the examples include fullerenes, carbon nanofibers, carbon nano tubes (CNT), carbon black, and graphene [42]. Further modification of nanocarrier materials is usually required to enhance the properties such as water stability, nontoxic nature, ease of ligation to biological moieties, and biodegradability [43].

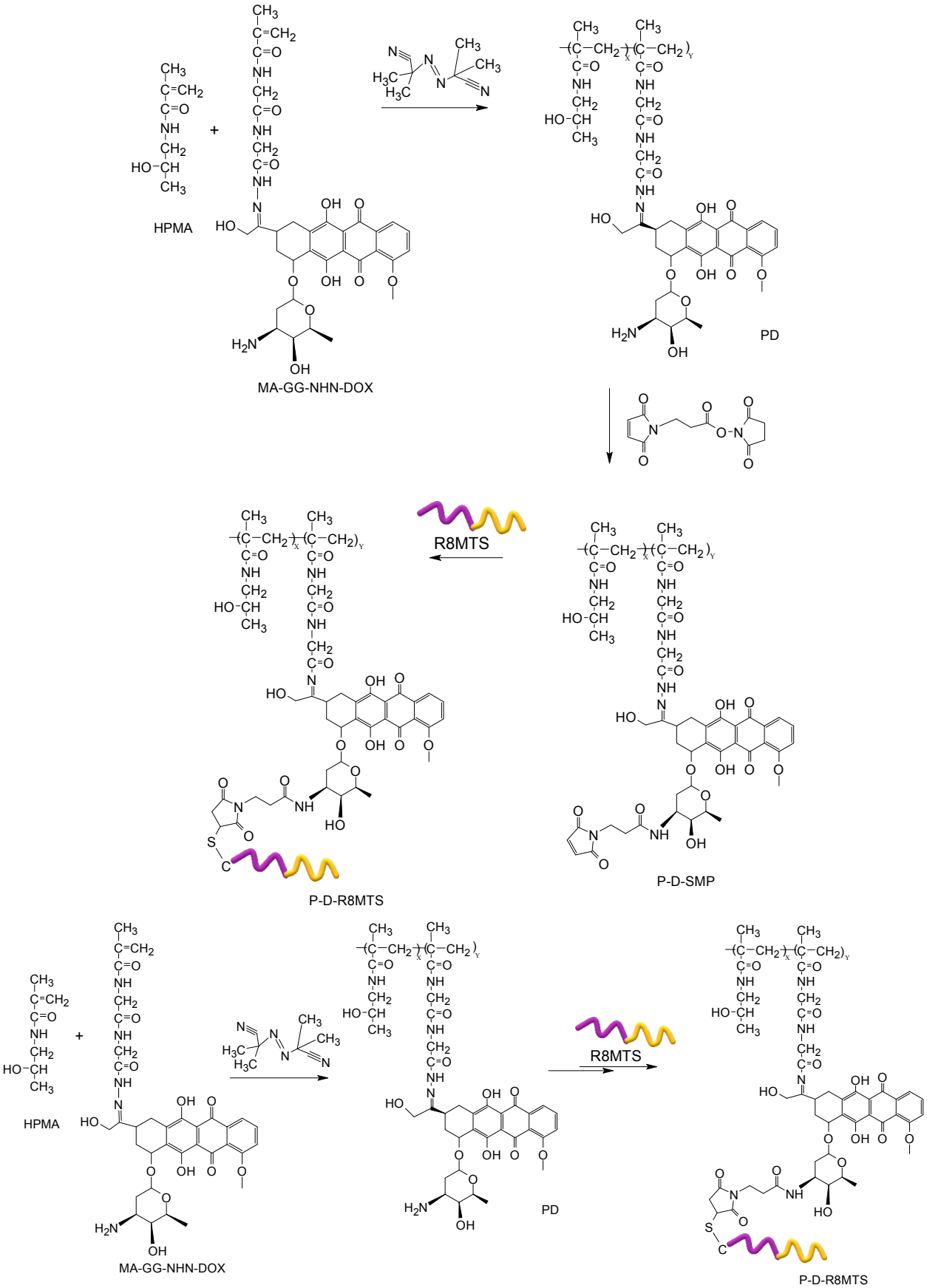
3. PEPTIDE-BASED NANOCARRIERS FOR MITOCHONDRIAL TARGETING

3.1. Mitochondrial Targeting Sequences and Polymer/Copolymer-Based Nanocarriers

Mitochondrial targeting sequences (MTS) are endogenous peptides that transport proteins synthesized in the cytoplasm to mitochondria by interacting with the mitochondrial import machinery that is attached to the outer and inner mitochondrial membranes [44]. Although MTS sequences have the ability to target mitochondria, they are reported to have poor penetration through the cell membrane. To overcome this issue, some recent studies have suggested combining a MTS peptide with a cell-penetrating peptide [35]. Li *et al.* reported a mitochondrial targeted drug delivery system (P-D-R8MTS) in which they combine a MTS peptide (ALD5MTS) with a cell penetrating peptide (octaarginine or R8) and conjugate the peptide assembly with copolymers featuring the anticancer drug doxorubicin [44]. They synthesize and characterize P-D-R8MTS, P-D-R8, P-D-MTS, and PD formulations for this study. For the synthesis of P-D-R8MTS, they conjugate this peptide assembly with N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers and then load the anticancer drug doxorubicin (DOX). The radical solution copolymerization strategy is employed for the synthesis of HPMA copolymer-DOX conjugates (PD) with a pH-sensitive hydrazone (HPMA and MA-GG-NHN=DOX using a molar ratio of 92.5:7.5). This conjugate is attached with N-succinimidyl-3-maleimidopropionate (SMP) using a molar ratio of 1:4 in dimethylformamide. Then the maleimide group of P-Dox-SMP complex is linked to the peptide conjugate R8MTS *via* C-S bond (Scheme **1**) [44].

The zeta potential values are more positive for P-D-R8 and P-D-R8MTS when compared to PD and PD-MTS because of the more cationic nature of the R8 peptide owing to the positively charged amino acid arginine. The drug release efficiency was tested at different pH levels. It was found that the release of doxorubicin was only 20% at physiological pH while almost 80% at pH 6.5.

The *in vitro* studies were performed on mouse breast cancer cell line 4T1 and the human breast cancer cell line MDA-MB-231. The flow cytometry and CLSM analysis showed that the cellular uptake was highest for P-D-R8 and P-D-R8MTS. After testing different inhibitors of the uptake pathways, the authors conclude that the mechanism for the internalization of the drug conjugate is energy-dependent endocytosis (caveolae and clathrin mediated) [44] for all polymers peptide conjugates. The CLSM imaging and line profiling also show that most of the copolymer peptide conjugate (P-D-R8MTS) is released into the cytoplasm and further localized in the mitochondria (2.50-fold higher than PD). The study suggested that after mitochondrial localization, there is the highest ROS generation for the P-D-R8MTS conjugate and the highest upregulation of cytochrome c is observed, leading to the start of intrinsic apoptosis. The expression levels of matrix metalloproteinase -2 (MMP-2, the enzyme responsible for ECM degradation) show that it is downregulated in both 4T1 cells and MDA-MB-231 cells when treated with the designed copolymer peptide conjugates. Hence, tumor metastasis is suppressed while the cytotoxicity of the drug conjugates is higher in 4T1 cells than in MDA-MB-231 cells owing to the heterogeneity of the tumor cell lines. The *in vivo* studies were performed in 4T1 tumor-bearing mice. The results indicated that P-D-R8MTS had 86.8% higher tumor growth inhibition compared to saline, while other copolymers of P-D-R8, PD, and P-D-MTS and free doxorubicin showed 66.7%, 61.2%, 58.6% and 38.3% inhibition, respectively. The mitochondrial accumulation of the copolymer peptide conjugate was highest for P-D-R8MTS. The *in vivo* lung metastasis investigation revealed that P-D-MTS and P-D-R8MTS inhibited lung metastasis of 4T1 tumor-bearing mice. The study revealed that the mitochondrial targeting sequence along with the cell penetrating peptide and copolymer conjugate efficiently targeted the mitochondria and significantly reduced tumor growth by inducing apoptosis [44]. Some other studies developed only polymer-based nanocarriers having the ability to specifically target the mitochondria. These comprise of cisplatin-loaded multifunctional polymer micelles having cell membrane/mitochondrion-targeting abilities [45], α-cyclodextrin - based PR polymeric prodrug of polyrotaxane-poly(doxorubicin)-*co*-poly[(ethylene glycol) methyl ether methacrylate



Scheme 1. Synthesis of P-D-R8MTS nanocarrier [44]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

[46], hyaluronic acid-d-α-tocopherol succinate-(4-carboxybutyl)triphenyl phosphonium bromide (HA-TS-TPP)-based nanocarriers for the delivery of lapatinib [47], theragnostic nanoparticles (NPs) with inner core of copolymer pluronic F127 and encapsulated near-infrared (NIR) heptamethine cyanine dye (me-IR825) for mitochondrial targeting and imaging [48,49] and HPMA copolymer backbone attached to glycyrrhetinic acid-Dox conjugate followed by the gelatin nanoparticles attachment (for tumor-cells internalization) leading to mitochondrial targeting at the tumor site [50].

