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Review

Mitochondrial targeting theranostic nanomedicine and molecular biomarkers for efficient cancer diagnosis and therapy

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ABSTRACT

Mitochondria play a crucial part in the cell's ability to adapt to the changing microenvironments and their dysfunction is associated with an extensive array of illnesses, including cancer. Mitochondrial dysfunction has been identified as a potential therapeutic target for cancer therapy. The objective of this article is to give an in-depth analysis of cancer treatment that targets the mitochondrial genome at the molecular level. Recent studies provide insights into nanomedicine techniques and theranostic nanomedicine for mitochondrial targeting. It also provides conceptual information on mitochondrial biomarkers for cancer treatment. Major drawbacks and challenges involved in mitochondrial targeting for advanced cancer therapy have also been discussed. There is a lot of evidence and reason to support using nanomedicine to focus on mitochondrial function. The development of a delivery system with increased selectivity and effectiveness is a prerequisite for a theranostic approach to cancer treatment. If given in large amounts, several new cancer-fighting medicines have been created that are toxic to healthy cells as well. For effective therapy, a new drug must be developed rather than an old one. When it comes to mitochondrial targeting therapy, theranostic techniques offer valuable insight.

1. Introduction

Cancer is a serious public health concern in almost every country and most people have been affected by cancer globally [1,2]. The failure of cell's uncontrolled proliferation and cell death mechanisms in certain parts of the body causes the onset of this disease [3]. To date, most cancer patients have been treated with several therapies including radiation, surgery, endocrine and chemotherapy, etc. Furthermore, anti-angiogenesis agents, novel antibodies, viral therapies, and, small compounds are emerging therapeutics that make treatments more tumor-specific and less hazardous to the patients [4,5]. As a result, many anti-cancerous medications have been discovered to trigger cell death by targeting specific elements of tumor tissues. Usually, cancer tissues prior to the surgical removal, the patients must be subjected to chemotherapy for reducing the tumor size, followed by the surgical removal and then radiation therapy has to be performed to kill leftover tumors. However, because of their non-selectivity, many therapeutic techniques may have flaws and have negative effects on normal tissues. To overcome these significant constraints, new and focused treatment techniques are required [6].

Among these treatment methods, mitochondrial targeting of drugs is becoming an emerging field of study because of their promising outcomes in preclinical studies. Mitochondria are an attractive target for intracellular drug delivery since mitochondrial dysfunction may lead to a variety of human illnesses. Mitochondrial function is essential for apoptosis, calcium metabolism, oxidative damage, obesity, and diabetes [7]. Additionally, physiologically active compounds can be distributed selectively into organelles [8]. The mitochondria have a

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double-membrane structure made up of the mitochondrial outer membrane (MOM), the mitochondrial inner membrane (MIM), the interstitial membrane (ISM), and the matrix. The matrix comprises mitochondrial DNA (mtDNA), which helps in oxidative phosphorylation (OXPHOS) by encoding an essential protein. Because it limits introns and self-repair mechanisms, mtDNA is more prone to mutation than nuclear DNA (nDNA) [9]. Mutations in mtDNA have an impact on the respiratory chain, ensuing in the formation of reactive oxygen species (ROS). Excess ROS generation degrades mtDNA as well as proteins, cellular lipids, and nDNA. Enduring revelation to ROS can result in cellular demise. However, if the mutant cells last, they have the potential to develop as cancer cells [10].

There is evidence that many mutations in mtDNA cause a range of malignant tumors, including colon, prostate, and breast cancer [11]. Mitochondria play such a significant role in tumor development and movement that mitochondrial-targeted anti-tumor treatment is extremely required for the management of cancer. Alternatively, the mitochondrial membrane has relatively limited permeability to MIM and MOM only allowing molecules smaller than 1500 Da and 5000 Da through the membrane, respectively [12]. In this case, drug delivery to the mitochondria is quite difficult. Mitochondria differ from other subcellular structures in their complex dual-membrane structure [13]. The double membrane construction is intended to maintain some of the organelle's key activities, such as ATP synthesis, electron transport chain and cellular respiration. Cancer cells generate glucose differently than healthy cells. Glucose is transformed to pyruvate in the cytoplasm via glycolysis and subsequently oxidized with oxygen in the tricarboxylic acid cycle (TCA) in normal cells. This metabolism generates CO_2, NADH (a reduced form of NAD^+ that is utilized as fuel for ATP synthesis via OXPHOS), and modest amounts of lactic acid. Under anaerobic conditions, glucose is bio-transferred to pyruvate through glycolysis. Due to hypoxia, pyruvate cannot be further oxidized, so it is converted to lactic acid. This causes the cells to produce a high quantity of lactic acid [14]. Regardless of oxygen availability, most cancerous cells consume sugar by glycolysis, resulting in excessive lactate generation; this is called the Warburg effect/aerobic glycolysis, and it promotes mitochondrial dysfunction, producing a favorable environment for cells for development and proliferation into cancerous cells. This confirms that mitochondrial metabolism is disrupted by mtDNA alterations, resulting in an adapted microenvironment for cancer cells and tumor growth. This metabolic defect upsurges the generation of ROS in the mitochondria and alters oxidative stress activities. As a result, altering the function of transcription factors such as Fos, Jun and hypoxia-inducible factor (HIF), that alters gene expression and promotes cancer cell growth [15]. Furthermore, metabolic end products serve as raw materials for the synthesis of proteins, nucleotides, and lipids, all of which contribute to cancer formation [16]. The acidity of malignant cells is more than that of healthy cells, which is essential for their proliferation. The formation of lactic acid by anaerobic glycolysis is the primary source of this acidic environment [17].

Targeting offers optimized therapeutic results and decrease undesired side effects by distinguishing the targeted areas from other locations (i.e., nontarget sites). Molecular imaging and diagnostic have been integrated into theranostic system to better understand the locations and severity of the disease before prescribing the pharmaceuticals. Payloads in delivery vehicles include drugs and imaging or diagnostic molecules for the targeted imaging and therapy of cancer. Additionally, future drug delivery system (DDS) have piqued the curiosity of "Theranostics" which includes the targeted delivery of imaging and therapeutic agents to the tumors [18]. Through nanocarriers to control the mitochondrial microenvironment is an effective way to treat cancer. These nanocarriers target mitochondria through different genetics and physiological processes, including phagocytosis, macro-pinocytosis, clathrin-related endocytosis, receptor and protein mediation, membrane fusion and mitochondrial trans-membrane-mediated accumulation that triggers the accumulation of glutathione when exposed to triphosphine from ROS-accelerated hydrophobic peptide-mediated cells [19].

In a study, Zhang et al. developed an aggregation-induced emission (AIE)-targeted theranostic system that is capable of cancer- targeted imaging and treatment. Probes based on tetraphenylethylene (TPE), which are lipophilic, showed no emission in water; nevertheless, when aggregated, the triphenyl phosphine TPP-tagged TPE had a red emission. These analogs demonstrated preferential absorption in cancer cells (HeLa, MDA-MB-23, and NIH-3T3) over the reference. Because cancer cells have a greater mitochondrial membrane potential, this effect occurs. HeLa and MDA-MB-231 cells evaluated in the MTT experiment exhibited greater dark toxicity (IC_{50} = 6.31 mM and 4.03 mM, respectively) than other examined controls. The substances that were examined have the potential to damage the mitochondrial membrane, which is thought to be the cause of the dark toxicity. According to cell-based ROS production assays, under white light illumination (0.25 W/cm^2, 8 min), was capable of ROS creation and generated concentration-dependent photo-induced toxicities in HeLa (IC_{50} = 0.69 mM) and MDA-MB-231 (IC_{50} = 2.45 mM). Imaging-guided combination cancer therapies that don't rely on conventional drug conjugation might benefit from multifunctional systems that don't have a tendency to develop drug resistance [20].

In another study, Luo et al. established a molecular-based "structure inherent targeting and treatment" system. A series of cationic heptamethine analogs with different groups on the N-alkyl side chains. Researchers measured the NIR fluorescence contrast index, the photothermal effect, and the ability of the produced chemicals to generate ROS. With indocyanine green (ICG) as a reference, the compound was shown to have the best molar extinction coefficient (231,370 M^{-1} cm^{-1}, λabs/λemm = 779/800 nm in PBS), photothermal effect (ΔT ~ 28 °C), and ROS production capability among the investigated compounds (6-folds). Cellular uptake and co-localization experiments demonstrated that the lipophilic compound was taken up by cancer cells by energy-dependent organic-anion carrying polypeptide transporters but not by typical cellular endocytosis (clathrin, actin, and caveolae-mediated). Human cancer cell lines (A516, H460, MCF-7, and 4T1) examined with the lead chemical demonstrated concentration-dependent photo-induced toxicity (808 nm laser, 105 W/cm^2, 5 min). Calcein AM and propidium iodide assays show that photodynamic therapy (PDT) and photothermal therapy (PTT) act together to improve cytotoxicity. Additionally, in subcutaneous A516 and orthotopic 4T1 tumor models, the synergetic diagnostic (fluorescence/thermal imaging) and phototherapeutic (PDT/PTT, 808 nm, 0.8 W/cm^2, 5 min) effectiveness of a single laser was proven, with substantial tumor suppression and enhanced survival rates [21].

Recently, many types of nanocarriers enabling tumor targeting have been developed as a novel cancer therapy approach. Because of their nanoscale dimension, nanocarriers have a higher surface area and unique mechanical, electrical, photonic, and magnetic characteristics. Specialized nanocarriers for targeted cancer therapy have been established to address the challenges of regular drug delivery methods. Using multifunctional nanostructures as drug carriers, cancer cells can successfully overcome multidrug resistance [22]. Nanoparticles (NPs) have been proven to decrease adverse effects, enhance pharmacokinetic characteristics, and give a longer half-life in medication administration. In the present review, we have discussed the different approaches for mitochondrial targeting. It also highlights the necessity of nanomaterials and therapeutic techniques to target cancerous cells and address numerous conceptual benefits of the theranostic nanomedicine system for cancer therapy.

2. Molecular mitochondrial targeting for advanced cancer therapy

Mitochondria-targeted treatments are becoming successful in the field of cancer therapy. Much emphasis has been paid in developing mitochondrial-targeted therapeutic systems, as the mitochondria is the

primary power source of cancer cells. Cancer cells have unique mitochondria from normal tissues. And the unique characteristics of cancer cells' mitochondria might be used to target cancer cells' mitochondria with specialized medications. Several molecular targets and their key prospects have been illustrated in the Fig. 1 **and** Table 1. One or more of these mitochondrial characteristics can be used to develop a diversity of different medication delivery methods. Mitochondria accomplish a variability of roles related to channels, proteins, and enzymes, including the formation of energy in the form of ATP, appropriate glucose metabolism, the destruction of ROS, and so on. Some of them are discussed below:

2.1. The function of Hexokinase II target

The mitochondria can be targeted with the help of the enzyme Hexokinase-II because it is involved in the cell's metabolic process. Furthermore, cancer cells generate ATP solely through aerobic glycolysis, which can be used as effective drug targeting cancer cells. In glycolysis, hexokinase (HK) aids in the breakdown of glucose into glucose-6-phosphate (G-6-P). There are several isoforms, including HK-I, HK-II, HK-III, and HK-IV. HK-II is the most commonly active in cancer cells among the four subtypes [23]. The binding of HK-II takes place with the voltage-dependent anion channel 1 (VDAC1) in the outer membrane of the mitochondria and enhances VDAC1's interaction with the adenine nucleotide translocase in the inner membrane. Such interaction facilitates the combination of aerobic glycolysis and oxidative phosphorylation (OXPHOS), which acts as a link between both metabolic processes. HK-II also assists in the conversion of ADP to ATP in the mitochondria and speeds up glycolysis [24]. As a result, HK-II inhibition can result in the cessation of aerobic glycolysis and, ultimately, cancer cell death as illustrated in Fig. 2**.** HK-II can be identified using glucose comparisons such as 3-bromopyruvate (3-BPA) and 2-deoxy-D-glucose (2-DG). When 2-DG enters the cell, it competes with glucose and is phosphorylated by HK-II into 2-deoxyglucose phosphate (2-DGP), rendering it ineffective in subsequent glycolysis steps. As a result, 2-DGP accumulates in cells, causing HK-II products inhibition [25]. HK-II and mitochondria work together to prevent apoptosis. By converting HK-II sulfhydryl groups, 3-BPA is transformed into an alkylating agent. This mutation causes HK-II to be released from the mitochondria, resulting in apoptosis and cell death. According to Bao et al., 13 steroid extracts were obtained from *Ganoderma sinense* mushrooms, (22E, 24R)-6-methoxyergosta-7,9 (11), 22-triene-3, 5-diol had strong HK-II binding ability, which was verified in vitro by several techniques such as thermophoresis, inhibition of the enzyme, and testing on cells. As it inhibits HK-II, it