3.2. Mitochondrial Penetrating Peptides and Carbon-Based Nanocarriers

As mitochondrial targeting sequences usually lack higher cellular uptake, Battigelli *et al.* reported carbon nanotube carriers to carry the peptide cargo across the cell membrane [51]. Carbon-based nanomaterials including nanoparticles, fullerenes, carbon nanotubes (CNTs), and graphene are an emerging class of delivery systems in the field of nanomedicine [52]. Out of the two classes of carbon nanotubes (single-walled carbon nanotubes and multiwalled carbon nanotubes) [53], Battigelli *et al.* used multiwalled carbon nanotubes to conduct their study. The MTS peptide used was N-terminal region of subunit VIII of human cytochrome c oxidase that is an enzyme complex of the mitochondrial respiratory chain. They modified the C-terminal of the peptide by adding cysteine to later attach to multiwalled carbon nanotubes (MWCNTs) *via* irreversible disulfide linkage. The N-terminal of the peptide was attached to a fluorescent sulforhodamine B probe for imaging purposes. The final modified peptide sequence used in the study was KMSVLTPLLLRGLTGSARRLPVPRAKC [23]. They also functionalized the MWCNTs by first oxidizing and then attaching azomethine ylides through a 1,3-dipolar cycloaddition reaction to an amine on the carbon nanotubes. The addition of maleimide to the amine group of the nanotube led to the covalent linkage of the nanotube with the peptide. The quantification studies of the amino-functionalized nanotubes, peptide-functionalized nanocarriers MTS–MWCNTs, and the control (amino-functionalized nanotube with rhodamine) revealed that MTS-MWCNTs were obtained in good amounts (20 μmol/g) although rhodamine loading was less than the functionalized nanotubes (40 μmol/g) attributing to the more steric hindrance in the nanotubes having MTS. The *in vitro* cell studies performed with macrophages or HeLa cells show high cell viability. Confocal microscopy indicated that MTS–MWCNTs specifically localized in the mitochondria, while the MTS alone or MWCNTs having rhodamine did not show colocalization within the mitochondria. TEM analysis of the mitochondria isolated from the liver of CD1 mice also revealed that MTS–MWCNTs crossed the cell membrane and were localized in the mitochondrial membrane. They suggested a combination of active phagocytosis and a passive nanoneedle mechanism for the cellular uptake of the nanotube carrier. This study introduced covalent functionalization of carbon-nanotubes with MTS peptide resulting in better mitochondrial targeting of the nanocarrier [51]. Although this mitochondrial targeting strategy can be used for transporting certain payloads or imaging agents to the mitochondria of the tumor site, *in vivo* studies are needed to evaluate the overall effect in the physiological environment as the carbon-based nanocarriers often offer higher cytotoxicity [54] and the release mechanism of the nanocarrier out of the living system also needs to be further explored.

Carbon materials have also been explored as theranostic probes for plasmonic photothermal therapy (PPT) and surface-enhanced Raman spectroscopy (SERS) applications. Qi and coworkers reported the design of an organelle-targeting SERS nanoprobe consisting of porous Ag/Au nanoshells (Ag/Au NS) and carbon dots (CD) along with nucleus targeting signal peptide (GGVKRKKKPGGC) and mitochondrial targeting signal peptide (MLALLGWWWFFSRKKC) for nuclear and mitochondrial targeting, respectively [55]. A cell penetrating peptide [RGD peptide (RGDRGDRGDRGDPGC)] was also used for both nucleus and mitochondria to enhance the cellular uptake of peptides [34]. The study revealed differences in molecular stress response in PPT-induced hyperthermia cell death among cancerous HeLa and normal L929 and H8 cells by site-specific single-cell SERS detection (Fig. **3**). It was found that PPT-induced cell apoptosis in HeLa cells is mainly regulated through cellular thermal stress-responsive proteins such as metabolism-related Tyr and Phe were also found to be significantly higher in HeLa cells, while the cell death in L929 and H8 was found to be related to DNA [55].

Other carbon-based materials such as graphene have also been investigated for targeted delivery of drugs to mitochondria by different research groups. Some properties that make graphene and graphene oxide (GO) stand out among other materials are high surface area, 2D-planar structure, mechanical and chemical constancy, exalted conductivity and biocompatibility [43]. In graphene, the sp2-hybrid orbitals of carbon-carbon atoms are in-plane σ (C═C) and account for the strength of graphene, while out-of-plane π (C═C) bonds account for the highly delocalized array of electrons. These electrons make weak polar interactions between the graphitic layers of the graphene sheets as well as with other molecules. This unique surface chemistry, along with other properties, makes graphene an excellent choice to be used as cargo for targeted delivery of anticancer and poorly soluble drugs, peptides, DNA, RNA, antibodies, *etc*. [56]. A study found that glycyrrhetinic acid (GA, an anti-inflammatory, antioxidant, and antiviral agent) drug attached to graphene oxide-DOX conjugates showed a specific accumulation in mitochondria and not in the nucleus showing that graphene might also have some mitochondrial targeting ability. This study did not use peptides as targeting moieties but instead evaluated the ability of drug-graphene conjugates to target mitochondria [57]. Another study reported that when magnetic γ-Fe_2O_3 was grafted on the surface of GO nanosheets followed by mitochondrial targeting peptide (MitP) functionalization to transport mitoxantrone drug (MTX), the designed formulation (GOMNP-MitP) showed a high accumulation in tumor mitochondria. The use of the peptide for mitochondrial targeting and applying an alternating magnetic field for controlled drug release enhanced the mitochondrial localization of MTX that ultimately induced cell death by activating apoptosis pathways [58]. Graphene-based nanoplatforms not only offer better mitochondrial accumulation when combined with mitochondrial targeting peptides or other targeting moieties, but also provide the opportunity to be used for

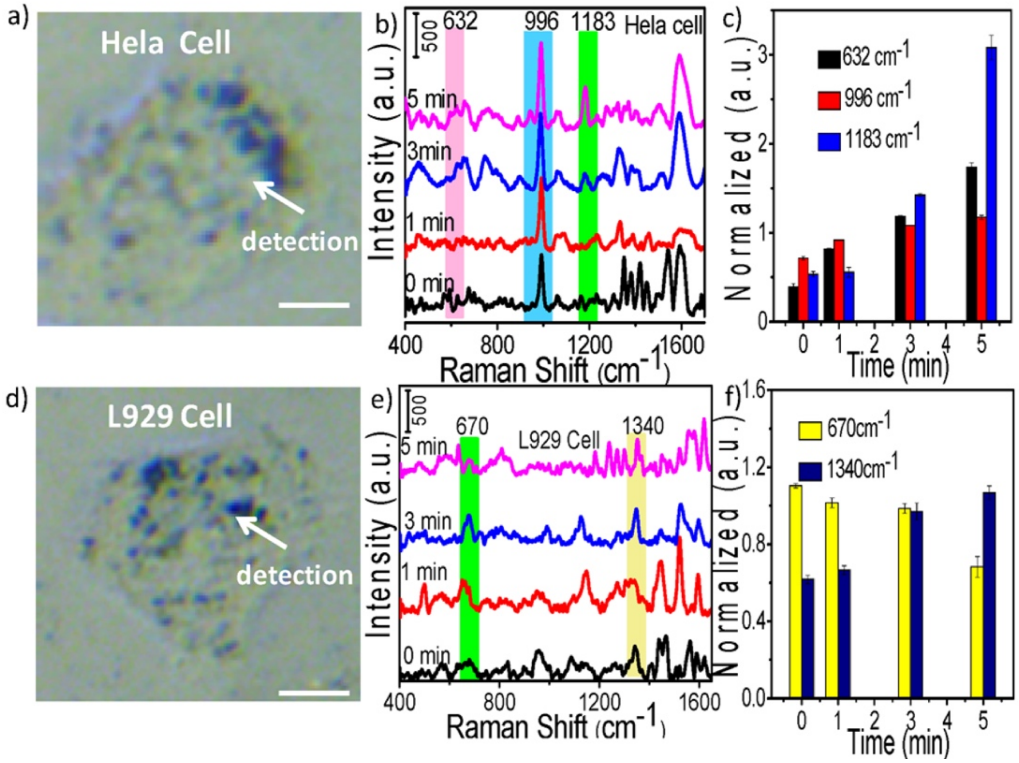


Fig. (3). "Mitochondria SERS detection**).** (**a** and **d**) Bright-field images of Hela and L929 cells incubated with MT-nanoprobes for 24 h (Scale bar**).** 5 μm). (**b**) Mitochondria site-specific time-dependent SERS spectra were recorded from single HeLa (**e**) and L929 cells under NIR 808 nm laser exposure at 2 W/cm^2 for 0, 1, 3, and 5 min, respectively. (**c** and **f**) SERS intensity variations of related Raman bands for HeLa and L929 cells with different irradiation times." Reprinted with permission from reference [55]. Copyright 2018, American Chemical Society, Washington, DC. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

photothermal therapy owing to the unique physiochemical properties and high thermal conductivity of the material [56].

3.3. Mitochondrial Penetrating Peptides and Organic (Liposomes/Micelle-Based) Nanocarriers

Lipid-based drug carriers offer high biocompatibility, low toxicity, longer circulation times, and better interaction with their target due to the lipophilic nature of the lipid carrier that helps to pass through the cell membrane and are biodegradable [59]. Yamada *et al.* developed a liposomal mitochondrial nanocarrier ("MITO-Porter") [60]. The MITO-Porter can effectively deliver cyto-toxic payloads into the mitochondria by membrane fusion. The designed cargo was taken up by the cell *via* micropinocytosis due to the surface modification of the liposomal nanocarriers with the cationic cell-penetrating peptide octaarginine (*R8*). The interaction of the MITO-Porter with the mitochondrial membrane occurs *via* electrostatic interaction with the negatively charged inner mitochondrial membrane leading to the delivery of cargo inside the mitochondria. The authors found an optimal lipid composition of the formulation dioleophosphatidyl ethanolamine (DOPE)/phosphatidic acid (PA)/stearylated R8 or DOPE/sphingomyelin (SM)/STR-R8 [molar ratio 9:2:1] for the maximum mitochondrial accumulation with low toxicity. Mito-porter was also reported to deliver the antioxidant Co-enzyme CoQ10 and the aminoglycoside drug gentamicin in the mouse liver and tumor mitochondria [60-62]. The next logical step was the development of a liposomal carrier modified with peptides to target the mitochondria. For this purpose, different mitochondrial peptides were synthesized: *R8*, SS (COOH-Dmt-$_D$-Arg-FK-NH_2), gramicidin S (GS), MPP with repeating sequences of positively charged arginine and lipophilic cyclohexylalanine residues (six amino acids chain) and circular peptide (GKGGRAKDGGK). They also modified SS peptide by combining two SS peptides that offered lower toxicity along with the higher mitochondrial accumulation in the isolated mitochondria and the rat liver homogenate compared to all other peptide liposomes formulations [63].

Juang *et al.* reported peptide-modified liposomes and solid lipid nanoparticles (SLN) for for the delivery of irinotecan and miR-200, respectively [64]. Irinotecan (Iri) is water-soluble camptothecin that is used as a therapeutic agent for the treatment of colorectal cancer. This drug causes cell death by forming a cleavable drug-DNA-topoisomerase I complex [65,66]. MicroRNAs (miR-200) are known to suppress the epithelial-mesenchymal transition (EMT) and ZEB expression (ZEB-1 is an EMT activator and its expression promotes metastasis by activating EMT) hence increasing the efficiency of certain anticancer drugs when used in combination [67]. However, irinotecan therapy suffers from MDR, while miRNA suffers from rapid enzymatic degradation, low cellular uptake, and poor endosomal escape [65,67].

To overcome these issues, authors reported the following peptide moieties; the *RF peptide* (GLKKLARLF-

HKLLKLGC) that belongs to the potent cell penetration peptides family, the *H peptide* (CVAASGVRSMH) to target the tumor site by binding to nerve/glial antigen 2 that is transmembrane proteoglycan and highly expresses in tumor neovasculature, and the *K peptide* (CKLAKLAK) for mitochondria-targeting at the tumor site. *K-peptide* and *RF peptides* are also known to induce cell death by releasing cytochrome c, leading to the activation of caspase-9 and caspase-3 and ultimately cell death at the tumor site [68]. A long-chain PEG 5000 polymer, O'-methyl polyethylene glycol, was conjugated to the lipid nanocarrier to form a pH-sensitive imine bond. This bond facilitates the release of the nanocarrier in the acidic environment of colon cancer HCT116 cells. Liposomes were prepared *via* the thin film hydration method by using DSPC, cholesterol, DSPE-PEG-peptide, and DSPE-omPEG (at a molar ratio of 1: 0.1: 0.1: 0.1: 0.1). The solid lipid nanoparticles were synthesized by the aqueous solvent diffusion method using l-α-Phosphatidylcholine (PC), cholesterol, DOTAP, DSPE-PEG-peptide, and DSPE-omPEG at a molar ratio of 1: 0.1: 0.1: 0.1: 0.1. The encapsulation efficiency of both Irinotecan and miRNA was above 88%. The TEM analysis showed no aggregation in the formulated liposome conjugates and zeta potential measurements gave negative values for liposome conjugates while positive values for SLN-conjugates. The pH-dependent release of the formulations was evaluated by comparing the % release with the formulations having no pH-sensitive bond and it was found that the percent release from omLip-RFKH was 46.86 ± 2.34% at pH 7.4, which was increased to 67.89 ± 1.88% at pH 6.5 while Iri/PEG Lip-RFKH showed pH-independent drug release. The *in vitro* experiments on HCT116 cells revealed that the cellular uptake for both (liposome and SLN) formulations was higher at 6.5 pH and occurred *via* endocytosis with high endosomal escape. The cytotoxicity of both formulations was high when tested on HCT116 cells and IEC-6 cells and a higher rate of apoptosis induction was also observed with both formulations compared to irinotecan and miRNA alone. It was also reported that miRNA inhibited the expressions of β-catenin, ZEB1, Vimentin, c-Myc, P-gp, MRP1, Rac1, KRAS, MRP2, and Slug compared to the other treatment groups that led to less % migration of the tumor. The *in vivo* studies in mice bearing the colorectal cancer CT26 show that the omLip-RFKH/iri and omSLN-RFKH/miR-200 formulations reduced the tumor size leading to the measured fluorescence. The serum levels of different biomarkers from liver, kidney, and heart (GPT, CRE, and CKMB) show a decrease in the serum levels, indicating less injury to the tissues/inflammation [64]. This study solves the issues of low cellular uptake, short circulation time, unwanted cytotoxicity, and metastasis control, but the conjugate-peptide interactions for mitochondrial localization and drug release mechanisms remain a question.

Sudipta, Mallick *et al*. and coworkers developed liposomes containing cholesterol and a mitochondria-penetrating peptide (MPP) based drug delivery system for the delivery of antimycin A to mitochondria in A549 cells. The liposomes were prepared using MPP (FRFK) and cholesterol. For the liposome formulation, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and Chol-FRFK were used at 1:1 molar ratio. The mitochondria accumulation was confirmed by confocal microscopy (Fig. **4**) [69].

The small size of the designed peptide-liposomal conjugate and cationic charge resulted in higher uptake and mitochondrial localization of Chol-FRFK/D. Higher mitochondrial localization of the drug conjugate Chol-FRFK/D-AMA resulted in significantly increased cytotoxicity of the drug compared to free drugs due to disruption of the inner membrane potential of mitochondria [69].

3.4. Mitochondrial Penetrating Peptides and Inorganic Nanocarriers

Inorganic nanoparticles have been used frequently in the field of nanomedicine for the targeted delivery of drug cargoes in tumor sites by many research groups. In this regard, a study by Luo *et al*. uses an antibiotic peptide $(KLAKLAK)_2$ modified with the mitochondrial targeting moiety TPP and attached to the surface of topotecan-loaded MSNs through disulfide bonds. The group further attached poly(ethylene glycol)-blocked-2,3-dimethylmaleic anhydride-modified poly(L-lysine) (PEG-PLL(DMA)) on the surface for charge reversal. Under acidic conditions, the DMA block degrades and removes the outer shielding layer. This triggers the uptake of nanocarriers in the HeLa cell lines and then the modified peptide is released from the nanocarrier by the overexpressed glutathione leading to mitochondrial targeting and cancer cell death [70,71].

A similar approach was employed by Cao *et al*. for mitochondrial targeting in cancer treatment. The research group synthesized a novel antibacterial peptide, RGD-hylin a1, with reduced hemolysis and loaded the peptide within the pores of the MSNs (RGD-hylin a1-MSN). The peptide was released from the nanocarrier in a pH-controlled manner in an acidic environment that induced the reduction of mitochondrial membrane potential, ultimately causing cell death. There were almost no cell deaths at physiological pH. In *vivo* studies revealed that the size and weight of the solid tumor were reduced (50–60%) after intravenous administration in tumor-bearing mice [71,72].