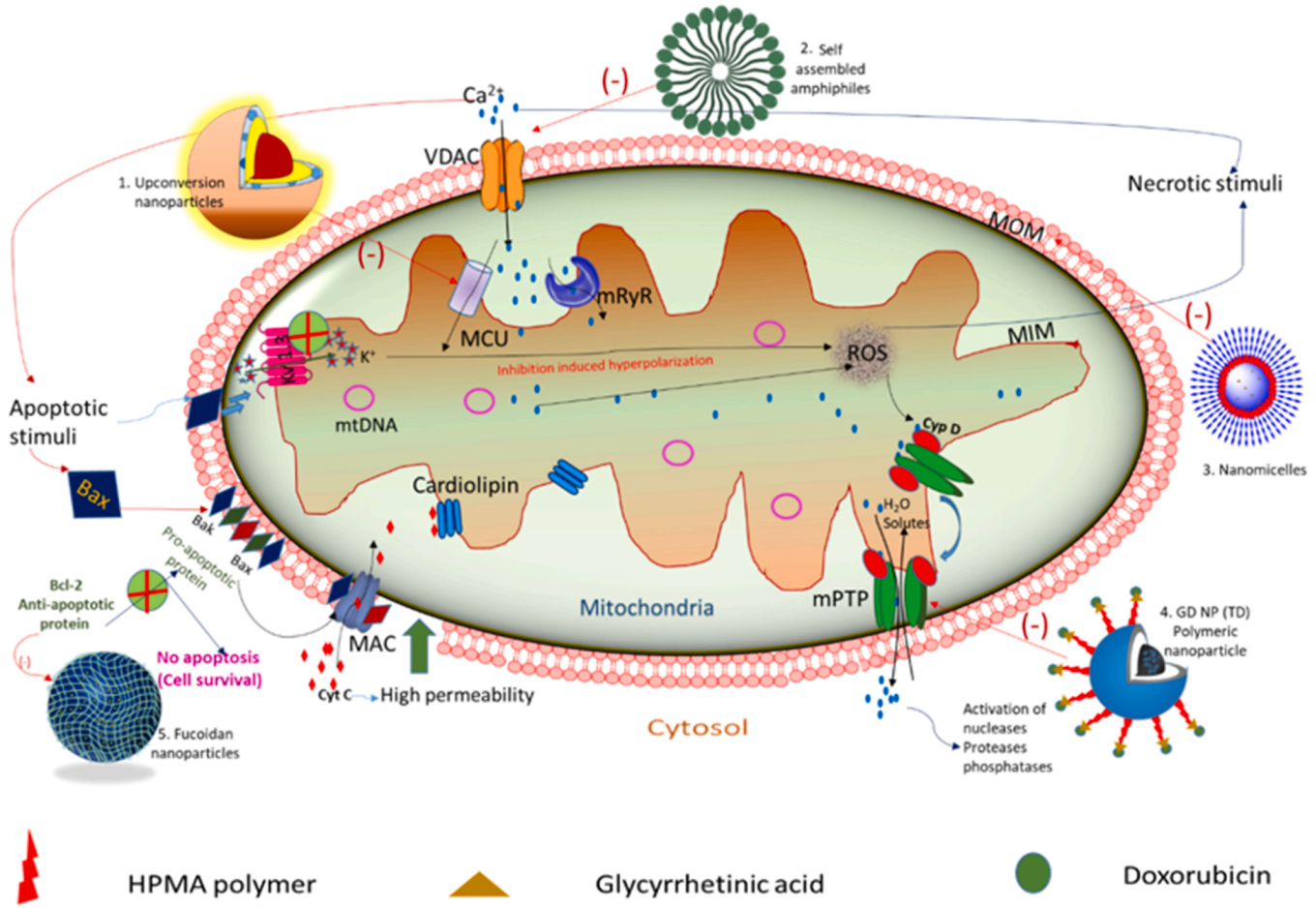

Fig. 1. Schematic illustration of several approaches involved in nanoparticles mediated mitochondrial targeting for cancer therapy: (1) Dysfunction and disruption of calcium channels by up-conversion of nanoparticles loaded with ruthenium red, which inhibits the Ca^{2+} channel proteins like MCU and restricts the uptake of Ca^{2+} from the cytoplasm leading to apoptosis. (2) self-assembled supramolecular PalpHK-pKV synthetic amphiphilic peptide engineered with hexokinase-II protein which binds to VDAC-1 and triggers the dissociation of the VDAC1–HK-II complex causes mitochondria-mediated A549 cancer cell apoptosis. (3) Paclitaxel (PTX)-loaded TPP-Pluronic F127-hyaluronic (PTX/TPH) nano-micelles induce mitochondrial outer membrane permeabilization (MOMP) by inhibiting anti-apoptotic Bcl-2, leads to release of cytochrome C and subsequent activation of caspase-3 and caspase-9, results in apoptosis. (4) Glycyrrhetinic acid-Doxorubicin conjugated with tripolyphosphate-Dox core self-assembled nanoparticle [GD-NP(TD)] enhances the specific penetration to mitochondria by opening the mPTPs resulting in substantial mitochondrial impairment, and ultimately inhibits the orthotopic tumor growth. (5) P-selectin targeted fucoidan nanoparticles loaded with venetoclax localizes at Bcl2 protein, inhibited the anti-apoptotic proteins, and initiate the process of apoptosis.

Table 1
Different molecular mitochondrial targets and their clinical outcomes.

Peptide	Type of cancer	Study design	Result	Ref.
Anti-cancer peptides targeting the VDAC pathway				
Antp-LP4	Chronic lymphocytic leukemia	In vitro	Decreases the quantity of cellular ATP and MMP, as well as HK-I, Bcl-2, and Bcl-xL anti-apoptotic actions and Cyt c release.	[134]
Tf-LP4	Chronic lymphocytic leukemia	In vitro	Cell death that has been induced	[31]
TAT-LP4	Chronic lymphocytic leukemia	In vitro	Inducing cell death is less effective.	[30]
TAT-HK	Liver cancer	Ex vivo	Depolarization of the mitochondrial membrane leads to an increase in cell death.	[135,136]
Anti-cancer peptides targeting the Ca^{2+} pathway				
MTD	Breast cancer, Colon carcinoma	Treating cells causes cellular swelling and necrosis rather than apoptosis.	By opening mPTP, penetrate any type of cytoplasmic membrane and trigger $Ca2^{+}$ leakage from mitochondria.	[137]
Peptides from the Bcl-2 family that are anti-tumor				
ABT-737	Breast cancer, cervical cancer	Preclinical (acute myeloid leukemia, lymphoma multiple myeloma, acute lymphocytic leukemia, acute lymphocytic leukemia, and SCLC)	AML blast, stem cells and progenitors are successfully killed by programmed cell death of malignant cells without damaging normal hematopoietic cells.	[138,139]
Navitoclax	Lymphatic cancer, breast cancer	Phase I clinical (CLL, lymphomas)	In preclinical trials, ABT-263, which has similar biological features to ABT-737 but has a longer oral half-life, increased the activity of other chemotherapeutic drugs.	[140–142]
ABT-199	Chronic lymphocytic leukemia	Preclinical (CLL)	Bcl-2 inhibition by pharmacological means is showing promise in the treatment of Bcl-2-dependent hematological malignancies.	[143]
ATAP	Prostate cancer	In vitro	Caspase-dependent apoptosis is induced, and MOMP is induced without the involvement of Bax and Bak.	[144,145]
Ant-BH3	Prostate cancer	NA	MOMP was caused by targeting Bcl-xL.	[146]
p15 tBid	Liver cancer	In vivo and in vitro	Cyt c and BAK oligomerization release are initiated, followed by apoptosis.	[147,148]

(*continued on next page*)

Table 1 (*continued*)

Peptide	Type of cancer	Study design	Result	Ref.
Obatoclax	Non-small cell lung cancer, multiple myeloma, lymphoma	Phase I/II clinical trials (Leukemia, lymphoma, multiple myeloma, and non-SCLC)	Added to carboplatin/ etoposide chemotherapy as a first-line treatment for small-cell lung cancer in its advanced stages.	[149]
TLSGA-FELSRDK	Ovarian Cancer	In vivo (SKOV3 ovarian cancer)	Induces early-stage apoptosis	[150]
Anti-cancer peptides targeting ROS pathway				
GO-203	Lymphomas	In patients with advanced solid tumors, GO-203–2c was given intravenously daily21 and was repeated every 28 days in a phase I clinical trial: lymphomas	The C-terminal protein of MUC1 is targeted by redox imbalance and an increase in ROS, which affects MUC1-C ROS suppression.	[151,152]
TAT-FHIT	Hepatocellular carcinoma	In vitro Hepatocellular carcinoma cells	Apoptosis is induced by the production of reactive oxygen species (ROS) and the stimulation of calcium absorption in the mitochondria.	[153]
Other peptides mitochondria-targeted cancer therapy				
R7-kla	Fibrosarcoma	Both in vitro and in vivo studies have been conducted (HT1080 human fibrosarcoma cell line)	When compared to kla, the target mitochondrial membrane showed better cytotoxicity.	[154]
RGD-4C-GG-D (KLAKLAK)2	Breast cancer	In vivo	MOMP was produced by targeting the mitochondrial membrane, which inhibited the growth of breast cancer in naked mice and caused endothelial cell death.	[155]
BHAP	Breast cancer	Both in vitro and in vivo studies have been conducted (selective internalization into HER-2 overexpressing human breast cancer cells)	The growth factor receptor signaling and mitochondrial function are both perturbed by the apoptosis-inducing peptide.	[156]
killerFLIP	leukemia, prostate and colon cancer	In vitro and in vivo	Cell death is triggered by activation of the apoptotic intrinsic route, which first affects plasma membrane permeability and later mitochondrial membrane permeability.	[157]
LL-37/hCAP-18	Colon cancer, oral squamous cell carcinoma	In vivo (human oral squamous cell carcinoma SAS-H1 cells colon cancer)	Induce apoptosis	[158,159]
Buforin II and Buforin IIb	Prostate cancer	In vivo (broad spectrum of cancer cells)	Caspase-9 activation and cytochrome c release result in oncolytic action.	[160]
Hunter-killer peptides	Lung, prostate and breast cancer	In vivo (breast, prostate, and lung carcinoma human xenografts)	Kill cancerous blood arteries without causing injury to the rest of	[161,162]

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Table 1 (*continued*)

Peptide	Type of cancer	Study design	Result	Ref.
			the circulatory system.	

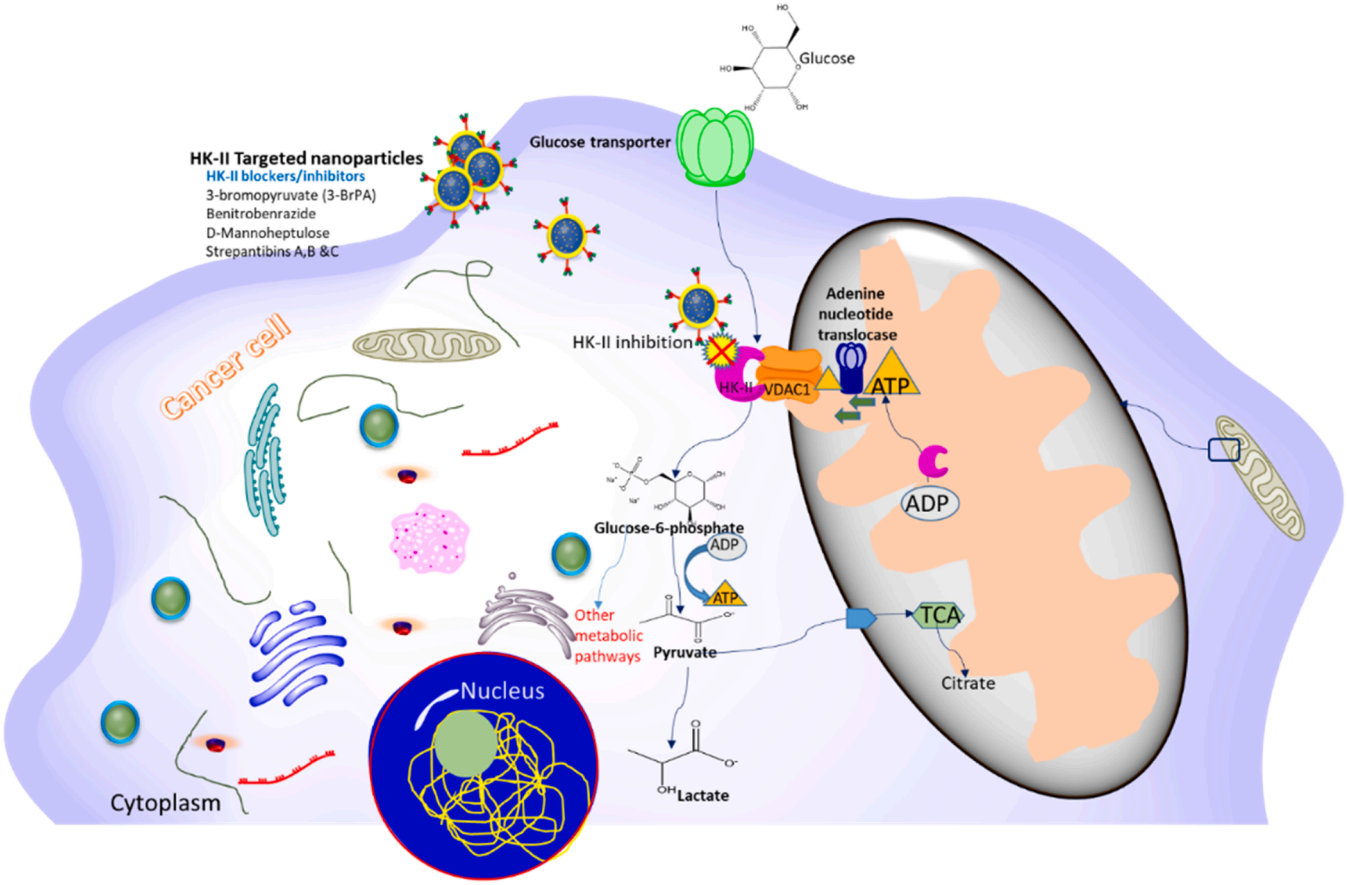

Fig. 2. Role of hexokinase-II inhibitors in cancer therapy: HK-II binds to the VDAC1 at MOM and enhances VDAC1's interaction with the adenine nucleotide translocase in the inner membrane. Such interaction facilitates the combination of aerobic glycolysis and oxidative phosphorylation and also assists the conversion of ADP to ATP in the mitochondria, subsequently speeds up glycolysis.

has anti-cancer potential for the treatment of cancer [26].

2.2. *Different ion channels available in mitochondria for targeting cancer cells*

2.2.1. *Outer membrane ion channels of mitochondria*

MOM ion channels control the entry of all bodily components and the exit of various apoptotic messengers. The outer translocation enzymes responsible for protein transport are the ion channels found in the MOM. This channel was designed to increase VDAC metabolites and cytochrome c release upon mitochondrial death. These three pathways aid in the setup of mPTP. By boosting cytochrome c release, mPTP promotes apoptosis [27]. According to MOM's ion channels, VDAC1 is often targeted for cancer therapy. VDAC is sometimes referred to as a mitochondrial porin. These channels in the mitochondrial inner membrane transport ions (Cl^{-1}, Na^{+}, Ca^{2+}, and K^{+}), NAD^{+}/NADH and ADP/ATP, cholesterol, fatty acids, and reactive oxygen species between the inner layer mitochondria and the cytosol. Mammals have three VDAC isoforms: VDAC1, VDAC2, and VDAC3. VDAC1 is a component of mitochondrial apoptosis in mammals. It inhibits apoptosis by interrelating with anti-apoptotic proteins, for instance, the glycolytic enzyme HK and B-cell lymphoma 2 (Bcl2). An apoptosis-inducing factor, cytochrome c was transported to the cytoplasm and promotes cell survival [24]. VDAC-1 inhibits protein-binding apoptosis, which increases mitochondrial inner membrane permeability. This will also allow pro-apoptotic proteins to get through the outer membrane and enter the cytoplasm, resulting in apoptosis [28]. Pro-apoptotic chemicals are compounds that inhibit the interaction of HK-II or VDAC1 and induce differentiation, culminating, in cell death. The combination of VDAC1-derived peptides, namely N-terminal-Antp and Antp-LP4, can selectively kill monocytes in patients with lymphoid leukemia without damaging normal cells [29,30]. The same research team also synthesized a single-molecule peptide called R-Tf-D-LP4 to improve cell specificity, recognition and, stability. The transferrin receptor internalization sequence (Tf) present in peptides improves peptide control in cancer cells because the transferrin receptor is overexpressed in cancer cells [31].

2.2.2. *Mitochondrial inner membrane ion channels*

The electrochemical gradient can be provided by the MIM that surrounds the substrate, which is required for the formation of ATP. Several ion channels in MIM are involved in ion exchange, ATP excretion, and mitochondrial cytochrome c release inside the cytoplasm. The apoptotic cascade may be regulated by controlling the proclamation of cytochrome c into the cytoplasm via these channels, thereby maintaining

mitochondrial homeostasis [32,33]. Recently, several researchers have developed many ion channels in the MIM that have been associated with cell death, such as ATP- dependent K^+ channels [34], voltage-gated K^+ channels [35], and calcium-activated potassium channels [36]. Cruz et al., analyzed the proteome of mouse liver MIM. The study includes multiple ion channels and several carrier proteins that unquestionably hold vital importance in mitochondrial function and so ought to be characterized at the molecular level. As a means of accomplishing this, they employed a novel strategy that included the utilization of highly pure inner membranes from mouse liver mitochondria, the extraction of membrane proteins with an organic acid, and tandem mass spectrometry using two-dimensional liquid chromatography. With the help of this method, they identify 182 proteins that are crucial to various biochemical processes, like the ion or substrate transport of the electron transport apparatus as well as the import of proteins into cells. The hydrophobicity values of up to 16 transmembranes predicted domains were included in the study, which covered the whole range of isoelectric point, molecular mass, and hydrophobicity values. For instance, 20 of the 182 proteins discovered were new or unrelated to the MIM. Using mammalian cells, some of these proteins were overexpressed, which validated their mitochondrial location and resulted in a significant modification of the mitochondrial network. There is a need for a more extensive analysis of the newly identified proteins in the MIM since this work gives the first proteome of the MIM [37].

2.2.3. Mitochondrial permeability transition pore (mPTP)

The mPTP is a pore that has been shown to improve MIM permeability. The mitochondrial outer membrane has a highly complicated structure that includes proteins such as HK-II and VDAC. The mitochondrial inner membrane, on the other hand, is made up of cyclophilin D (CyP-D) and adenine nucleotide translocase (ANT) [38,39]. A high concentration of calcium and ROS triggers these channels to open in the mitochondria. The opening of mPTP leads to depolarization, which further reduces the membrane potential of mitochondria and respiratory chain complexes, affecting ion homeostasis and ATP production [29]. There are two ways by which HK-II and VDAC prevent the formation of mPTP. First, the combination of VDAC and HK-II leads to a conformational alteration of VDAC, which causes ANT to undergo a structural alteration. This opens a route for ATP transport, but these modifications are incompatible with the development of mPTP. Second, the HK-II-VDAC interaction hinders Bax as well as other pro-apoptotic proteins from attaching to VDAC. This means that the oligomeric structure necessary for mPTP activation does not exist. Prevention of binding to VDAC by pro-apoptotic proteins like HK-II leads to the enlargement of mPTP, which could result in cell death if not prevented. Moreover, reduced CyPD seals mPTP pores whereas oxidized CyPD opens these pores. According to Folda et al., auranofin is an oxidant that promotes the oxidation of CyPD in human leukemia T cells [40].

2.2.4. Calcium uniporter complex in mitochondria

The level of intracellular calcium (Ca^{2+}) in the mitochondria is critical in the pathophysiology of the cell. The average level of Ca^{2+} regulates the tricarboxylic acid cycle and stimulates oxidative phosphorylation, whereas a high level of Ca^{2+} induces apoptosis by activating mPTP [41]. Upon exiting the endoplasmic reticulum and entering the cytoplasm, Ca^{2+} ions can traverse the two membranes and reach the mitochondria. It enters the mitochondria through the VDAC1 protein found in the MOM. It penetrates mitochondria, including the pore-forming components MCUa (mitochondrial calcium uniporter a), MCUb, and regulatory proteins such as mitochondrial calcium uptake 1 (MICU1) and MICU2. The complex enters the mitochondrial matrix with the assistance of the essential MCU regulator (EMRE) basic, which includes the MCU controller. MICU1 functions as an MCU activator, while MICU2 functions as an MCU inhibitor. MICU1 and MICU2 form heterodimers with MCU to regulate Ca^{2+} in the mitochondria under normal circumstances. When cytosolic Ca^{2+} levels rise, the dimer changes shape and MICU2 detaches, leaving the MCU-MICU1 complex behind. EMRE stabilizes this complex, allowing Ca^{2+} ions to enter the mitochondria. Ca^{2+} is important for ATP production in mitochondria. Due to the up-regulation of the MCU complex, many Ca^{2+} ions lead to cell apoptosis; the low Ca^{2+} level caused by the down-regulation of the MCU complex will result in low power generation efficiency. To sustain proper mitochondrial function, a balance must be maintained. As a result, up-and down-regulation of the MCU complex may be a viable method of killing cancer cells. Bortezomib treatment for multiple myeloma increases MCU complex expression and increases Ca^{2+} levels within mitochondria, ensuing in increased ROS production and tumor cell death [42].

2.2.5. Voltage-dependent potassium channels

Six transmembrane helices and a hole make up the voltage-dependent potassium channel (Kv). From Kv1 to Kv12 are the 12 families of the Kv channel, and each family contains several subtypes. The most frequent isoform seen in the central nervous system, T-lymphocytes, kidneys, epithelial cells, and other organs is Kv1.3 [43,44]. The presence of this protein in the plasma membrane regulates cell growth. However, the MIM appearance of Kv1.3 is involved in apoptosis because it can interact with the pro-apoptotic Bcl2-related X (Bax) in lymphocytes [45–47]. Compared with normal cells, Kv1.3 is more produced to help cancer cells proliferate, migrate and metastasize. Inhibition of these channels helps in the removal of malignancy. The majority of the penetration inhibitors (Psora4, PAP1, and clofazimine) that penetrate the plasma membrane and impact the MIM-Kv1.3 channel have been investigated. Inhibiting MIM Kv1.3 increases ROS generation, which opens the leaky junction, causing MIM depolarization, mitochondrial enlargement, cytochrome c release, and, eventually, death. In a study, Zaccagnino et al. reported the use of clofazimine to kill adenocarcinoma (PDAC) cells invitro and to decrease tumor development in-vivo by 50% in the orthotopic xenograft-PDAC-model of severe combined immunodeficiency (SCID) mice [48]. Adelman et al., investigated the linkage between episodic ataxia (EA)and voltage-dependent potassium channels. The voltage-dependent delayed rectifier, Kv1.1, on chromosome 12 has notably been linked to familial EA. Kv1.1 missense mutations have been found in six EA families, all of which are heterozygous. There are two homomeric channels formed by two EA subunits in Xenopus embryos, and these channels differ in how they open and close. The faster kinetics and higher C-type inactivation make the voltage dependence of V408A channels identical to wild-type channels, but F1 84C channels have a 20-millivolt positive voltage dependence. The remaining four EA subunits, when joined with wild-type subunits, do not produce functioning homomeric channels but rather lower the potassium flow in these channels. The findings suggest that EA is caused by a biological process in which the affected nerve cell's delayed rectifier function impairs its ability to efficiently repolarize after an action potential [49].

2.3. Function of Bcl-2 target in onco-therapies

Bcl2 is an anti-apoptotic protein from the B-cell lymphoma II families that contributes to tumor growth, progression, apoptosis prevention, and treatment resistance. It is possible to classify Bcl2 as pro- or anti-apoptotic based on the protein's structure and activity. Bcl2 (founding member), Bcl-XL, Bcl-W, Myeloid cell leukemia 1 (MCL1), Bcl2-related protein A1, and BCLB/Boo protein are all anti-apoptotic members [50]. Members of the pro-apoptotic family are separated into binary subfamilies: multi-domain pro-apoptotic "effectors" and "BH-3 only proteins" with just a short BH3 domain [51,52]. Pro-apoptotic and anti-apoptotic proteins can both influence cellular demise during apoptosis. The supply chain execution of the mitochondrial outer membrane is influenced directly by these proteins. When BAX/BAK is activated, it oligomerizes to form a pore, increasing mitochondrial permeability and finally leading to apoptosis. Bcl2, like anti-apoptotic proteins, inhibits BAK/BAX activation and therefore prevents cell

apoptosis, whereas BH3 protein increases BAK/BAX oligomerization and causes cell death [53]. Anti-apoptotic proteins rise in cancer, whereas pro-apoptotic proteins decrease. BH3 mimetics have recently been created and evaluated for anti-cancer efficacy. ABT263 (Navitoclax) is an orally active BH3 mimic that specifically inhibits Bcl2 and has demonstrated encouraging clinical outcomes. Venetoclax is an oral treatment that selectively targets Bcl2 and is beneficial in patients having chronic lymphocytic leukemia (CLL)/relapsed small lymphoid lymphoma (SLL) [54].

2.4. The electron transport chain (ETC) as a cancer therapeutic target

The ETC is necessary for mitochondrial oxidative phosphorylation, which produces energy. ETC is made up of five complexes found in the interior membranous side of mitochondria: Complexes -I, II, III, IV, V. ATP generation is the main function of these complexes, which is a type of energy. CI, CIII, and CIV help in the transportation of electrons from NADH and succinic acid to O_2, resulting in the production of water molecules. CI and CII transport electrons to CIII and subsequently to cytochrome c through the electron-carrier ubiquinone (UbQ). These electrons are sent to the CIV by cytochrome c, and the CIV utilizes them to transform molecular oxygen into water molecules. The produced energy is stored in the MIM as static electricity and proton gradients. CV (F1FOATPase) uses this energy to convert ADP and inorganic phosphate to ATP, thus providing basic energy substrates for cells [55,56]. ETC also generates ROS and ATP, whereas CI and CIII are the major locations of superoxide generation and signal maintenance. Apoptosis is caused by elevated amounts of ROS and superoxide. Cancer cells can perish by triggering apoptosis when CI, CII, and CIII are inhibited. In a study, Szczepanek et al., evaluated the relevance of mitochondrial STAT3 in preserving mitochondrial activity during ischemia was examined by the researchers. Electron transport chain complexes I and II in cardiac mitochondria expressed by MLS-STAT3E were reduced slightly in ischemia-induced cardiomyopathy. Complex I-dependent respiration rates in MLS-STAT3E hearts were protected from ischemia injury, whereas WT hearts were not. Cytochrome c was not released during ischemia when MLS-STAT3E was used. Ischemia had no effect on the formation of reactive oxygen species in MLS-STAT3E mitochondria in the same way as it did in WT mitochondria, perhaps because MLS-STAT3E was able to partially inhibit electron transport through complex I. Because of STAT3 overexpression, these findings suggest that mitochondrial STAT3 may have a protective role that is distinct from its usual role as a nuclear transcription factor [57].

2.5. Role of oxidative stress in targeting cancer cells

One of the mechanisms involved in the production of ATP is oxidative phosphorylation. This system is also in charge of regulating ROS in mitochondria. As a result, it controls apoptotic cell death. Targeting oxidative phosphorylation causes a rise in ROS generation and a decrease in ATP synthesis, triggering the apoptosis cascade mechanism and ultimately leading to the death of cells [58,59]. Using pro-oxidants, oxidative stress and the production of ROS are used to target. In a study, Xiao et al., reported that, rotenone can activate the NADPH oxidase 2 (NOX-2) complex, resulting in excessive ROS production via the PI3K/Akt/mTORC1 signaling pathway. The Cell Counting Kit-8 assay was used to detect the cytotoxicity of rotenone on CC cells, and the clone creation assay was used to confirm it. Transwell invasion and wound healing tests were used to investigate the effects of rotenone on CC cell invasion and migratory activity in vitro. In addition, reverse transcription-quantitative PCR, western blotting, and immunofluorescence assays were utilized to determine if rotenone influenced the epithelial-mesenchymal-transition (EMT) process. Western blotting was used to assess the expression levels of major PI3K/AKT pathway markers in colon cancer (CC) after rotenone treatment alone or in conjunction with a PI3K/AKT signaling activator. Finally, the anticancer effects of rotenone were studied in a subcutaneous xenotransplant tumor model that was given an intraperitoneal infusion of the drug. The findings show that rotenone treatment caused CC cell cytotoxicity, with larger effects detected as concentrations increased, and hindered cell growth when compared to untreated cells. Rotenone inhibited CC cell migration, invasion, and EMT in in vitro cell function assays compared to untreated cells. When rotenone-treated CC cells were compared to untreated cells, the phosphorylation levels of AKT and mammalian target of rapamycin (mTOR) were downregulated mechanically. In addition, the PI3K/AKT signaling activator insulin-like growth factor 1 (IGF-1) enhanced AKT and mTOR phosphorylation, which was inverted by rotenone therapy. According to cell function assays, rotenone therapy reduced IGF1-activated cell proliferation, migration, and invasion. These findings showed that rotenone inhibited the PI3K/AKT/mTOR signaling pathway, which inhibited CC cell proliferation and metastatic abilities. Furthermore, rotenone inhibited tumor growth and CC's ability to spread, as demonstrated in a xenograft mouse model [60].