Owing to the possible side effects and nonspecific targeting in chemotherapy, photothermal therapy is gaining attention because it is a noninvasive therapy with minimal nonspecific damaging, deeper penetration, and remote manipulation. Photothermal therapy uses agents that induce hyperthermia under the influence of NIR radiation at a targeted tumor site [73]. Ma *et al.* reported mitochondrial targeting peptide-based platinum nanoparticles because of the higher photobleaching resistance and excellent biocompatibility [74]. To eliminate the toxicity resulting from surfactants, the authors suggest the surface functionalization of nanoparticles with a peptide moiety to enhance the stability and biocompatibility of the nanoparticles. The peptide KPGKPGK was modified with the triphenylphosphonium ligand KPGKPK (TPP)K to enhance the mitochondrial homing ability and was adsorbed on the surface of Pt-NPs. The zeta potential measurement value of 4.02 mV indicated the positive character of the formulated peptide nanocarrier conjugate. The UV–vis spectra revealed that TPP-Pt possesses a high absorption curve at the 1000–1100 nm region. The higher cellular uptake in the HepG2 cells was observed when analyzed with CSLM and inductively coupled plasma (ICP)–MS. The mitochondrial localization was confirmed with Mito-Tracker

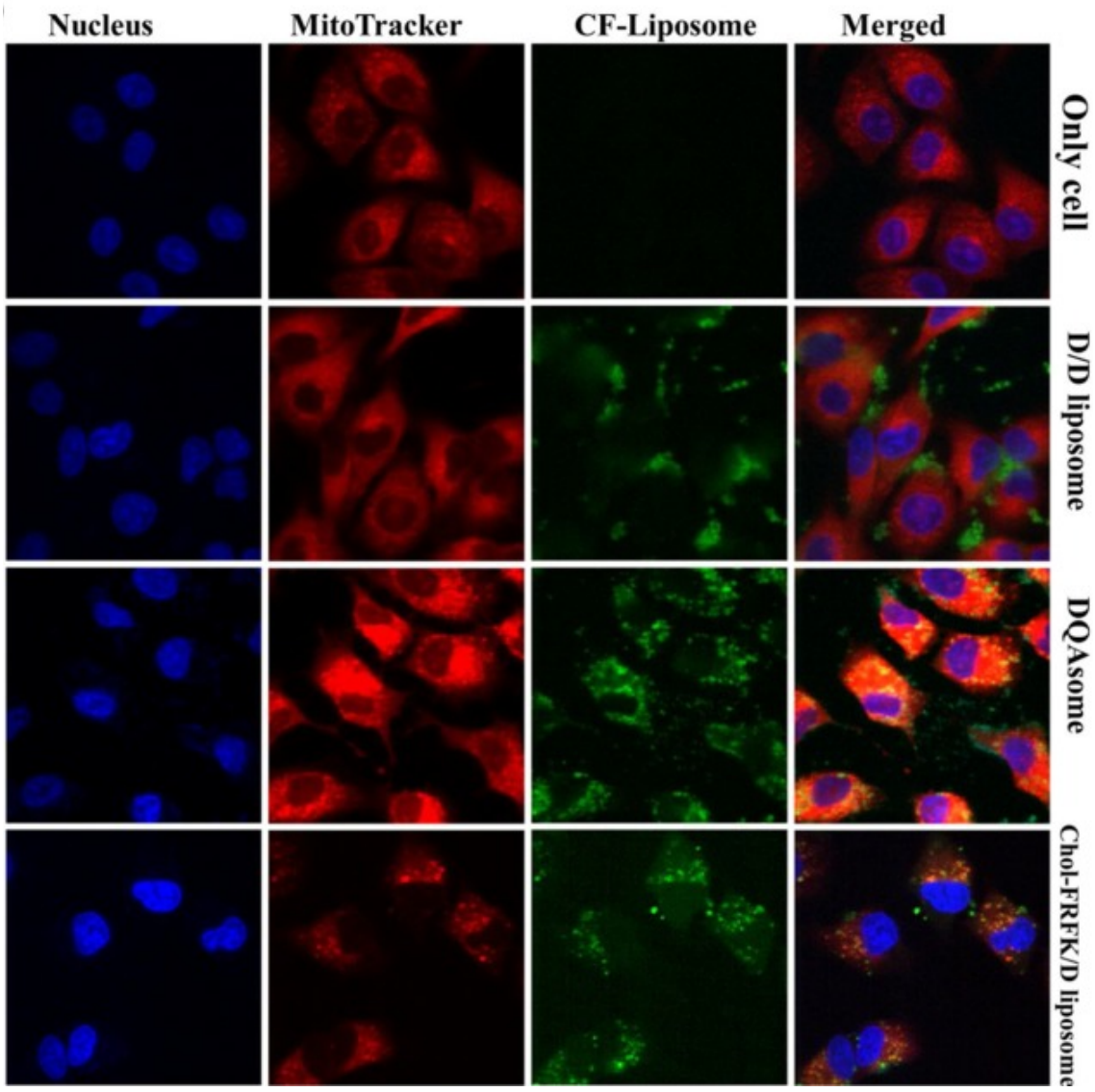


Fig. (4). Mitochondrial localization of Chol-FREK/D-liposome conjugates confirmed by confocal microscopy. Reprinted with permission from reference [69]. Copyright 2018, Colloids and Surfaces B). Biointerfaces, Elsevier, Amsterdam, The Netherlands. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Green and was attributed to the positive charge and the TPP moiety of the peptide, while small amounts were also present in the nucleus and lysosomes. The study also revealed that the cytotoxicity of the cancer cells was directly related to the irradiation of 1064 nm light. The specific targeting of mitochondria induced apoptosis leading to the death of cancerous cells. The intravenous injection of Cy7-labeled TPP-Pt had higher enrichment of the nanoparticles at the tumor site even after 12 h, and *ex vivo* analysis gave a high fluorescence at the tumor site. A thirteen-day photothermal therapy was performed on mice injected with TPP-Pt intravenously with the irradiation of 1064 nm light (4h). The size of the tumors and body weight data showed that the tumor growth was effectively inhibited in the mice that were irradiated with NIR light compared to the mice that were injected with the TPP-Pt nanoparticles but were not irradiated with light. The H&E and TUNEL assay of the tumor tissues showed a severe nucleus shrinkage that was an indication of tumor cell damage. The liver function test (ALT, AST, BUN, CR) was normal and nanoparticles were concluded to be nontoxic for the other tissues [74] (Fig. **5**).

Liu *et al.* developed silica-coated gold nanoparticles and functionalized the surface with the mitochondria-targeting peptide named RLA $(RLARLAR)_2$. The β-cyclodextrin was used for the capping of pores. This conjugate was loaded with indocyanine green for photodynamic and photothermal therapy. A charge-reversible polymer was utilized to facilitate dissociation in the acidic microenvironment of the tumor, thus exposing the RLA peptide for cell internalization followed by mitochondria accumulation in MCF-7 tumor cells. Upon irradiation of NIR light (808 nm), the drug conjugate induced tumor cell death by generating ROS species and local hyperthermia both *in vitro* and *in vivo* [71,75]. Among other cancer types, glioblastoma is very hard to treat because of the blood-brain barrier and therapeutics used for antiangiogenic therapy often come with MDR. A study by Agemy and coworkers addressed the issue by developing an

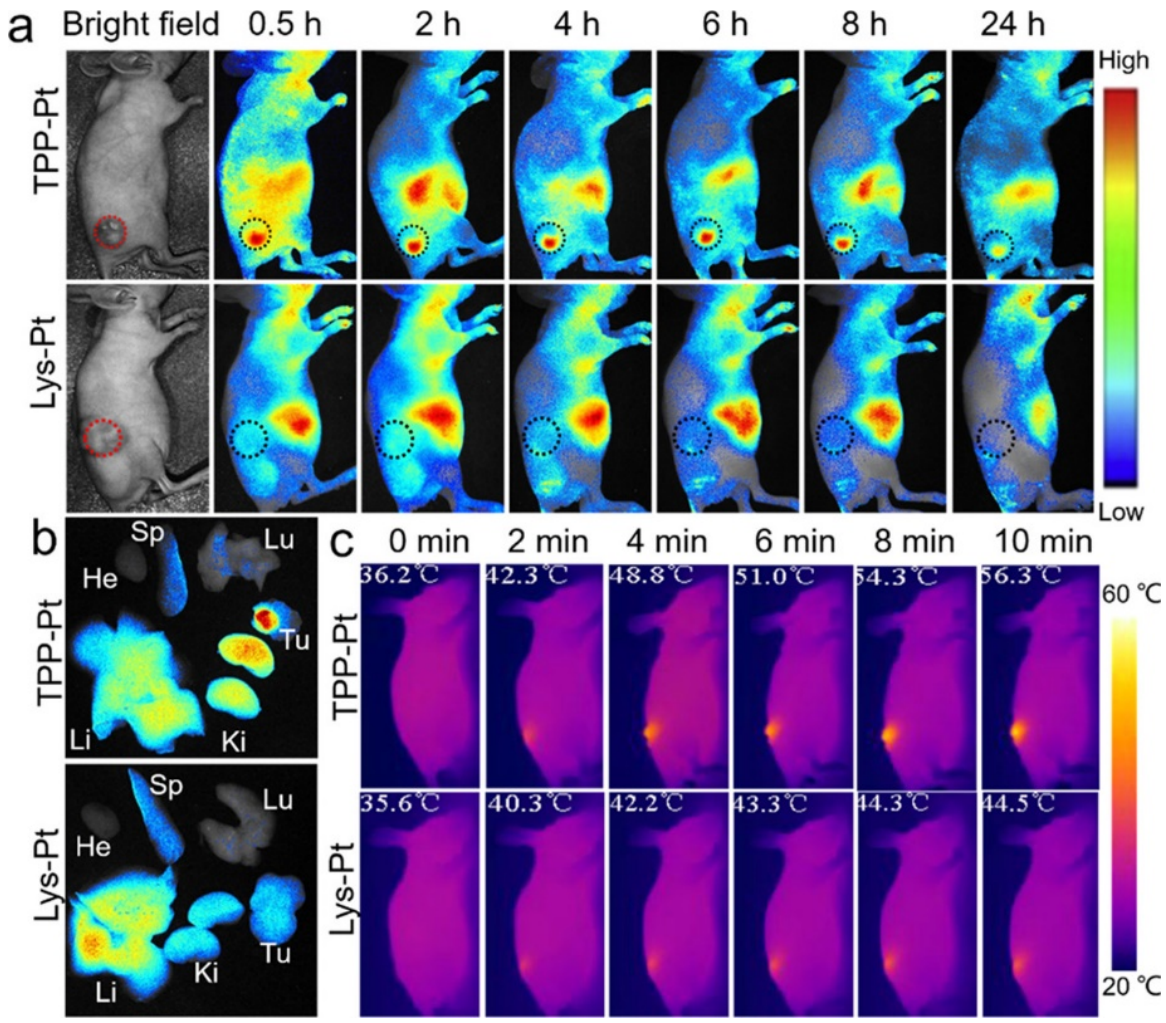


Fig. (5). (a) "*In vivo* imaging at different time points**).** 0, 0.5, 2, 4, 6, 8, 24 h. **(b)** Images of organs and tumors in samples at 24 h postinjection. **(c)** Infrared thermal images when irradiated with 1064 nm laser. Reprinted with permission from reference [74]. Copyright 2020, American Chemical Society, Washington, DC. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

iron oxide-based nanoplatform and functionalized nanocarrier with peptides for specific tumor and mitochondrial targeting. Authors used a mitochondrial homing peptide motif (Cys-Gly-Lys-Arg-Lys) that has the ability to target tumor vasculature as well as the mitochondria of tumor cells. This peptide was screened through the library of peptides homed to epidermal tumors in mice. Tumor blood vessels express various cell surface and extracellular matrix marker proteins that are not expressed on normal vessels and are readily available to bind with circulating ligands (such as peptides and antibodies). The tumor and mitochondrial targeting motif CGKRK targets the p32 protein (also called SF2-associated p32, p32/TAP, and gC1qR) that is a mitochondrial protein present in the mitochondria matrix and is overexpressed on the surface of tumors such as breast and prostate cancer. They further conjugate this homing peptide with a proapoptotic peptide $_D$[KLAKLAK]$_2$ that acts on mitochondria to generate anticancer effects by inducing apoptosis. This study also shows that upon systemic treatment of two glioblastoma-bearing mouse models (showing MDR to other therapeutic treatments) with the designed formulation, most tumors were eradicated in one mouse model while delayed in the second mouse model. The iron oxide nanoparticles also enabled the imaging of glioblastoma tumors in mice [76,77]. The Mitochondrial targeting peptide-based nanocarriers that are discussed here are summarized in Table **1**.

CONCLUSION

Mitochondria are an interesting target for cancer therapy because of their role in the cell survival processes, apoptosis, and the difference in the structure and function of the mitochondria of healthy and cancerous cells. The degree of mitochondrial dysfunction can be determined by factors including depletion of ATP levels in the defective tissue/site and/or increased ROS levels in tumor cells. All these characteristics have made scientists try different chemical moieties to target the mitochondria for cancer therapy. The use of nanocarriers (liposomes/polymers/carbon-based materials/inorganic particles) is an effective approach in this regard but suffers from low efficacy to target the mitochondria at a particular site and possible toxicities that arise due to the induction of oxidative stress, apoptosis, DNA destruction, and mutagenesis. The use of mitochondrial targeting peptides to functionalize these nanocarriers has improved the biocompatibility and mitochondrial targeting ability. These peptide moieties are

Table 1. Mitochondrial targeting peptide-based nanocarriers (discussed in this review) [22, 26, 44, 51, 55, 58, 63, 64, 69, 72, 74, 75, 76, 77, 78].

Mitochondrial Targeting Peptide	Nanocarrier	Anticancer Drug	Cellular Response/ Effect	Refs.
$_{D}$(KLAKLAK)$_2$-GRGD		Doxorubicin	Increased ROS, mitochondrial disruption, ATP depletion	[21]
(Fx-r-Fx-K-Fx-r-Fx-K) derivatives			Class of MPPs synthesized for higher mitochondrial accumulation	[25]
ALD5MTS (along with a CPP, R8)	HPMA copolymer	Doxorubicin	mitochondrial localization, ROS generation followed by upregulation of cytochrome c leading to intrinsic apoptosis	[43]
KMSVLTPLLLRGLTGSARRL-PVPRAKC (modified MTS)	Multi-walled carbon nanotubes		Active phagocytosis and a passive nanoneedle mechanism for uptake, low toxicity to cells	[50]
MLALLGWWWFFSRKKC (MTS, along-with a CPP RGDRGDRG-DRGDPGC)	Ag/Au nanoshell with carbon dots		Hyperthermia leading to activation of cell death mechanisms	[54]
MitP	γ-Fe2O3 grafted on the surface of GO nanosheets	Mitoxantrone	Reduction of mitochondrial membrane potential, Activation of apoptosis pathways	[57]
MITO-Porter (modified with mitochondrial targeting peptides R8, SS (COOH-Dmt-D-R-FK-NH2), gramicidin S (GS), MPP with repeating sequences of positively charged arginine and lipophilic cyclohexylalanine residues, and circular peptide (GKGGRAKDGGK), S2 (two SS peptides combined)	Lipososmes		Mitochondrial accumulation was dependent on overall cationic charge; S2 offered lowest toxicity	[62]
K peptide (CKLAKLAK) for mitochondrial targeting, along with a CPP RF peptide (GLKKLARLF-HKLLKLGC) and tumor targeting H peptide (CVAASGVRSMH)	modified liposomes and solid lipid nanoparticles (SLN	Irinotecan and miR-200	Increased efficiency of anticancer drugs	[63]
MPP (FRFK) modified with cholesterol	Liposome	Antimycin A	Increased mitochondrial accumulation leads to increased cytotoxicity	[63]
KLA peptide modified with TPP	Mesoporous silica nanoparticles	Topotecan	Mitochondrial targeting of drug resulting in cancer cell death	[68]
RGD-hyalin a1 (antimicrobial peptide for mitochondrial targeting)	Mesoporous silica nanoparticles		Low toxicity towards cells at physiological pH while induced apoptosis at lower pH	[71]
KPGKPK(TPP)K	Platinum nanoparticles		Photothermal therapy with NIR radiation resulted in significant tumor reduction	[73]
RLA peptide (RLARLAR)$_2$	Silica-coated gold nanoparticles	Indocyanine green for the photodynamic and photothermal therapy	Photothermal therapy with NIR radiation generated local hyperthermia generating ROS leading to cell death	[74]
CGKRK	Iron oxide nanoparticles	Proapoptotic peptide $_{D}$[KLAKLAK]$_2$ to generate anticancer effect	Enabled imaging, induced apoptosis, eliminated MDR	[75, 76]
SS-31 ($_{D}$-R-Dmt-K-F-NH$_2$) and derivatives			Mitochondrial accumulation and antioxidant activity	[77]

designed to have a high positive charge and lipophilic character to pass through the negatively charged and hydrophobic membranes of the mitochondria. The use of peptides as a targeting moiety often suffers from low bioavailability, so conjugating nanocarriers with peptides enhance the bioavailability of the peptides. Among all four classes of functionalized-nanocarriers discussed, the use of organic nanocarriers (liposomes, micelles) always offers more biocompatibility and less toxicity due to their biodegradable and non-immunogenic nature.

As mitochondria are an emerging target for cancer therapy, a very deep analysis of the effects of nanocarriers on the complex processes taking place within the mitochondria is still a challenge. The detailed study of the pathways responsible for the entry of the designed conjugates and their mechanism of inducing cell death at the organelle level is also challenging. More *in vivo* studies are needed to evaluate the possible toxicities associated with the designed nanocarriers in a biological system along with the retention time of the nanocarriers inside the body and the release mechanisms of the nanocarriers.