3. Cellular entry mechanism of novel drug delivery systems (NDDS) and therapeutic barriers for mitochondria

NDDSs can enhance the tumor microenvironment, internalize cells, and deliver drugs intracellularly via passive or active targeting. By altering the penetration and retention of nanoparticles (NPs), it is possible to influence their internalization and subcellular location by altering NP's size, shape, and surface charge [61]. Because of their increased affinity for organelles like mitochondria and nuclei, positively charged ultra-small NPs can increase the permeability of the cell's inner membranes. Antibodies, ligands, etc., are commonly used in active targeting because they have a particular interaction with the receptor and hence have a greater impact than standard therapeutic procedures. Because of our continued interest in nanoformulations that target specific subcellular organelles. Most cancer cells overexpress an extensive variety of targets that can be targeted in order to increase the amount of medication accumulated around them, including folate receptors, transferrin receptors (Tf), and antigens. Passive or active targeting can bring NPs to the cell surface, but cancer cells mostly take them up via endocytosis [62]. In order to enter the cell, distinct NDDSs use different cell endocytosis pathways. This ensures that they enter the cell in certain intracellular areas. Endocytosis mechanisms will be briefly reviewed to help with nanoformulation purpose predictions in cells. Various endocytosis process such as clathrin-mediated (CME), caveolae-mediated (CVME), macropinocytosis, and phagocytosis are shown in Fig. 3. CME and CVME are the primary absorption mechanisms for several different nanoformulations in this group. Large NPs (120 nm and larger) are mostly absorbed by CME, and particular ligand-modified nanoformulations can considerably boost the effectiveness of this endocytosis process [63]. To reach the lysosomal lumen, nanoformulations must first go through the early endosomal, late endosomal, and then lysosomal lumen trafficking pathways. While endosome/lysosomal degradation is expected to occur for nanoformulations that target other cytoplasmic locations, they should be tailored to sustain their biological function. The use of acid-resistant carrier materials and pH buffering solutions. However, nanoformulations with a particle size of less than 60 nm often rely on CVME for cell penetration. When these NPs are covered with caveolae, they frequently don't get into lysosomes and end up in the endoplasmic reticulum (ER) or Golgi. In order to achieve subcellular enrichment, anticancer NPs must first be endocytosed [64]. A lot of attention is paid to endocytic pathways while designing novel delivery methods for subcellular targeting. In an in-depth study and investigation of these subjects for subcellular targeting, endocytic pathways show a vital character in the development of novel delivery methods.

Even though direct delivery of medicinal drugs to the mitochondria is required, the definite mission is further problematic due to the mitochondria's very intricate nature, which consists of four parts: OM, IM, IMS, and MM Fig. 3. Developing efficient nanocarriers that can target

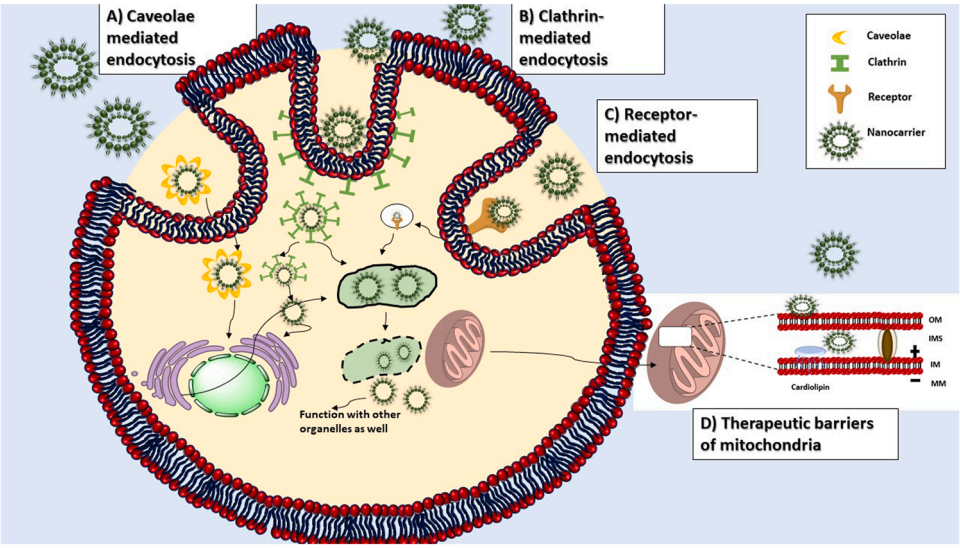

Fig. 3. Endocytosis and intracellular distribution of nano formulations are depicted in this diagram. A) Caveolae mediated endocytosis; B) Clathrin-mediated endocytosis; C) Receptor mediated endocytosis; D) Therapeutic barriers of mitochondria. OM, outer membrane; IMS, intermembrane space; IM, inner membrane; MM, mitochondrial matrix.

mitochondria is hampered by the difficulty of overcoming biological membrane barriers. The cytomembrane, cytoplasm, OM, and IM of mitochondria are the key obstacles for mitochondria's targeted nanocarriers. Once the nanocarriers have been intravenously administered, they may then spread into the extravascular environment of tissues and organs and cause harm there [65]. As nanocarriers pass through the cellular membrane in various ways, early and late endosomes are successively created by particles and are united with lysosomes to make the inclusion in the late endosome destroyed. An escape from the endosome is necessary for nanocarriers in order to avoid degradation by enzymes and acid in lysosomes. In contrast, the cytoplasm is very viscous because it is crammed with numerous tiny molecules, ions, and macromolecular, including proteins, RNA, and DNA. This makes the cytosol a significant diffusion barrier for nanocarriers to pass inside cells. As an example, fluorescein isothiocyanate's diffusion efficiency in the cytosol of Swiss 3T3 fibroblasts was only 28 % as fast as in free aqueous circumstances [66]. The phospholipid membrane of the mitochondria is composed of two separate layers, one on either side of the other. The OM and IM of mitochondria are the major barriers to nanocarriers that are developed for targeting MM. For the most part, the IMS is composed of voltage-dependent anion channels, which do not have transmembrane potentials. In the IM of mitochondria, a unique phospholipid, cardiolipin (CL), a two-tailed diphosphatidylglycerol lipid, was found, which adds another layer of complexity to these organelles and greatly influences

Table 2
Summary of certain particular mitochondria-targeting ligand nanocarriers and anticancer drug conjugates.

Nanocarriers/Drug delivery systems	Technique of preparation	Targeting ligands	Therapeutics agent	Therapeutic applications	Ref.
Liposomes	Film dispersion-active loading method	Dequalinium	Topotecan	Overcome the multidrug resistance in breast cancer MCF-7 cells and in lung metastatic model by cytochrome c mediated apoptosis.	[163]
Micelles	Dialysis method	TPP-PEG	Celastrol	The generation of ROS, and cytochrome release–mediated apoptosis via celastrol micelles with mitochondrial targeting	[164]
Micelles	Solvent exchange method	TPP	Doxorubicin	Induced cell death through mitophagy	[165]
HPMA copolymers	Radical precipitation copolymerization method	SS20	α-tocopheryl succinate	Using a cell-penetrating peptide in combination with mitochondrial targeting, significantly increased the distribution of HPMA copolymers in mitochondria, indicating an effective technique for enhancing mitochondrial targeting efficiency.	[166]
Self-assembled cyanostilbene nanoparticles	Multi-step approach	TPP	Doxorubicin	Self-assembled cyanostilbene nanoparticles offer a promising technique for prospective image-guided treatment as well as a site-specific distribution system for cancerous cells.	[167]
Prodrug nanocarriers	Iron-mediated ROS generation	TPP	Artesunate	Apoptosis and cytotoxicity were seen in cells treated with prodrug nanocarriers, which resulted in MMP depletion. As a result, artesunate nanocarriers offered an optimistic and promising alternative therapy aimed at combating cancer.	[76]
Polymeric nanocarriers	Co-solvent evaporation method	Biotin	Doxorubicin	The multifaceted nanomaterials have been shown to be an effective technique for delivering doxorubicin to MCF-7/ADR cancer cells and reversing MDR.	[103]
Liposomes nanocarriers	Reverse phase-evaporation method	PD-L1	R162	This nanoplatform enables systemic tumor elimination with great certainty attributable to multimodal imaging for therapy guidance. By altering redox equilibrium, this approach may provide important insights for cancer care.	[101]
Mesoporous silica nanoparticles	Co-condensation method	TPP	Chlorin e6	The drug loaded mesoporous silica nanoparticles successfully inhibit the molecular pathways of mitochondria. Thus, it inactivates ATP production and promotes ATP depletion.	[168]
Carbon dots	liquid-solid-solid synthetic route	TPP	N/A	In the treatment of mitochondrial illnesses and the early detection and therapy of cancer, this bio-nanoplatform might be useful	[97]

the numerous mitochondrial functions. Because of the respiratory chain's huge transmembrane electrochemical gradient and the resulting pH gradient (acidic on the outside) and high membrane potential (negative on the inside), mitochondria are primarily responsible for ATP generation via OXPHOS. These therapeutic compounds are unable to traverse the complex IM of mitochondria, which is why most nanocarriers are incapable of passing through negative membrane potential (160–180 mV) [67]. That's why this negative membrane potential is specific to mitochondria, as evidenced by this study's findings: Electropotential, ion trapping, and complex formation with CL may be used to selectively accumulate nanocarriers at a mitochondrial membrane potential [68].

4. Nanotherapeutics based approaches for mitochondrial targeting

It has been shown that nanotechnology-based techniques (Table 2) can assist medications in retaining and enhancing their therapeutic effects. Because of this, nanotechnology has a major impact on medication delivery by enhancing the pharmacokinetics of several pharmaceuticals and the biodistribution of others drugs. There has also been a significant advancement in the progress of DDS and mitochondrial treatments that use this method of delivery. When traveling via the mitochondria, medications transferred to the cell surface face many difficulties. Small drug molecules, on the other hand, greatly benefit from nanotechnology-based delivery systems. Nano formulations also have the virtue of solubilizing hydrophobic pharmaceutical compounds, extending their half-life, and reducing their side effects and immunogenicity [69]. One of the most important advantages of nanotechnology-based techniques is tailored medication delivery, which improves treatment efficacy. Various types of nanoparticles were seen in the publications to be particularly effective in delivering cargo into the mitochondrial matrix and directing the liberation of these cargos in multiple portions of the mitochondrial matrix in cancerous cells [70].

4.1. Peptide's based NPs

Using peptide-based nanocarriers to deliver anticancer medicines has received a lot of attention. Additionally, peptide nanocarriers have a number of advantages, notably their minimal cytotoxicity and abundance of renewable resources. It is also possible to change the surface of peptide nanocarriers with diverse ligands in order to target specific tumors with site-specific drug conjugation. Furthermore, cell-penetrating peptides (CPPs) are becoming increasingly popular as delivery systems since they generate a plethora of nano complexes based on the formulation circumstances and the features of CPPs that are employed in their formulation [71]. Bae et al. designed a cationic mitochondria-targeting sequence hybrid oligopeptide (MTS-H3R9) having anti-tumor activity as well as a dual role as a mitochondrial targeting carrier. When compared to unmodified MTS, MTS-H3R9 demonstrated significant cell penetration and internalization activity, better mitochondrial targeting ability in HeLa cells, and successful endosomal escape. In 3D spheres and 2D cell culture models, MTSH-3R9 causes cell death by boosting ROS generation and lowering mitochondrial membrane potential, while also enhancing anti-cancer effectiveness. In cancer treatment, MTS-H3R9 has the potential to operate as both a medication carrier and an anti-cancer agent [72]. Xiao et al. developed a novel cell-penetrating peptide, mitochondrial targeting peptides (MTPs), which were subsequently functionalized with diverse targeting ligands using resin-based solid--phase peptide synthesis (SPPS). This linear MTP has two hydrophobic naphthylalanine (Nal) residues and three positively charged arginines that have been found to have highly efficient mitochondrion targeting. So, this peptide-based formulation functions by selectively accumulating in cancer cells' mitochondrion and inducing mitochondrial depolarization by decreasing mitochondrial membrane potential, ensuing in cellular death by apoptosis [73].

4.2. Prodrug based NPs

In terms of anticancer drug delivery, prodrug-based nano assemblies have a lot of advantages. These include increased drug availability and increased loading efficiency, as well as resistance to recrystallization when they are encapsulated. To address physical chemistry, biopharmaceuticals, and pharmacokinetics problems, prodrugs must be enzymatically and chemically converted into active parent molecules. These limitations include restrictions on stability, solubility, presystemic metabolism, permeability, and targeting of cancer cells [74]. In a study, Wang et al., established a multi-prodrug delivery system for mitochondrial-targeted camptothecin (CPT) to deliver anticancer medication. In the system, an amphiphilic poly-prodrug of Dextran P has been utilized to deliver drugs. Intracellular reducing agents, such as glutathione, dissolve the disulfide bonds of polymeric prodrugs, allowing the active component to be released. When compared to non-targeted prodrugs, the CPT molecule caused more DNA damage and mitochondrial apoptosis in the multi-prodrug system, which enhanced apoptosis and cell death. Polymeric prodrugs targeted to mitochondria are more effective in inhibiting tumor development and promoting cancer cell death [75]. In another study, Chen et al. developed a polymer-drug conjugate that avoids the difficulties associated with polymer-drug conjugates. Artesunate-N, N-bis (octadecyl) L-glutamine diamide (ARTLGC12) and long-chain methoxy polyethylene glycol distearoyl phosphatidylethanolamine (mPEGDSPE) are prodrugs modified to produce mitochondria-targeting nanoparticles. The produced nanoparticles further surface conjugated with (TPP) and that nanoparticles has substantial cytotoxicity in several tumor cell lines including cisplatin-resistant cancer cells. [76].

4.3. Immunotherapy for cancer via NPs

Cancer immunotherapy has recently become a standard of care in the fight against the disease. A fundamental advantage of immunotherapy is that it not only cures the underlying tumor, but also inhibits metastasis and recurrence. Current cancer immunotherapies do not always adequately transport tumor antigens into current cancer immunotherapies, resulting in very modest therapeutic advantages. Solid tumors, unlike lymphomas, generate an immune-suppressive tumor microenvironment to elude anti-cancer immunity. Nanoparticles based on biomaterials may help to overcome some of cancer immunotherapy's current drawbacks. Immunotherapy methods are focused on stimulating or complementing the immune system with a range of substances such as antibodies, vaccines, and lymphokines [77].

Light stimulation may stimulate the host's immune response, and encapsulating ZnPc (Zinc phthalocyanine) in a biodegradable polymer resulted in poly-(D, L-lactide-co-glycolide)-b-poly (ethylene glycol)-TPP NPs (TZnPcNP) (PLGA-b-PEG-TPP). Cancer cells stimulate dendritic cells to generate large amounts of interferon-gamma. The autocrine action of interleukins IL-12 and IL-18 was caused by the exceptional ex-vivo stimulating capacity of dendritic cells in this tumor cell supernatant. Ex-vivo research demonstrated that the chemical produced by TZnPcNP-treated cancer cells might be used to create cancer vaccines [78]. Cancer immunotherapy based on checkpoints has recently emerged as a potential cancer therapeutic method. However, clinical applicability has been impeded owing to nanocarriers' low tumor penetration and immune response activation. Foreign chemicals and aberrant cells are readily recognized and eliminated by our system. Tumor cells, on the other hand, may readily evade the immune system and live on. The PD-1/PD-L1 checkpoint is a contributing factor. Tumor cells with overexpressed PD-L1 can attach to T cells with PD-1, limiting the interaction between antigen-presenting cells (APCs) and T cell receptors and inhibiting T cell assault on tumors. PD-1/PD-L1 checkpoint blockage can unleash and reactivate cytotoxic T cells to achieve tumor cytotoxicity by blocking immune-suppressive pathways using PD-1 or PD-L1 monoclonal antibodies. Many monoclonal antibodies, including

durvalumab, pembrolizumab, and nivolumab, have been authorized by the FDA for the treatment of non-small cell lung cancer and melanoma in recent clinical trials.