LIST OF ABBREVIATIONS

ATP = Adenosine 5'-Triphosphate

HIF = Hypoxia-Inducible Factor

FH = Fumarate Hydratase

ROS = Reactive Oxygen Species

OMM = Membrane of Mitochondria

IMM = Inner Mitochondrial Membrane

CONSENT FOR PUBLICATION

All authors consent to the publication of this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Medical Definition of Cancer. Available from: https://www.medicinenet.com/cancer/definition.htm

[2] Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2021. *CA Cancer J. Clin.,* **2021**, *71*(1), 7-33. http://dx.doi.org/10.3322/caac.21654 PMID: 33433946

[3] Healthline. Benign and Malignant Tumors: How Do They Differ? Available from: https://www.healthline.com/health/cancer/difference-between-benign-and-malignant-tumors

[4] WHO. Cancer Today. Available from: http://gco.iarc.fr/today/home

[5] Kalubowilage, M.; Covarrubias-Zambrano, O.; Malalasekera, A.P.; Wendel, S.O.; Wang, H.; Yapa, A.S.; Chlebanowski, L.; Toledo, Y.; Ortega, R.; Janik, K.E.; Shrestha, T.B.; Culbertson, C.T.; Kasi, A.; Williamson, S.; Troyer, D.L.; Bossmann, S.H. Early detection of pancreatic cancers in liquid biopsies by ultrasensitive fluorescence nanobiosensors. *Nanomedicine,* **2018**, *14*(6), 1823-1832. http://dx.doi.org/10.1016/j.nano.2018.04.020 PMID: 29782949

[6] Udukala, D.N.; Wang, H.; Wendel, S.O.; Malalasekera, A.P.; Samarakoon, T.N.; Yapa, A.S.; Abayaweera, G.; Basel, M.T.; Maynez, P.; Ortega, R.; Toledo, Y.; Bossmann, L.; Robinson, C.; Janik, K.E.; Koper, O.B.; Li, P.; Motamedi, M.; Higgins, D.A.; Gadbury, G.; Zhu, G.; Troyer, D.L.; Bossmann, S.H. Early breast cancer screening using iron/iron oxide-based nanoplatforms with sub-femtomolar limits of detection. *Beilstein J. Nanotechnol.,* **2016**, *7*, 364-373. http://dx.doi.org/10.3762/bjnano.7.33 PMID: 27335730

[7] Udukala, D.N.; Wendel, S.O.; Wang, H.; Yapa, A.S.; Covarrubias-Zambrano, O.; Janik, K.; Gadbury, G.; Troyer, D.L.; Bossmann, S.H. Early detection of non-small cell lung cancer in liquid biopsies by ultrasensitive protease activity analysis. *J. Cancer Metastasis Treat.,* **2020**, *6*(Spec.Iss.), 25. http://dx.doi.org/10.20517/2394-4722.2020.45

[8] Wang, H.; Udukala, D.N.; Samarakoon, T.N.; Basel, M.T.; Kalita, M.; Abayaweera, G.; Manawadu, H.; Malalasekera, A.; Robinson, C.; Villanueva, D.; Maynez, P.; Bossmann, L.; Riedy, E.; Barriga, J.; Wang, N.; Li, P.; Higgins, D.A.; Zhu, G.; Troyer, D.L.; Bossmann, S.H. Nanoplatforms for highly sensitive fluorescence detection of cancer-related proteases. *Photochem. Photobiol. Sci.,* **2014**, *13*(2), 231-240. http://dx.doi.org/10.1039/c3pp50260k PMID: 24096539

[9] Kimman, M.; Jan, S.; Kingston, D.; Monaghan, H.; Sokha, E.; Thabrany, H.; Bounxouei, B.; Bhoo-Pathy, N.; Khin, M.; Cristal-Luna, G.; Khuhaprema, T.; Hung, N.C.; Woodward, M. Socioeconomic impact of cancer in member countries of the Association of Southeast Asian Nations (ASEAN): The ACTION study protocol. *Asian Pac. J. Cancer Prev.,* **2012**, *13*(2), 421-425. http://dx.doi.org/10.7314/APJCP.2012.13.2.421 PMID: 22524800

[10] USA Today NEWS. Life after cancer: More survivors live longer, face new health challenges. Available from: https://www.usatoday.com/in-depth/news/50-states/2019/02/13/life-after-cancer-survivors-oncology-survivorship-plans-long-term-health/2794121002/

[11] The Genetics of Cancer. Available from: https://www.cancer.net/navigating-cancer-care/cancer-basics/genetics/genetics-cancer

[12] Peters, J.M.; Gonzalez, F.J. The Evolution of Carcinogenesis. *Toxicol. Sci.,* **2018**, *165*(2), 272-276. http://dx.doi.org/10.1093/toxsci/kfy184 PMID: 30629266

[13] American Cancer Society. Types of Cancer Treatment. Available from: https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types.html

[14] Cancer Research UK. Side effects of cancer drugs. Available from: https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/cancer-drugs/side-effects

[15] Vander Heiden, M.G.; Cantley, L.C.; Thompson, C.B. Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science,* **2009**, *324*(5930), 1029-1033. http://dx.doi.org/10.1126/science.1160809 PMID: 19460998

[16] Weinberg, S.E.; Chandel, N.S. Targeting mitochondria metabolism for cancer therapy. *Nat. Chem. Biol.,* **2015**, *11*(1), 9-15. http://dx.doi.org/10.1038/nchembio.1712 PMID: 25517383

[17] Pustylnikov, S.; Costabile, F.; Beghi, S.; Facciabene, A. Targeting mitochondria in cancer: Current concepts and immunotherapy approaches. *Transl. Res.,* **2018**, *202*, 35-51. http://dx.doi.org/10.1016/j.trsl.2018.07.013 PMID: 30144423

[18] Dong, L.; Neuzil, J. Targeting mitochondria as an anticancer strategy. *Cancer Commun. (Lond.),* **2019**, *39*(1), 63. http://dx.doi.org/10.1186/s40880-019-0412-6 PMID: 31653274

[19] Panieri, E.; Santoro, M.M. ROS homeostasis and metabolism: A dangerous liason in cancer cells. *Cell Death Dis.,* **2016**, *7*(6), e2253. http://dx.doi.org/10.1038/cddis.2016.105 PMID: 27277675

[20] Wang, H.; Zhang, F.; Wen, H.; Shi, W.; Huang, Q.; Huang, Y.; Xie, J.; Li, P.; Chen, J.; Qin, L.; Zhou, Y. Tumor- and mitochon-

dria-targeted nanoparticles eradicate drug resistant lung cancer through mitochondrial pathway of apoptosis. *J. Nanobiotechnology,* **2020**, *18*(1), 8. http://dx.doi.org/10.1186/s12951-019-0562-3 PMID: 31918714

[21] Han, K.; Zhu, J.Y.; Jia, H.Z.; Wang, S.B.; Li, S.Y.; Zhang, X.Z.; Han, H.Y. Mitochondria-targeted chimeric peptide for trinitarian overcoming of drug resistance. *ACS Appl. Mater. Interfaces,* **2016**, *8*(38), 25060-25068. http://dx.doi.org/10.1021/acsami.6b06522 PMID: 27595983

[22] Jean, S.R.; Tulumello, D.V.; Riganti, C.; Liyanage, S.U.; Schimmer, A.D.; Kelley, S.O. Mitochondrial targeting of doxorubicin eliminates nuclear effects associated with cardiotoxicity. *ACS Chem. Biol.,* **2015**, *10*(9), 2007-2015. http://dx.doi.org/10.1021/acschembio.5b00268 PMID: 26038829

[23] D'Souza, G.G.; Wagle, M.A.; Saxena, V.; Shah, A. Approaches for targeting mitochondria in cancer therapy. *Biochim. Biophys. Acta,* **2011**, *1807*(6), 689-696. http://dx.doi.org/10.1016/j.bbabio.2010.08.008 PMID: 20732297

[24] Kang, H.C. Mitochondria-targeting theranostics. *Biomater. Res.,* **2018**, *22*(1), 34. http://dx.doi.org/10.1186/s40824-018-0145-7 PMID: 30455977

[25] Horton, K.L.; Stewart, K.M.; Fonseca, S.B.; Guo, Q.; Kelley, S.O. Mitochondria-penetrating peptides. *Chem. Biol.,* **2008**, *15*(4), 375-382. http://dx.doi.org/10.1016/j.chembiol.2008.03.015 PMID: 18420144

[26] Battogtokh, G.; Cho, Y.Y.; Lee, J.Y.; Lee, H.S.; Kang, H.C. Mitochondrial-targeting anticancer agent conjugates and nanocarrier systems for cancer treatment. *Front. Pharmacol.,* **2018**, *9*, 922. http://dx.doi.org/10.3389/fphar.2018.00922 PMID: 30174604

[27] Liew, S.S.; Qin, X.; Zhou, J.; Li, L.; Huang, W.; Yao, S.Q. Smart design of nanomaterials for mitochondria-targeted nanotherapeutics. *Angew. Chem. Int. Ed. Engl.,* **2021**, *60*(5), 2232-2256. http://dx.doi.org/10.1002/anie.201915826 PMID: 32128948

[28] Lu, P.; Bruno, B.J.; Rabenau, M.; Lim, C.S. Delivery of drugs and macromolecules to the mitochondria for cancer therapy. *J. Control. Release,* **2016**, *240*, 38-51. http://dx.doi.org/10.1016/j.jconrel.2015.10.023 PMID: 26482081

[29] Wang, Z.; Guo, W.; Kuang, X.; Hou, S.; Liu, H. Nanopreparations for mitochondria targeting drug delivery system: Current strategies and future prospective. *Asian J Pharm Sci,* **2017**, *12*(6), 498-508. http://dx.doi.org/10.1016/j.ajps.2017.05.006 PMID: 32104363