In a study, Chen et al., designed a tumor-responsive mitochondrial nano complex in conjunction with a photosensitizer and repressed siRNA-programmed cell death ligand-1 (PDL-1) for a synergistic grouping of immunotherapy and PDT. This study combines cytokine-induced killer (CIK) cells with PD-1 inhibition before transfusion to improve the efficacy of CIK therapy in patients with non-small cell lung cancer (NSCLC). Natural killer (NK) cell treatment does not appear to produce this effect [79,80].

4.4. Stimuli based NPs in targeting cancer cells

Stimuli-responsive mitochondria DDSs have all the benefits of Stimuli-DDSs and Mitochondria-DDSs, including the ability to respond to stimuli, (1) The ability to focus on many levels at once. Tumor cells and cellular mitochondria organelles are sequentially delivered medicines by stimuli-mitochondria-DDSs; (2) Nontarget tissue locations do not receive or leak medicine from stimuli-DDSs when they are activated by the stimulus trigger. A system developed by mitochondria targeting delivers medications to the target organelle mitochondria using stimuli-mitochondria-DDSs after they enter cells, (3) This approach is capable of maximizing treatment efficacy while minimizing side effects. To maximize efficacy while minimizing nonspecific toxicity, drug molecules should accumulate as much as possible at the specific target organelle while spending as little time as possible in healthy cells and other organelles. A number of additional steps must occur prior to the drug molecule reaching the mitochondria, including removal from circulation, accumulation in the target tissue, passage through the cellular membrane barrier, and evasion of lysosomal endocytosis. It appears that stimuli-DDS can improve the stability of nanocarriers and limit systemic nontarget medication release. Stimuli-DDS's ability to respond to both endogenous (En) and exogenous (Ex) stimuli (also known as bio stimulators, which include parameters like pH, redox potentials, enzymes, and glucose) has the potential for a wide range of biological applications. Stimulus-responsive DDS releases medications when certain stimuli are engaged (such as ultrasound, redox trigger, light, external magnetic fields, pH, or enzymes). These technologies help to deliver medicine to the tumor site and prevent leakage of loaded drugs from the nanocarrier. Zhang et al., carried out work in which they employed IR780 as a sonosensitizer to create nano-droplets. In vitro studies have shown that IR780 nanodroplets show mitochondrial targeting, and the excessive production of ROS in mitochondria induces apoptosis, and the death of cancer cells is observed [81]. In a study, Zhou et al., prepared paclitaxel-loaded lipopolymer hybrid nanoparticles composed of a reduction-responsive amphiphilic polymer (DLPESS PEG 4000), a TPP-carrying amphiphilic polymer (C_{18} PEG 2000 TPP), and PLGA. Once the nanoparticle penetrates cancer cells, PEG 4000 will be removed due to the decrease of GSH conditions, thereby improving the targeting of mitochondria and apoptotic cell death [82]. Shah et al. developed magnetic core-shell nanoparticles (MCNPs) to deliver a pro-apoptotic peptide used in the management of malignancy, for mitochondria targeting, e.g. amphiphilic tail-anchored peptide (ATAP). By using an external magnetic field and attaching internalized RGD, MCNP-ATAP targets metastatic breast cancer cells and malignant brain cells (a tumor targeting peptide). Death caused by apoptosis MCNP-ATAP increases penetrability through MOM, leading to mitochondrial dysfunction [83].

4.5. Miscellaneous examples of nanovesicular system used for the treatment of mitochondrial dysfunction

NPs have attracted great attention in recent years, especially for the treatment of cancer, because of their capability of enhancing the safety, pharmacokinetic profile, and bioavailability of chemotherapeutic drugs. Miscellaneous nanocarriers used for the mitochondrial targeting includes supramolecular peptides, micelles, polymeric materials, etc. Nanoparticles that fall outside of these categories are being identified for use in the detection and treatment of cancer. On the other hand, nanoparticles should be used with caution because the harmful consequences of nanoparticles have not been thoroughly studied. More study is needed on the usage of nanoparticles in the medical field and their toxicity profile.

4.5.1. Supramolecular peptide

There is a great deal of complexity in the composition and structure of supramolecular living matter; it often resides outside of equilibrium. The biological process of making things uses enzyme reactions, supramolecular templates, compartmentalization, and confinement to make structures of different sizes in a wide range of settings. In a study, Liu et al., reported supramolecular peptide that penetrates cells and targets mitochondria. In order to increase the apoptotic stimuli, the technique adopted tries to decrease the mitochondrial VDAC1–HK-II connection. The HK-II protein's N-terminus, which interacts with VDAC1, is used in peptide engineering. To begin, a cell-penetrating peptide is synthesized by anchoring a positively charged segment (pKV) to a specified 15-aminoacid sequence (pHK-pKV). A further step is to attach Pal, a long lipid chain, to pHK's N-terminus so the HK-II scaffold may be delivered into cells more efficiently. Synchrotron small-angle X-ray scattering (Bio-SAXS) and cryogenic transmission electron microscopy (cryo-TEM) imaging are used to examine the self-assembly capabilities of these two synthetic peptides, and the results show the production of nano-assemblies with ellipsoidal forms. An amphiphilic peptide model for the discovery of partial induction can be seen by circular dichroism (CD) spectroscopy. In human NSCLC A549 cells, confocal imaging reveals the precise mitochondrial location of Pal-pHK-pKV assemblies. The cytotoxicity and apoptotic experiments show that Pal-pHK-pKV self-assembled reservoirs are more bioactive than pHK-pKV, resulting in significant A549 cell death. A substantial decrease in cytotoxicity was seen when non-cancerous NCM460 cells were treated with Pal-pHK-pKV. Conjugates made of self-assembled lipopeptides (HK-II derived) show promise in cancer therapy, according to the study's findings [84].

4.5.2. Nanomicelles

Drug delivery can be improved by using micelles, which are nanoscale medication carriers that boost drug effectiveness. Moreover, to target MOM, paclitaxel (PTX)-loaded TPP-Pluronic F127-hyaluronic (PTX/TPH) nanomicelles was produced by Wang et al., which enters the acidic lysosomes through micropinocytosis, that results in the degradation of hyaluronic acid (HA) by hyaluronidase and completed lysosomal escape, finally localizing to mitochondria. Once MOMP was achieved, caspase-3 and caspase-9 were activated, which led to the release of cytochrome C and the death of the cancer cells. This was accomplished by suppressing the anti-apoptotic Bcl-2 protein [85].

Zhu et al. described an anticancer method based on the depletion of energy in cancer cells by inducing excessive mitophagy. TPGS and dc-IR825 have been used to create nanomicelles that target mitochondria. By inflicting mitochondrial damage on cancer cells, the TPGS/dc-IR825 nanomicelles promote the activation of two distinct autophagic pathways: mitophagy and ATP shortage-triggered autophagy. As a result, the creation of micrometer-sized vacuoles and a degradation blockage is caused by mitophagy/autophagy activities that are significantly more than the degradative capacity of autolysosomes. According to immunofluorescence and western blot studies, respectively, there was a severe ATP depletion in nanomicelle-treated cancer cells, which eventually leads to cell death. It has also been shown that the intravenously administered nanomicelles had a significant antitumor effect by promoting excessive mitophagy/autophagy and energy depletion in tumor cells. Because of the photothermal and photodynamic effects of dc-IR825, more near-infrared laser treatment makes the nanomicelles work better against cancer in vitro and in vivo [86].

4.5.3. Polymeric materials

Drug bioactivity and solubility can be improved by using polymeric carriers. Additionally, these carriers are acquiring promising prospects in drug discovery since they may stabilize medications and localize their action to increase therapeutic effectiveness and specificity. Controlled and sustained administration, longer bioactivity, and increased dissolution are all advantages of using polymeric carrier systems. These carriers also have promising potential in drug discovery since they can stabilize medications and localize their effects to increase the therapeutic effectiveness and specificity of the therapeutic agent. However, for mPTP targeting a Glycyrrhetinic acid-Doxorubicin conjugated with tripolyphosphate-Dox core self-assembled nanoparticle [GD-NP(TD)] was designed by Lin et al. This eventually limits tumor growth in the orthotopic tumor by opening mPTPs, resulting in considerable mitochondrial impairment that leads to increased apoptosis, reduction of energy input, and inactivation of multiple metastasis-associated proteins [87]. The Bcl2 targeting had been best demonstrated by Tannan et al., they prepared P-selectin targeted fucoidan nanoparticles loaded with venetoclax localizes at Bcl2 protein, inhibited the anti-apoptotic proteins and initiate the process of apoptosis. Small compounds S63845 and venetoclax, which target both MCL1 and BCL2 proteins, produce long-lasting remissions in mice harboring human diffuse large B-cell lymphoma tumors. However, they are associated with hematologic damage and weight loss. S63845 or venetoclax was encapsulated into nanoparticles that target P-selectin, which is more abundant in tumor endothelial cells. The nanoparticles were shown to preferentially target lymphoma tumors over essential organs in in vivo and ex vivo imaging studies. After administering nanoparticle medicines, mass spectrometry confirmed tumor enrichment of the medication while decreasing plasma concentrations. A further advantage of nanoparticles is that they can reduce drug dosages by as much as 3.5–6.5 times, resulting in longer-lasting remissions and lessening the risk of side effects. So, nanoparticles that deliver BH3 mimetic mixtures to lymphoma and other tumors could help toxic drugs used to treat cancer [88].

Liu et al., combine HA with cholesterol-poly(ethylene glycol)2k-NH2 (poly(ethylene glycol)2k-NH2) and the mitochondria-acting fluorescent cyanine dye, IR825-NH2 (abbreviated as HA–IR825–Chol). In addition to its increased photostability and good photothermal characteristics, the HA-IR825-Chol can promptly and substantially infiltrate CD44-overexpressed cancer cells and preferentially concentrate in the mitochondria of the cells. Damage to mitochondria caused by near-infrared laser irradiation can lead to cytochrome release and cell death. It is also possible to include the chemotherapeutic agent 10-hydroxycamptothecin (HCPT) into the hydrophobic cores of these nanoparticles for combination chemophotothermal treatment. An increase in cell uptake and simultaneous mitochondrial and nuclear localization of HA-IR825-Chol results in the release of cytochrome c from mitochondria and an elevation of cleaved caspase-3, which both contribute to the cell apoptosis/death process. The remarkable tumor-targeting ability of HA-IR825-Chol/HCPT has been demonstrated in vivo, ensuring that the chemo-photothermal treatment is effective in eradicating tumors. An inherently mitochondrial nanocarrier has been developed for precise subcellular structure-localized drug delivery, and the quick and large endocytosis of the nanoagents by Chol may provide a strong technique for increasing the efficacy of nanomedicines [89].

5. Theranostic nanocarriers in mitochondrial targeting

Therapeutics and diagnostics are used in theranostics to boost the effectiveness and distribution of a medicine to the tumor region. Chemotherapy efficacy may be evaluated using theranostics, which can measure tumor growth and provide real-time feedback on the effectiveness of the treatment. The applications of nanotechnology to medicine present a chance to enhance the safety, efficiency, and sensitivity of traditional medical therapies [90]. A nano-based drug delivery system focuses on the utilization of nanostructures and nanomaterials for the targeted transport of a therapeutic or diagnostic chemical and for its release in a controlled way. In addition, the utilization of large-size structures in drug administration is hard, because of their low bioavailability and stability, unwanted effects, restricted targeted drug delivery, and therapeutic efficiency. Recently, numerous ways of medication delivery have been proposed to alleviate these restrictions through the synthesis and manufacture of smart nanomaterials [91].

5.1. Carbon-based nanotheranostics

Carbon-based nanomaterials such as carbon nanotubes (CNTs), graphene derivatives, and carbon dots (C-dots) have piqued the interest of researchers over the last decade due to their outstanding effect in biological domains like targeted drug delivery, chemo-photothermal therapy, and biological imaging in real-time. They share some of the same physical and chemical properties as supramolecular stacking, photoluminescence, high adsorption capacity, and biocompatibility. Tumor cells are frequently killed by these substances by damaging the mitochondria. Chemically functionalized carbon nanotubes assemble tumor-specifically, have minimal toxicity, and are biocompatible [92, 93]. Multi-walled carbon nanotubes (MWCNTs) based on peptides were among the first CNTs to target mitochondria. According to the confocal laser scanning microscopy, transmission electron microscopy, and fluorescence imaging, the Hela cell lines and macrophages and mitochondria can be targeted by functionalizing the MWCNTs with mitochondrial targeting sequence (MTS). The results demonstrated that MTS functionalized MWCNTs did not cause substantial cytotoxicity, suggesting that they have a bright future as a nano-formulation carrier [94].

Multifunctional nanographene triggered by near-infrared (NIR) mitochondrial targeting was created by Wu et al. which in situ triggers the photodynamic cancer therapy approach. They devised a method based on the TPP modification of graphene that might function as a carrier for photosensitive dye (IR820) to mitochondrial components. Following NIR laser irradiation, the photoactive chemical generates ROS and photothermal heat, which induces mitochondrial collapse and ultimately kills cancer cells. In-vivo treatment of the immunostimulatory chemical DP-CpG greatly boosts the synthesis of tumor necrotic factor, interferon, and proinflammatory cytokine-like interleukin-6, as well as the tumor's immunogenicity. In-vivo, the combination of IR 820's photothermal action with DP-immunostimulatory CpG's activity resulted in significant suppression of tumor formation (tumor inhibition rate of roughly 88 %) [95]. Hua et al. developed a one-step hydrothermal for the treatment of chitosan, ethylenediamine, and mercaptosuccinic acid to yield a new form of fluorescent carbon quantum dots with intrinsic mitochondrial targeting capabilities. To achieve mitochondrial imaging and photodynamic cancer therapy using the CDs as-prepared, no additional mitochondriotropic ligand changes are required (such as triphenylphosphine, TPP). Unlike conventional mitochondrial probes like MitoTrackers, which require wash-in and wash-out procedures, our CDs may be imaged for long periods of time with no cytotoxicity and are incredibly straightforward to synthesize. Because of its gratifying anticancer efficacy and the fact that mitochondria are vulnerable to the reactive oxygen species created during chemo, light, or radiation, mitochondria-targeted cancer therapy has garnered a lot of interest. A photosensitizer rose was attached to the CDs to see if mitochondria-targeted medication delivery was possible. The CDs-RB nanomissiles derived from rose bengal (RB) were able to efficiently enter cells [96].

In a study, Zhang et al. synthesized magnetic-based mesoporous silica particles ($Fe3O4@mSiO_2$), and the surface was modified via triphenylphosphine (TPP) and coupled with fluorescent carbon dots (CDs). When tested on numerous cell lines, the $Fe_3O_4@mSiO_2$ TPP/CDs nanoplatform exhibits enormously low cytotoxicity and programmed cell death. This nanoplatform is unique in that it combines long-term cellular imaging, mitochondrial targeting, and magnetic field-enhanced cellular absorption into a single device. Flow cytometry and

confocal laser scanning microscopy confirmed the time-dependent colocalization of mitochondria in all cell lines, whereas the multicolored fluorescence of the Fe_3O_4@$mSiO_2$TPP/CDs remained brilliant and persistent after co-incubation for 24 h. The efficiency of uptake by A549 and HFF cell lines in a coincubation setup was also improved quickly by applying a static magnetic field of 0.30 T that has been presented in Fig. 4A, 4B. In the treatment of mitochondrial illnesses and the early detection and therapy of cancer, this bio-nanoplatform might be useful [97].

Whereas another study performed by Zheng et al. for brain tumor cells, they used D-glucose and L-aspartic acid as starting ingredients in the pyrolysis process to make a novel sort of carbon dot (CD-Asp) with targeted activity against brain malignant glioma. Even without any additional targeting molecules, the CD-Asp in its as-prepared form demonstrates outstanding biocompatibility and full-color emission that may be tuned to the desired intensity. CD-Asp biodistribution was clearly visible in-vivo fluorescence images 15 min after tail vein injection. They have the capacity to effortlessly enter the blood-brain barrier (BBB) and accurately target the tumor tissue, as demonstrated by a much greater fluorescent signal in the glioma location than in the normal brain. Glioma is not a specific target for these other CD molecules: the CDs derived from glucose, aspartic acid, or glutamic acid have no or poor selectivity for this cancer. To diagnose glioma non-invasively, CD-Asp might serve as an imaging and targeting agent. As shown in Fig. 4C, five minutes after the insertion of the dose via the tail vein, CD-Asp was found in the brain, suggesting that CD-Asp might effortlessly cross the BBB and penetrate under brain tissue. Fluorescence intensity peaked after 15 min, then decreased progressively as time went on, demonstrating that CD-Asp may enrich at glioma when it is removed from the body. Imaging of 3-D renewal was possible 20 min post- insertion, and the result (Fig. 4D) demonstrated that the fluorescent concentration in the glioma location was substantially better than that in usual brain tissue, showing that CD-Asp might localize at the glioma position. In contrast, ex vivo imaging (Fig. 4E) of the brain further demonstrates that CD-Asp targets the glioma location. In contrast, 200 mg/kg of CD-Asp, CD-G, CD-A, and CD-Glu were injected intravenously into mice via the tail vein, and the entire body's bright dispersion was observed using an in vivo imaging system at different intervals of time: 0.5, 1, 2, 4, 6, and

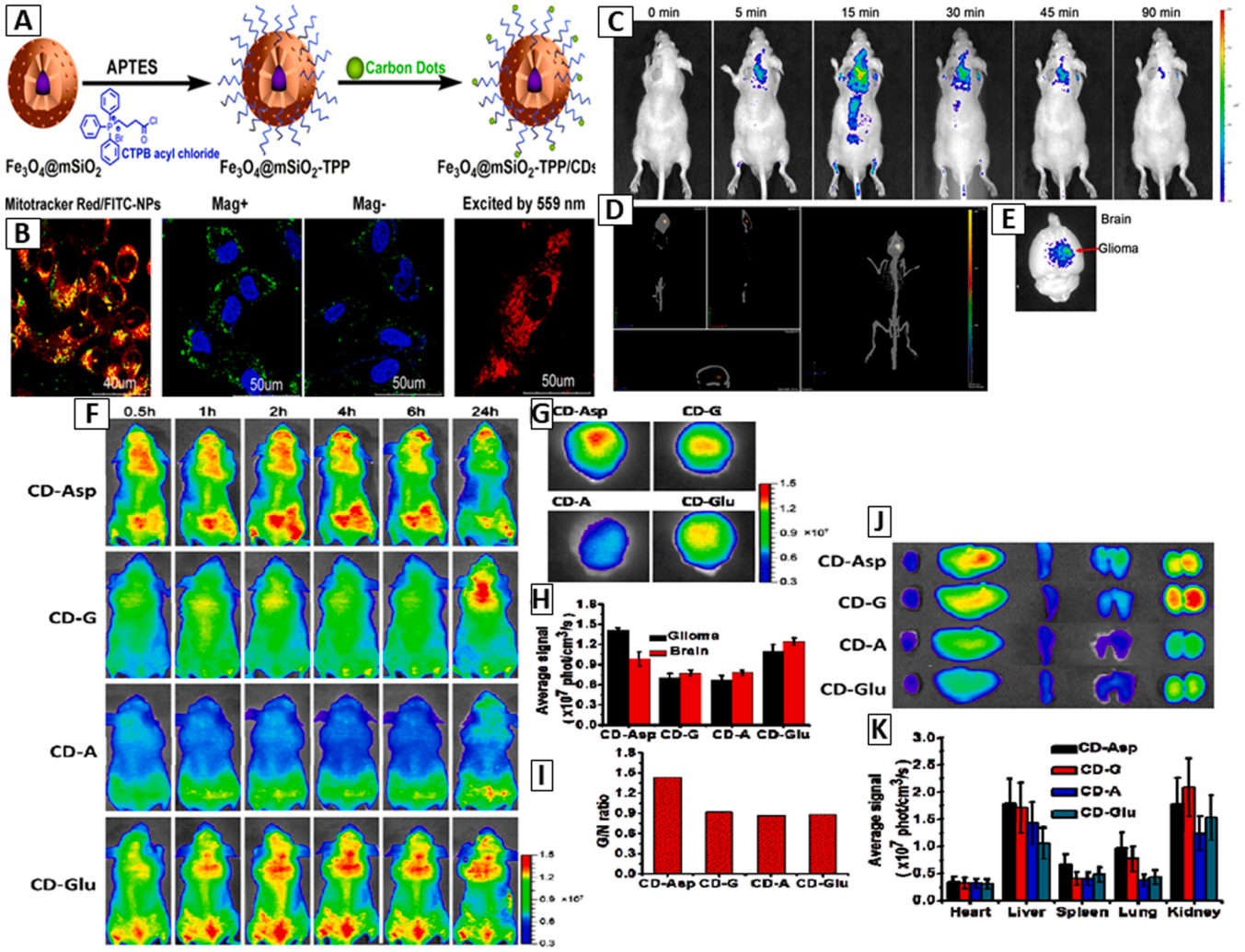

Fig. 4. A) The Fe_3O_4@$mSiO_2$TPP/CDs nanoplatform synthesis approach is depicted schematically; B) CLSM images on various cancer cell lines treated with Fe_3O_4@$mSiO_2$-TPP/CDs for 24 h; C) Allocation of CD-Asp in the body over time following injection; D) A 20-min post-injection 3D reconstruction of CD-Asp brain distribution; E) Brain imaging 90 min after CD-Asp infusion; F) At six different time points injections were administered into glioma bearing mice; G) Imaging of a glioma-bearing brain under ex vivo conditions; H) Brain and glioma fluorescence intensity semi-quantitative measurements; I) G/N ratio of CD-Asp, CD-G, CD-A and CD-Glu groups; J) Imaging shows normal tissues 24 h after the injection of CD-Asp, CD-Asp, CD-G, CD-A and CD-Glu; K) Liver, lung, spleen, and kidney semi-quantitative fluorescence intensity.

B) **Reproduced with the permission from Ref.** [97]**, graphical abstract. (ACS Publication 2015).** K) **Reproduced with the permission from Ref.** [98]**, Fig. 4 and** Fig. 5**. (ACS Publication 2015).**

24 h. A comparison of CD-Asp to CD-G, CD-Glu, and CD-A is shown in Fig. 4F, 4G, and 4H. The CD-Asp group had a substantially greater concentration of CD-Asp in the brain than the other three groups. For CD-Asp and other CDs, the glioma/normal brain ratio (G/N ratio) (Fig. 4I) was employed to straight comparison the glioma targeting competence. There is only a clear glioma targeting efficiency for CD-Asp in the G/N ratios of 1.42, 0.91, and 0.86, 0.88 for CD-G, CD-A, and CD-Glu. They are in line with prior LSCM investigations. For this reason, glioma diagnosis may be made using CD-Asp, which is a fluorescent imaging agent that specifically targets C6 cells and can identify glioblastoma. CD-Glu, CD-Asp, CD-G, and CD-A all had similar fluorescence intensities in normal tissues (Fig. 4J), showing that CD-targeting Asp's function did not affect the distribution of CDs in the healthy cells, as shown in the fluorescence concentrations of usual organs [98].

5.2. Liposomes as nanotheranostics

Liposomes are globular vesicles with an inner hydrophilic core that are enclosed in a lipid bilayer. For hydrophobic and water-soluble medicines, lipid bilayers and core regions can be loaded. Biswas et al. created a novel PEG-PE (PEGylated PEGylethanolamine) with TPP conjugation bonded to the PEG block's trailing edge (TPP-PEG-PE). The mitochondrial targeting ability, cytotoxicity, and effective transport of paclitaxel (PTX) to cancer cells of these modified liposomes were investigated. PTX-loaded PEG-PE-TPP liposomes showed stronger anti-tumor efficacy and PTX-mediated cytotoxicity than PTX-loaded non-targeted liposomes (PL-PTX) in both in vitro and in vivo settings [99]. A novel liposome loaded with doxorubicin (DOX) has selective targeting properties to cancer cells via folic acid (FA) and mitochondrial targeting

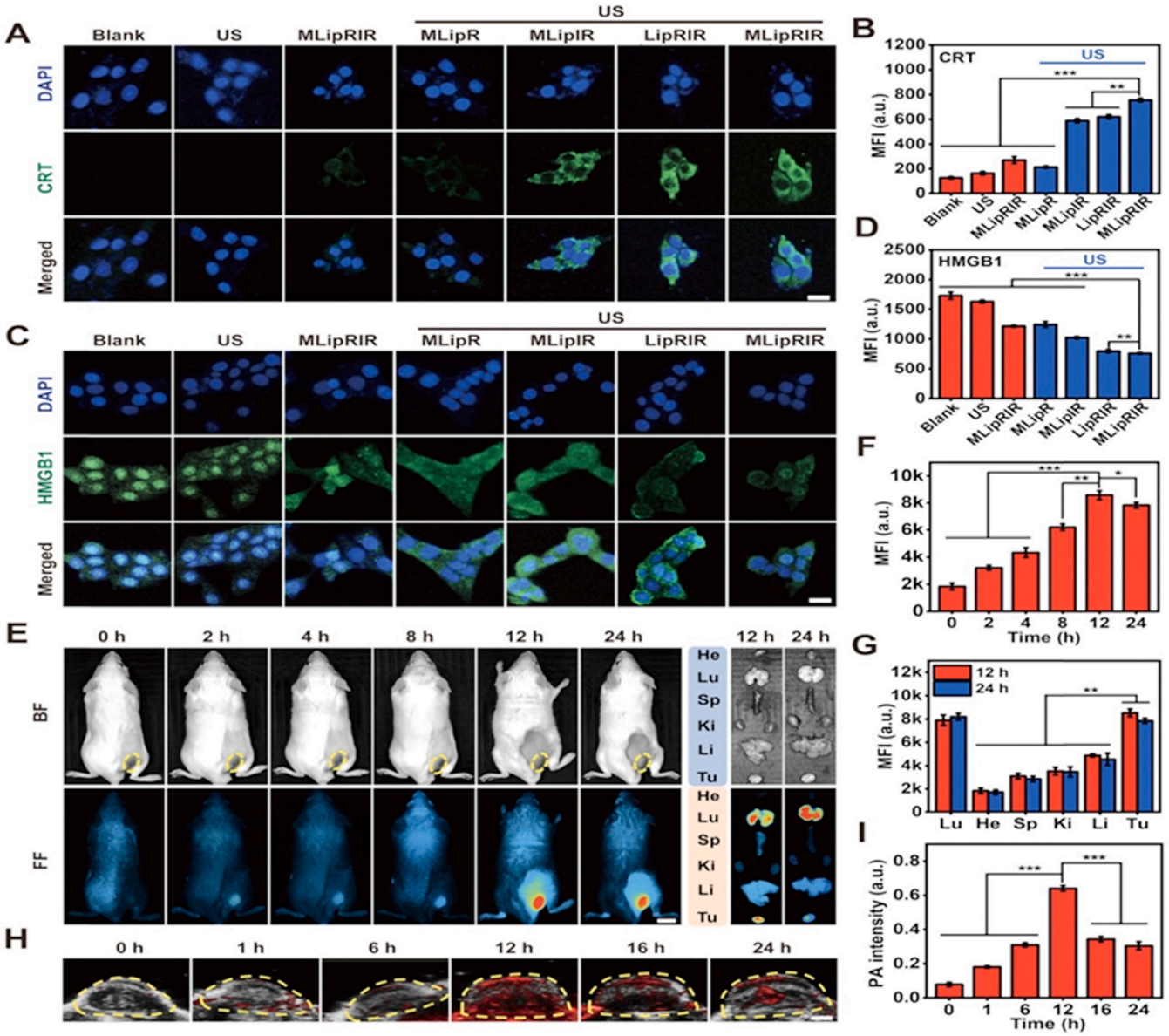

Fig. 5. Improved MLipRIR NP biodistribution in vivo and enhanced release of damage-associated molecular patterns (DAMP) in vitro; A) Exposure of 4T1 cells to Calreticulin (CRT) and subsequent treatment choices seen in this confocal microscopy image; B) MFI (mean fluorescence intensity) of CRTs exposed to radiation; C) Confocal microscopy picture demonstrating the release of HMGB1 from 4T1 cells following various treatment regimens; D) Residual HMGB1 MFI in 4T1 cell nuclei; E) Images of tumor-bearing animals at 24 h following i.v route of 3 mg/mL MLipRIR NPs and ex vivo microscopy of vital organs and excised tumor (He, Li, Sp, Lu Ki, Tu - heart, liver, spleen, lung, kidney; tumor); F) Time-varying MFI values of the tumor area corresponding to (E); (G) MFI of vital organs and excised tumor harvested at 12 and 24 h corresponding to E; H) At 0, 6, 16, and 24 h after injection, pictures of the associated tumorous tissue were taken using the PA technique; I) PA pictures of the PA signal collected at 12 and 24 h intervals corresponding to H.
Reproduced with the permission from Ref. [101]**,** Fig. 4**. (Ivyspring 2021).**

via TPP cation. DOX-loaded liposomes were tested for cytotoxicity, ROS generation, and cellular absorption using the KB cell. These dual-targeted liposomes were more cytotoxic and produced more ROS than non-targeted liposomes [100].