[30] Constance, J.E.; Lim, C.S. Targeting malignant mitochondria with therapeutic peptides. *Ther. Deliv.,* **2012**, *3*(8), 961-979. http://dx.doi.org/10.4155/tde.12.75 PMID: 22946430

[31] Jeena, M.T.; Kim, S.; Jin, S.; Ryu, J.H. Recent progress in mitochondria-targeted drug and drug-free agents for cancer therapy. *Cancers (Basel),* **2019**, *12*(1), E4. http://dx.doi.org/10.3390/cancers12010004 PMID: 31861339

[32] Astruc, D. Introduction to nanomedicine. *Molecules,* **2015**, *21*(1), E4. http://dx.doi.org/10.3390/molecules21010004 PMID: 26791291

[33] Covarrubias-Zambrano, O.; Yu, J.; Bossmann, S.H. Nano-inspired technologies for peptide delivery. *Curr. Protein Pept. Sci.,* **2020**, *21*(4), 379-400. http://dx.doi.org/10.2174/1389203720666191202112429 PMID: 31793426

[34] Klimpel, A.; Neundorf, I. Bifunctional peptide hybrids targeting the matrix of mitochondria. *J. Control. Release,* **2018**, *291*, 147-156. http://dx.doi.org/10.1016/j.jconrel.2018.10.029 PMID: 30367921

[35] Pucci, C.; Martinelli, C.; Ciofani, G. Innovative approaches for cancer treatment: Current perspectives and new challenges. *Ecancermedicalscience,* **2019**, *13*, 961. http://dx.doi.org/10.3332/ecancer.2019.961 PMID: 31537986

[36] De Leo, V.; Milano, F.; Agostiano, A.; Catucci, L. Recent advancements in polymer/liposome assembly for drug delivery: From surface modifications to hybrid vesicles. *Polymers (Basel, Switz.),* **2021**, *13*(7), 1027.

[37] Fazio, E.; Spadaro, S.; Corsaro, C.; Neri, G.; Leonardi, S.G.; Neri, F.; Lavanya, N.; Sekar, C.; Donato, N.; Neri, G. Metal-oxide based nanomaterials: Synthesis, characterization and their applications in electrical and electrochemical sensors. *Sensors (Basel),* **2021**, *21*(7), 2494. http://dx.doi.org/10.3390/s21072494 PMID: 33916680

[38] Speranza, G. Carbon nanomaterials: Synthesis, functionalization and sensing applications. *Nanomaterials (Basel),* **2021**, *11*(4), 967. http://dx.doi.org/10.3390/nano11040967 PMID: 33918769

[39] Wang, H.; Yapa, A.S.; Kariyawasam, N.L.; Shrestha, T.B.; Kalubowilage, M.; Wendel, S.O.; Yu, J.; Pyle, M.; Basel, M.T.; Malalasekera, A.P.; Toledo, Y.; Ortega, R.; Thapa, P.S.; Huang, H.; Sun, S.X.; Smith, P.E.; Troyer, D.L.; Bossmann, S.H. Rationally designed peptide nanosponges for cell-based cancer therapy. *Nanomedicine,* **2017**, *13*(8), 2555-2564. http://dx.doi.org/10.1016/j.nano.2017.07.004 PMID: 28754467

[40] Zheng, W.; Zhou, Q.; Yuan, C. Nanoparticles for oral cancer diagnosis and therapy. *Bioinorg. Chem. Appl.,* **2021**, *2021*, 9977131. http://dx.doi.org/10.1155/2021/9977131 PMID: 33981334

[41] Ealias, A.M.; Saravanakumar, M.P. A review on the classification, characterisation, synthesis of nanoparticles and their application. *IOP Conf. Ser.: Mater. Sci. Eng.,* **2017**, *263*, 1-15.

[42] Daniyal, M.; Liu, B.; Wang, W. Comprehensive review on graphene oxide for use in drug delivery system. *Curr. Med. Chem.,* **2020**, *27*(22), 3665-3685. http://dx.doi.org/10.2174/1381612825666190201129 6290 PMID: 30706776

[43] Li, Q.; Yang, J.; Chen, C.; Lin, X.; Zhou, M.; Zhou, Z.; Huang, Y. A novel mitochondrial targeted hybrid peptide modified HPMA copolymers for breast cancer metastasis suppression. *J. Control. Release,* **2020**, *325*, 38-51. http://dx.doi.org/10.1016/j.jconrel.2020.06.010 PMID: 32598957

[44] Wei, G.; Wang, Y.; Huang, X.; Yang, G.; Zhao, J.; Zhou, S. Induction of mitochondrial apoptosis for cancer therapy *via* dual-targeted cascade-responsive multifunctional micelles. *J. Mater. Chem. B Mater. Biol. Med.,* **2018**, *6*(48), 8137-8147. http://dx.doi.org/10.1039/C8TB02159G PMID: 32254933

[45] Bai, S.; Zhang, X.; Ma, X.; Chen, J.; Chen, Q.; Shi, X.; Hou, M.; Xue, P.; Kang, Y.; Xu, Z. Acid-active supramolecular anticancer nanoparticles based on cyclodextrin polyrotaxanes damaging both mitochondria and nuclei of tumor cells. *Biomater. Sci.,* **2018**, *6*(12), 3126-3138. http://dx.doi.org/10.1039/C8BM01020J PMID: 30362476

[46] Lee, S.Y.; Cho, H.J. Mitochondria targeting and destabilizing hyaluronic acid derivative-based nanoparticles for the delivery of lapatinib to triple-negative breast cancer. *Biomacromolecules,* **2019**, *20*(2), 835-845. http://dx.doi.org/10.1021/acs.biomac.8b01449 PMID: 30566834

[47] Pan, G-Y.; Jia, H-R.; Zhu, Y-X.; Sun, W.; Cheng, X-T.; Wu, F-G. Cyanine-containing polymeric nanoparticles with imaging/therapy-switchable capability for mitochondria-targeted cancer theranostics. *ACS Appl. Nano Mater.,* **2018**, *1*(6), 2885-2897. http://dx.doi.org/10.1021/acsanm.8b00527

[48] Pan, G-Y.; Jia, H-R.; Zhu, Y-X.; Sun, W.; Cheng, X-T.; Wu, F-G. Correction to cyanine-containing polymeric nanoparticles with imaging/therapy-switchable capability for mitochondria-targeted cancer theranostics. *ACS Appl. Nano Mater.,* **2020**, *3*(1), 935. http://dx.doi.org/10.1021/acsanm.9b02381

[49] Liu, Y.; Zhou, Z.; Lin, X.; Xiong, X.; Zhou, R.; Zhou, M.; Huang, Y. Enhanced reactive oxygen species generation by mitochondria targeting of anticancer drug to overcome tumor multidrug resistance. *Biomacromolecules,* **2019**, *20*(10), 3755-3766. http://dx.doi.org/10.1021/acs.biomac.9b00800 PMID: 31465208

[50] Battigelli, A.; Russier, J.; Venturelli, E.; Fabbro, C.; Petronilli, V.; Bernardi, P.; Da Ros, T.; Prato, M.; Bianco, A. Peptide-based carbon nanotubes for mitochondrial targeting. *Nanoscale,* **2013**, *5*(19), 9110-9117. http://dx.doi.org/10.1039/c3nr02694a PMID: 23903095

[51] Magrez, A.; Kasas, S.; Salicio, V.; Pasquier, N.; Seo, J.W.; Celio, M.; Catsicas, S.; Schwaller, B.; Forró, L. Cellular toxicity of carbon-based nanomaterials. *Nano Lett.,* **2006**, *6*(6), 1121-1125. http://dx.doi.org/10.1021/nl060162e PMID: 16771565

[52] Zeinabad, H.A.; Zarrabian, A.; Saboury, A.A.; Alizadeh, A.M.; Falahati, M. Interaction of single and multi wall carbon nanotubes with the biological systems: Tau protein and PC12 cells as targets. *Sci. Rep.,* **2016**, *6*(1), 26508. http://dx.doi.org/10.1038/srep26508 PMID: 27216374

[53] Zhang, Y.; Petibone, D.; Xu, Y.; Mahmood, M.; Karmakar, A.; Casciano, D.; Ali, S.; Biris, A.S. Toxicity and efficacy of carbon

nanotubes and graphene: The utility of carbon-based nanoparticles in nanomedicine. *Drug Metab. Rev.,* **2014**, *46*(2), 232-246. http://dx.doi.org/10.3109/03602532.2014.883406 PMID: 24506522

[54] Qi, G.; Zhang, Y.; Xu, S.; Li, C.; Wang, D.; Li, H.; Jin, Y. Nucleus and mitochondria targeting theranostic plasmonic surface-enhanced raman spectroscopy nanoprobes as a means for revealing molecular stress response differences in hyperthermia cell death between cancerous and normal cells. *Anal. Chem.,* **2018**, *90*(22), 13356-13364. http://dx.doi.org/10.1021/acs.analchem.8b03034 PMID: 30234969