Ren et al. prepared the lipid bilayer of mitochondrial targeting liposomal nanoparticles (MLipRIR NPs) containing IR780 (a hydrophobic sonosensitizer), which is used for ultrasound (US)-activated tumor dyshomeostasis treatment and immunogenic cell destruction (ICD). The glutaminolysis in mitochondria is disrupted, leading to a decreased enzymatic activity of glutathione peroxidase (GPx) from MLipRIR NP-R162. When exposed to US radiation, loaded IR780 produces large quantities of ROS, which not only disrupts mitochondrial respiration but also destroys local glutathione, causing apoptosis. Lipid peroxides accumulate in tumor cells as a result of GSH depletion and GPx inactivation. ICD can be activated by such intracellular redox dysregulation, which can lead to both primary and distant tumor suppression with the help of immunological checkpoint inhibition. Multimodal imaging for treatment guiding may help to ensure that this nanoplatform can effectively eradicate tumors throughout the body. By altering redox homeostasis, this cutting-edge paradigm may give important insights into cancer treatment. Cells treated with "MLipIR," "LipRIR," and "MLipRIR" under US irradiation showed increased Calreticulin (CRT) activity, as shown by the bright green fluorescence emission **(**Fig. 5**A)**. The CRT level was 5.1 times higher in the "MLipRIR + US" group than in the blank control **(**Fig. 5**B)**. As shown by the green fluorescence in the cytoplasm in Fig. 5C, all of the "MLipRIR," "MLipR + US," and "MLipIR +US" groups, all had moderate translocation and release of nuclear HMB1 into the cytoplasm, as shown by the green fluorescence in Fig. 5C. As seen by total fluorescence degradation in both nucleic and cytoplastic areas, cells treated with "LipRIR + US" and "MLipRI + US" released more HMGB1 than those treated with "LipRIR + US" or "MLipRI + US" (HMGB1 levels reduced by 54.0 % or 56.1 %, respectively) **(**Fig. 5**D)**. For up to 12 h after delivery, a gradual rise in the local fluorescence intensity revealed drug enrichment in the tumor site (Fig. 5E). The tumor's fluorescence intensity peaked at 12 hrs and then began to decline at 24 h postinjection **(**Fig. 5**F)**. Very high retention of MLipRIR NPs in the tumor site was further demonstrated by ex vivo fluorescence imaging, with a peak level of up to 12 h after injection. By semi-quantifying the mean fluorescence intensity (MFI), solid tumors were discovered to have a considerable uptake of MLipRIR NPs. This could be because of efforts to make mitochondrial respiration and the EPR mechanism for circulation last longer **(**Fig. 5**G)**. A PA graph of solid tumors was developed after injecting MLipRIR NPs intravenously into a mouse model **(**Fig. 5**H)**. The peak PA signal strength 12 h after the injection was what caused the most MLipRIR NPs to gather at the tumor site **(**Fig. 5**I)**. Fluorescence and PA imaging showed that 12 h after injection was the best time for US irradiation [101].

5.3. Polymeric nanotheranostics

Polymeric nanocarriers made of biodegradable and biocompatible polymers are the most promising medication delivery technology. These polymers may encapsulate both water-soluble and water-insoluble pharmaceuticals, and enhances aqueous solubility, retention time, and bioavailability of the loaded pharmaceuticals. Enhanced drug stability and also more controlled drug release are only two of the major advantages of polymeric nanoparticles. The most often utilized biodegradable and biocompatible hydrophobic polymeric blocks are poly (lactic-co-glycolic acid) and polycaprolactone. PEG is the most widely used hydrophilic block polymer approved by the Food and Drug Administration (FDA). The PEG has distinct properties, such as increasing the time of retention in the body and allowing for further functionalization with the medication or target molecule [65]. Pan et al. developed the polymeric nanoparticles by physically encasing the NIR heptamethine cyanine dye me-IR825 in the inner core of the micelle-forming copolymer Pluronic F127 (PF127). The PF127/me-IR825 NPs showed two fluorescence emissions at 610 nm and 845 nm (stimulated by 550 nm and 780 nm). Early-stage cancer diagnosis with high fluorescent contrast and in vitro mitochondrial fluorescence imaging were all possible uses for the first. The latter was employed for NIR fluorescence imaging in vivo. The NPs could also be used for in vivo photoacoustic imaging at 808 nm excitation. A high power density 808 nm laser irradiation resulted in good photothermal tumor ablation in vitro and in vivo. PTT treatment of the NPs also degraded me-IR825 in the NPs inner core into biocompatible compounds, ensuring the NPs post-treatment biosafety. For biomedical uses, they can be employed as a nanoplatform because of their great cancer/normal cell differentiation capacity, easy fabrication, good colloidal dispersibility/stability, and outstanding in vivo dual-modal imaging-guided treatment results [102].

In an attempt to improve multidrug resistance (MDR) in breast cancer therapy, Li et al. created a multifunctional nanoparticle system based on methoxy polyethylene glycol-poly(L-histidine)-D-Vitamin E succinate (MPEG-PLH-VES) copolymers. Biotin was added to the MPEG-PLH-VES nanoparticles (NPs) for targeted medication delivery. This copolymer had no discernible effect on the ADR/MCF-7cell's expression levels of P-gp, but it had a considerable impact on mitochondrial membrane potential loss, intracellular ATP decrease, and P-gp ATPase inhibition in these cells. In an aqueous solution, the particle size of the MPEG-PLH-VES NPs was increased by an acidic pH. Drug encapsulation was found to be around 90%, average particle size was found to be approximately 130 nm, and the drug release profile was pH-responsive in an acidic environment for MPEG-PLH-VES/biotin PEG-VES NPs DOX-encapsulated (MPEG-PLH/VES/B). CLSM study demonstrated that DOX-loaded NPs were able to transport the drug into MCF/ADR cells and enable their escape from endo-lysosomal entrapment (Fig. 6A). After administration of MPEG-PLH-VES NPs and MPEG-PLH-VES/B NPs had a high concentration of DiR molecules in the liver at 2 h, however the accumulation in tumors was restricted. In spite of this, the tumor's fluorescence intensity appeared to rise over time steadily. For both MPEG-PLH-VES and MPEG-PLH-VES/B NPs, fluorescence accumulation in tumors was confirmed 12 h after injection (Fig. 6B). More importantly for this study, the fluorescence signals from the MPEG-PLH-VES/B NPs in tumor tissue were more intense than those from MPEG-PLH-VES NPs. A larger tumor accumulation of MPEG-PLH-VES/B NPs than of MPEG-PLH-VES NPs was verified by ex vivo fluorescence pictures of the dissected tumor (Fig. 6C). The reticuloendothelial system in the livers and spleens is thought to be responsible for the significant liver and spleen accumulation of the two DiR-loaded NPs. Dox-loaded MPEG-PLH-VES/B NPs had a greater cytotoxicity on MCF-/ADR cells compared to free DOX solution in an in-vitro evaluation of in vitro cytotoxicity. Researchers found that when MCF-7/ADR tumors were transplanted into mice, the MPEG-PLH-VES/B NPs aggregated in the tumor location more densely at a certain time period. To get around the problem of multidrug resistance (MDR) in breast cancer therapy, researchers developed a multifunctional nanoparticulate system made of methoxy poly(ethylene glycol)-poly(L-histidine)-D—Vitamin E succinate (MPEG-PLH-VES) copolymers. In order to distribute drugs to specific locations, the MPEG-PLH-VES nanoparticles (NPs) were functionalized with the biotin domain. Only a small but substantial impact on P-gp ATPase activity and loss of mitochondrial membrane potential was seen in the MCF-7/ADR cells treated with the MPEG-PLH-VES copolymer compared to its effect on P-gp expression in MCF-7/ADR cells. MPEG-PLH-VES nanoparticles were shown to have an acidic pH, which induces an increase in particle size in water. Drug encapsulation effectiveness was found to be around 90 %, average particle size was found to be approximately 130 nm, and the drug release profile was pH-responsive in an acidic environment for MPEG-PLH-VES/biotin PEG-VES NPs DOX-encapsulated (MPEG-PLH-VES/B). The CLSM experiments showed that the DOX-loaded NPs were highly successful at transporting DOX into MCF-/ADR cells and allowing the drug to escape endo-lysosomal entrapment. Dox-loaded MPEG-PLH-VES/B NPs had a greater

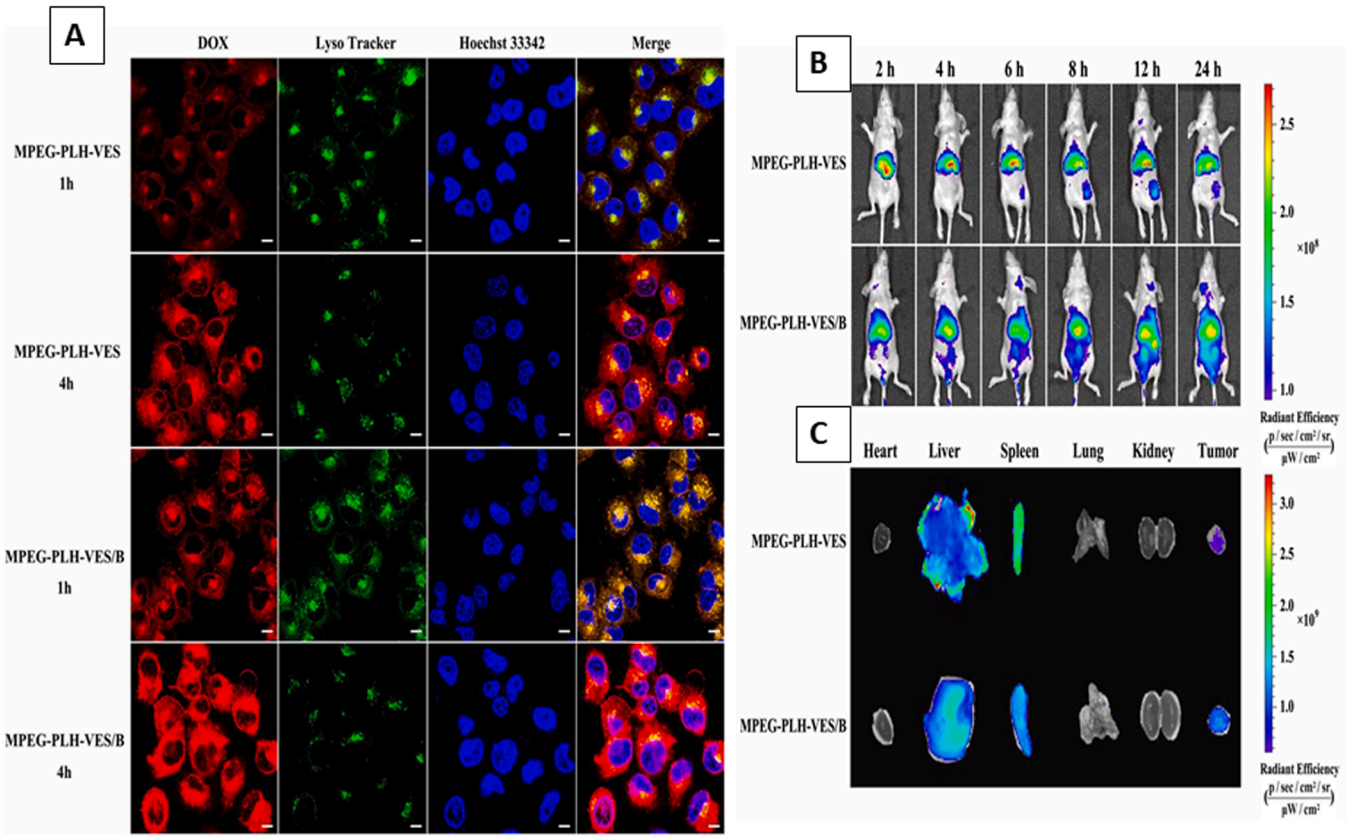

Fig. 6. A) MPEG-PLH-VES/B and DOX NPs, placed in MCF-7/ADR cells and incubated at 4hrs; B) Experiments on mice injected with DiR-loaded MPEG-PLH-VES and MPEG-PLH-VES/B NPs at 24 h post-injection; C) At 24 h post-injection, nude mice carrying the MCF-7/ADR tumor underwent ex vivo imaging of DiR-loaded NPs in the heart, liver, spleen, lung, kidney, and tumor respectively.
Reproduces with the permission from Ref. [103], Fig. 7 **and** 8**. (ACS Publications 2018).**

cytotoxicity on MCF-/ADR cells compared to free DOX solution in an in vitro evaluation of cytotoxicity [103].