[55] Karki, N.; Rana, A.; Tiwari, H.; Negi, P.; Sahoo, N.G. Theranostics application of graphene-based materials in cancer imaging, targeting and treatment. In: *Tumor progression and Metastasis*; Lasfar, A.; Cohen-Solal, K., Eds.; IntechOpen Ltd., **2020**; pp. 1-25. http://dx.doi.org/10.5772/intechopen.91331

[56] Zhang, C.; Liu, Z.; Zheng, Y.; Geng, Y.; Han, C.; Shi, Y.; Sun, H.; Zhang, C.; Chen, Y.; Zhang, L.; Guo, Q.; Yang, L.; Zhou, X.; Kong, L. Glycyrrhetinic acid functionalized graphene oxide for mitochondria targeting and cancer treatment *in vivo*. *Small,* **2018**, *14*(4), 1703306. http://dx.doi.org/10.1002/smll.201703306 PMID: 29205852

[57] Zhu, H.; Zhang, B.; Zhu, N.; Li, M.; Yu, Q. Mitochondrion targeting peptide-modified magnetic graphene oxide delivering mitoxantrone for impairment of tumor mitochondrial functions. *Chin. Chem. Lett.,* **2021**, *32*(3), 1220-1223. http://dx.doi.org/10.1016/j.cclet.2020.09.003

[58] Pathak, K.; Keshri, L.; Shah, M. Lipid nanocarriers: Influence of lipids on product development and pharmacokinetics. *Crit. Rev. Ther. Drug Carrier Syst.,* **2011**, *28*(4), 357-393. http://dx.doi.org/10.1615/CritRevTherDrugCarrierSyst.v28.i4.20 PMID: 21967401

[59] Yamada, Y.; Nakamura, K.; Abe, J.; Hyodo, M.; Haga, S.; Ozaki, M.; Harashima, H. Mitochondrial delivery of Coenzyme Q10 *via* systemic administration using a MITO-Porter prevents ischemia/reperfusion injury in the mouse liver. *J. Control. Release,* **2015**, *213*, 86-95. http://dx.doi.org/10.1016/j.jconrel.2015.06.037 PMID: 26160304

[60] Abe, J.; Yamada, Y.; Harashima, H. Validation of a strategy for cancer therapy: Delivering aminoglycoside drugs to mitochondria in HeLa cells. *J. Pharm. Sci.,* **2016**, *105*(2), 734-740. http://dx.doi.org/10.1002/jps.24686 PMID: 26523487

[61] Yamada, Y.; Akita, H.; Kamiya, H.; Kogure, K.; Yamamoto, T.; Shinohara, Y.; Yamashita, K.; Kobayashi, H.; Kikuchi, H.; Harashima, H. MITO-Porter: A liposome-based carrier system for delivery of macromolecules into mitochondria *via* membrane fusion. *Biochim. Biophys. Acta,* **2008**, *1778*(2), 423-432. http://dx.doi.org/10.1016/j.bbamem.2007.11.002 PMID: 18054323

[62] Kawamura, E.; Yamada, Y.; Harashima, H. Mitochondrial targeting functional peptides as potential devices for the mitochondrial delivery of a DF-MITO-Porter. *Mitochondrion,* **2013**, *13*(6), 610-614. http://dx.doi.org/10.1016/j.mito.2013.08.010 PMID: 24012978

[63] Juang, V.; Chang, C.H.; Wang, C.S.; Wang, H.E.; Lo, Y.L. pH-responsive PEG-shedding and targeting peptide-modified nanoparticles for dual-delivery of irinotecan and microrna to enhance tumor-specific therapy. *Small,* **2019**, *15*(49), e1903296. http://dx.doi.org/10.1002/smll.201903296 PMID: 31709707

[64] Gottesman, M.M.; Fojo, T.; Bates, S.E. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat. Rev. Cancer,* **2002**, *2*(1), 48-58. http://dx.doi.org/10.1038/nrc706 PMID: 11902585

[65] Basel, M.T.; Balivada, S.; Shrestha, T.B.; Seo, G.M.; Pyle, M.M.; Tamura, M.; Bossmann, S.H.; Troyer, D.L. A cell-delivered and cell-activated SN38-dextran prodrug increases survival in a murine disseminated pancreatic cancer model. *Small,* **2012**, *8*(6), 913-920. http://dx.doi.org/10.1002/smll.201101879 PMID: 22238072

[66] Suliman, M.A.; Zhang, Z.; Na, H.; Ribeiro, A.L.; Zhang, Y.; Niang, B.; Hamid, A.S.; Zhang, H.; Xu, L.; Zuo, Y. Niclosamide inhibits colon cancer progression through downregulation of the Notch pathway and upregulation of the tumor suppressor miR-200 family. *Int. J. Mol. Med.,* **2016**, *38*(3), 776-784. http://dx.doi.org/10.3892/ijmm.2016.2689 PMID: 27460529

[67] Jiang, L.; Li, L.; He, X.; Yi, Q.; He, B.; Cao, J.; Pan, W.; Gu, Z. Overcoming drug-resistant lung cancer by paclitaxel loaded dual-functional liposomes with mitochondria targeting and pH-response. *Biomaterials,* **2015**, *52*, 126-139. http://dx.doi.org/10.1016/j.biomaterials.2015.02.004 PMID: 25818419

[68] Mallick, S.; Thuy, L.T.; Lee, S.; Park, J.I.; Choi, J.S. Liposomes containing cholesterol and mitochondria-penetrating peptide (MPP) for targeted delivery of antimycin A to A549 cells. *Colloids Surf. B Biointerfaces,* **2018**, *161*, 356-364. http://dx.doi.org/10.1016/j.colsurfb.2017.10.052 PMID: 29100129

[69] Luo, G.F.; Chen, W.H.; Liu, Y.; Lei, Q.; Zhuo, R.X.; Zhang, X.Z. Multifunctional enveloped mesoporous silica nanoparticles for subcellular co-delivery of drug and therapeutic peptide. *Sci. Rep.,* **2014**, *4*(1), 6064. http://dx.doi.org/10.1038/srep06064 PMID: 25317538

[70] Gisbert-Garzarán, M.; Lozano, D.; Vallet-Regí, M. Mesoporous Silica Nanoparticles for Targeting Subcellular Organelles. *Int. J. Mol. Sci.,* **2020**, *21*(24), E9696. http://dx.doi.org/10.3390/ijms21249696 PMID: 33353212

[71] Cao, J.; Zhang, Y.; Shan, Y.; Wang, J.; Liu, F.; Liu, H.; Xing, G.; Lei, J.; Zhou, J. A pH-dependent antibacterial peptide release nanosystem blocks tumor growth *in vivo* without toxicity. *Sci. Rep.,* **2017**, *7*(1), 11242. http://dx.doi.org/10.1038/s41598-017-11687-y PMID: 28894233

[72] O'Neal, D.P.; Hirsch, L.R.; Halas, N.J.; Payne, J.D.; West, J.L. Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. *Cancer Lett.,* **2004**, *209*(2), 171-176. http://dx.doi.org/10.1016/j.canlet.2004.02.004 PMID: 15159019

[73] Ma, Z.; Zhang, Y.; Zhang, J.; Zhang, W.; Foda, M.F.; Dai, X.; Han, H. Ultrasmall Peptide-Coated Platinum Nanoparticles for Precise NIR-II Photothermal Therapy by Mitochondrial Targeting. *ACS Appl. Mater. Interfaces,* **2020**, *12*(35), 39434-39443. http://dx.doi.org/10.1021/acsami.0c11469 PMID: 32805937

[74] Liu, J.; Liang, H.; Li, M.; Luo, Z.; Zhang, J.; Guo, X.; Cai, K. Tumor acidity activating multifunctional nanoplatform for NIR-mediated multiple enhanced photodynamic and photothermal tumor therapy. *Biomaterials,* **2018**, *157*, 107-124. http://dx.doi.org/10.1016/j.biomaterials.2017.12.003 PMID: 29268142

[75] Agemy, L.; Friedmann-Morvinski, D.; Kotamraju, V.R.; Roth, L.; Sugahara, K.N.; Girard, O.M.; Mattrey, R.F.; Verma, I.M.; Ruoslahti, E. Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma. *Proc. Natl. Acad. Sci. USA,* **2011**, *108*(42), 17450-17455. http://dx.doi.org/10.1073/pnas.1114518108 PMID: 21969599

[76] Agemy, L.; Kotamraju, V.R.; Friedmann-Morvinski, D.; Sharma, S.; Sugahara, K.N.; Ruoslahti, E. Proapoptotic peptide-mediated cancer therapy targeted to cell surface p32. *Mol. Ther.,* **2013**, *21*(12), 2195-2204. http://dx.doi.org/10.1038/mt.2013.191 PMID: 23959073

[77] Zhao, K.; Zhao, G.M.; Wu, D.; Soong, Y.; Birk, A.V.; Schiller, P.W.; Szeto, H.H. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J. Biol. Chem.,* **2004**, *279*(33), 34682-34690. http://dx.doi.org/10.1074/jbc.M402999200 PMID: 15178689