5.4. Inorganic nanotheranostics

Inorganic NPs have various benefits over organic NPs owing to their homogeneity and smaller particle size. So far, many inorganic materials have been used to create mitochondrial targeting NPs. A rod-shaped hydroxyapatite nanoparticle was created by Sun et al. via aqueous precipitation method (about 10 nm in width and 50 nm in length). Hydroxyapatite (HAP) is biocompatible and have the highly drug-loading capacity, which is a component of mammalian hard tissue. HAP NPs enter tumor cell mitochondria and trigger death via altering mitochondrial membrane potential and cytochrome c leakage. Both normal human bronchial epithelial cells (16 HBE) and lung cancer cell lines (A549) transport HAP NPs via caveolae-mediated endocytosis. A549 cells, on the other hand, take in more NPs, resulting in a prolonged elevation in intracellular calcium rather than a transient increase in normal human bronchial epithelial cell lines. This resulted in enhanced cellular absorption and intracellular calcium, which reduced tumor development by around 40 % in lung cancer and has no side effects in a naked mouse model [104,105]. TPP-Au nanoparticles aggregated largely in the mitochondria of tumor cells, according to Ma et al. and the resulting coupling effect of plasma interparticle was responsible for hyperthermia in mitochondrial and anti-tumor activity. Normal cells, on the other hand, accumulate just a small quantity of TPP-Au NPs, which is insufficient to produce the plasmonic effect and hyperthermia. As a result, normal tissue would not be affected by irradiation. In the in-vivo investigation it was discovered that the temperature rise after irradiation was roughly four times that of normal tissue, implying that TPP-Au NPs demonstrated high cancer selectivity treatment while having fewer adverse effects [106].

5.5. Dendrimers as nanotheranostics

Dendrimers are star-shaped, hyperbranched, nanometer-sized macromolecules. It possesses unique properties such as biodegradability, high encapsulation efficiency and water solubility, low toxicity, prolonged retention duration, surface modification, and specificity. Biswas et al. created a 5th generation mitochondrial target PAMAM dendrimer (G-(5)-D). TPP was subsequently conjugated to G-(5)-D-Ac surfaces by cationic charge of acid amine coupling, thereby neutralizing the G-(5)-D-Ac's cationic charge fraction [107]. PTX treatment failure is exacerbated by MDR to the cancer cells. A mitochondrial membrane potential (MMP)-2-activated, glucose transporter-mediated, and conjugate of the mitochondrial target was developed by Ma et al. to address this problem. TPP is co-modified with an amide and PTX disulfide link in the PAMAM dendrimer core of the conjugate. After that, an MMP-sensitive peptide was attached to a long-lasting PEG layer. Through the TPP-followed route, the conjugate was found to enter mitochondria and release PTX via disulfide bond breakage. Toxic to MCF-7/ADR cells, the chemical accumulates preferentially in mitochondria and is more toxic, effectively reversing MDR in these cells [108].

Liu et al. formulated azoreductase sensitive DNA nanotrain that can activate hypoxia imaging and boost photodynamic treatment effectiveness. As a fluorescent mitochondrion-targeted molecule (Cy3) and an azoreductase-responsive element (BHQ2), Cy3 and BHQ2 were covalently linked to DNA hairpin monomers as dyes. It was discovered that the photosensitizer 5,10,15,20-tetrakis(4-N-methylpyridiniumyl) porphyrin might be carried via a DNA hairpin monomer's long,

guanine-rich tail. Cy3 fluorescence and TMPyP4 singlet oxygen ($^1O_2$) production were successfully suppressed by BHQ2 through the fluorescence resonance mechanism during the commencement of DNA hairpin monomer and instigation probe. Cy3 fluorescence and TMPyP4 $^1O_2$ production will be greatly recovered once the programmable nanotrain enters cancer cells and the azo bond in BHQ2 is converted to amino groups by high expression of azoreductase under hypoxic circumstances. To further increase PDT efficacy, the TMPyP4-loaded nanotrain would aggregate in cancer cells mitochondria due to Cy3's mitochondrion-targeting feature. These DNA nanotrains-based multifunctional nanoplatforms might be employed for activatable imaging and high-performance PDT in hypoxia-related medicinal fields. For the blank group (MCF-7 cells not treated with the nanotrain), no fluorescence was observed, but vivid Cy3 fluorescence pictures were acquired upon treatment with the nanotrain at 10 % and 1 % O_2, as shown in Fig. 7a. Nanotrain-incubated MCF-7 cells with 1 % O_2 treatment had 5.2-fold higher Cy3 fluorescence intensity than cells with 10 % O_2 treatment. The fluorescent signal of DCF obtained from MCF-7 cells under light irradiation was minimal after treatment with nanotrain@TMPyP4 for 4 h under 21 % O_2. When MCF-7 cells were treated with nanotrain@TMPyP4 under 10 % and 1 % O_2 and then exposed to light, DCF fluorescence was extremely bright. In this experiment, it was shown that light irradiation activated nanotrain@TMPyP4 to create $^1O_2$ (Fig. 7b). As demonstrated in Fig. 7c, 7d, the fluorescence intensity of free Cy3 overlapped well with that of mitotracker green, whilst pearson's coefficient (PC) was 0.93 (Fig. 7f). The nanotrain's Cy3 fluorescence and mito-tracker green fluorescence exhibited a clear overlap when cultured with MCF-7 cells for four hours at 1 % oxygen concentration (Fig. 7c, 7e). The colocalization effect between the nanotrain and mito-tracker green, as shown in Fig. 7f, suggested that the nanotrains were distributed in the mitochondria (PC = 0.89). These findings confirmed that tagged Cy3 was able to promote the accumulation of nanotrains into mitochondria selectively. Nanotrain@TMPyP4 injections at the tumor site increased the fluorescence signal significantly over time, as seen in Fig. 7g, compared to UBnanotrain@TMPyP4 as a control. When nanotrain@TMPyP4-treated animals were injected, fluorescence intensity in the tumor was approximately 3.2-fold greater than that in the non-neoplastic area 120 min after injection (Fig. 7h). The nanotrain@TMPyP4-treated group showed the most notable tumor inhibitory effects by correlating the tumor volume of the TMPyP4-only and UC nanotrain@TMPyP4-treated groups in Fig. 7i, suggesting that nanotrain@TMPyP4 not only generates $^1O2$ for tumor therapy in vivo under light radiation and moreover demonstrates improved therapeutic potential. Each treatment group showed no significant changes in body weight, indicating that there were no serious adverse effects (Fig. 7j). Fig. 7k shows that the nanotrain@TMPyP4 group showed the most significant decrease in tumor weight when compared to the TMPyP4-only and UCnanotrain@TMPyP4-treated groups [109].

6. Molecular markers of mitochondria

The National Cancer Institute (NCI) defines a biomarker as "a biological molecule detected in blood, other body fluids, or tissues that is an indicator of normal or aberrant activity, or of a condition or disease".

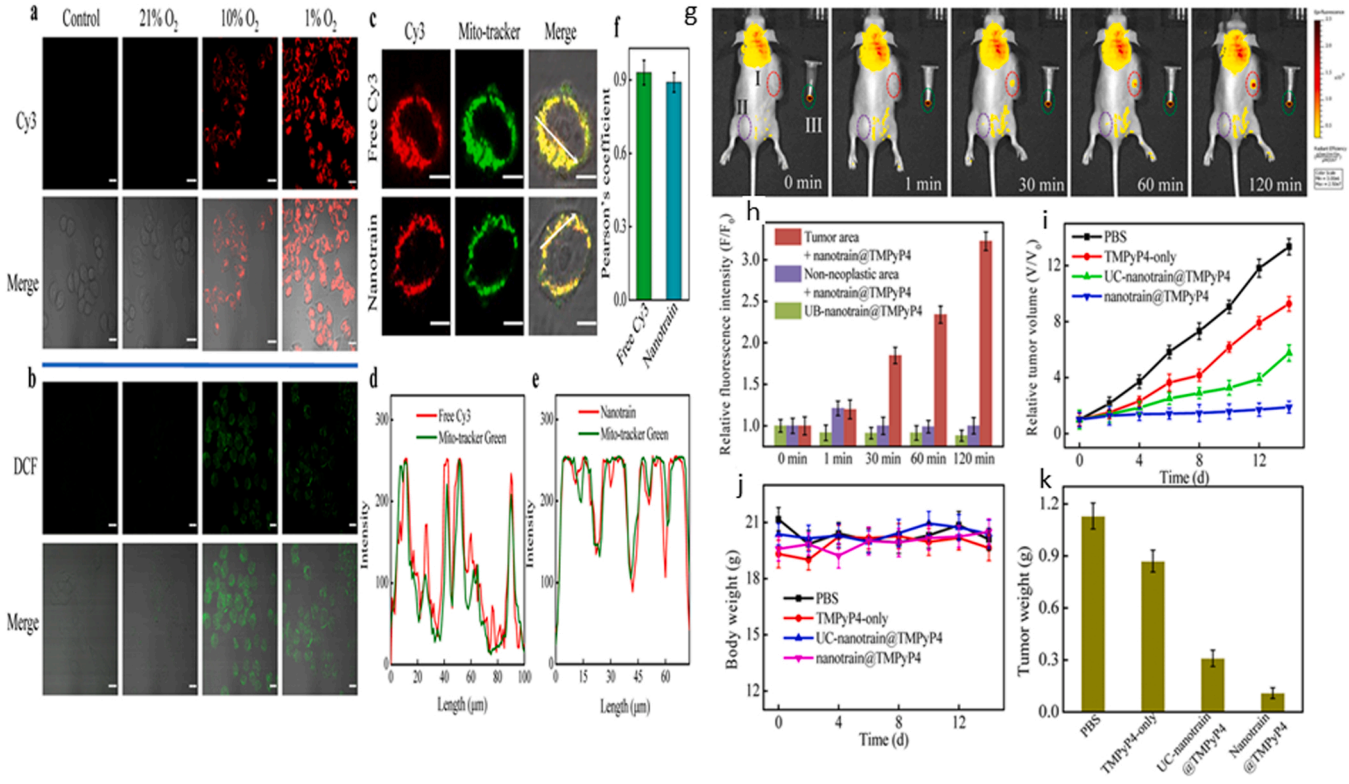

Fig. 7. A) MCF-7 cells treated with the nanotrain (50 nM); B) MCF-7 cells were treated with nanotrain@TMPyP4 (1 M) and it was treated with DCFH-DA and exposed to light; C) Free Cy3 (1 μM) and the nanotrain (50 nM) colocalization experiments with Mito-tracker Green were performed in MCF-7 cells; D) White arrow sections in unfixed Cy3-treated cells show a fluorescence intensity profile; E) White arrow patches in cells treated with nanotrains show a fluorescence intensity profile; f) Pearson correlation coefficient was used to examine the relationship between the colocalization coefficients of free Cy3 and the nanotrain with Mito-tracker Green; g) Fluorescence photos of MCF-7 tumor-bearing mice following nanotrain@TMPyP4 injection; h) MCF-7 tumor-bearing mice's fluorescence intensity as a function of therapy. It uses TMPyP4 as a command and control platform; i) Curve of tumor development relative to control in MCF-7 tumor-bearing mice treated with various methods; j) Mice with MCF-7 tumors and their body weight fluctuated in response to various treatments; (k) Results from 14-day post-treatment tumor weight measurements for mice in different groups.
Reproduces with the permission from Ref. [109], Fig. 4 and 6. (ACS Publications 2021).

Biomarkers possess a wide variety of applications in cancer theranostics such as risk analysis, diagnosis, screening, prognosis determination, therapy response prediction and tracking of disease progression. It plays a significant role in every stage of a disease like cancer. A biomarker can be employed to determine how effective treatment for a disease will work [110]. The list of several biomarkers shown in Fig. 8A; Box 1, and cellular localization of mitochondrial diagnostic and prognostic biomarkers shown in Fig. 8B.

6.1. Markers for the mitochondrial membrane

6.1.1. Markers of mitochondrial destruction and multiplication

Porin (VDAC) is frequently employed as a marker for mitochondrial abundance because mitochondrial membrane proteins can be used to assess the tumor's mitochondrial load. There was no discernible drop in mitochondrial content in renal cancer when comparing it to a control kidney using Western blot quantification of porin levels. In renal cancer tissues, however, citrate synthase activity, a common indicator of mitochondrial burden, was two-fold lower. A 5-fold increase in citrate synthase activity and an increase in mitochondria have been found in oncocytomas [111].

6.1.2. Channels for the import to mitochondria

The import of cytosolic proteins is critical for mitochondrial function. The intricate protein structures create a pathway for preproteins to move from the cytosol to the mitochondrial matrix. Translocase of the outer mitochondrial membrane (TOMM) and translocase of the inner mitochondrial membrane (TIMM) are the two proteins that make up these channels, which are responsible for transporting electrons across the mitochondrial membrane. Breast cancer cells are stained by TOMM20, whereas the surrounding lymph node stroma is not [112]. It's possible that western blot and immunohistochemistry could confirm that the TIMM17A protein level in breast cancer cells has increased fivefold based on proteomic research. Both in situ and invasive ductal carcinomas of the breast show significant staining, whereas the neighboring normal epithelial and stromal cells are negative. TIMM17A is negative in all normal breast tissues. Invasive carcinomas have much greater levels of quantitative RT-PCR than healthy breast tissue. When compared to the housekeeping gene cytokeratin 19, a recent study confirmed increased levels of TIMM17A mRNA. Two separate investigations have found that TIMM17A expression is associated with decreased disease-free and overall survival rates. So, TIMM17A seems to be a good way to diagnose and predict the outcome of breast cancer in women [113].

6.2. Markers of mitochondrial morphological characteristics

Transmission electron microscopy (TEM) is the most commonly used technique for studying mitochondrial morphology. Using these techniques, changes in mitochondrial morphology have been shown to be linked to changes in mitochondrial function. However, structural mitochondrial changes are heterogeneous and non-specific to cancers, as evidenced by mounting evidence of the role of mitochondrial dynamics in cancer development. In all of the cases where mitochondrial pleomorphism could be seen, normal mitochondria were also present. (a) increased mitochondrial mass as found in breast and pancreatic cancers

A) Box 1: Biomarkers of mitochondria

Antibody Name	Location	Function
HSP60 (Heat Shock Protein 60kDa)	Matrix	Imported polypeptide protein folding and assembly
Hexokinase 1	Outer membrane	Phosphorylates glucose, resulting in glucose-6-phosphate, which is then committed to the glycolytic pathway.
Prohibitin	Inner membrane	It's involved in normal cell cycle regulation, replicative senescence, cellular immortalization, and tumour suppression.
Mitofilin	Inner membrane / Cristae	One of the most widely distributed mitochondrial proteins. Contributes to the preservation of cristae morphology
HSP70 (Heat Shock Protein 70kDa)	Matrix	Folding, degradation, and connection with the presequence translocase-associated motor to induce preprotein import are all functions of this protein.
VDAC1	Outer membrane	VDAC is a voltage-dependent anion channel pore that permits ions to enter and exit the cytosol and mitochondria. PINK/Parkin-mediated autophagy requires this substrate.
TOMM20	Outer membrane	Together with TOM22, it acts as a transit peptide receptor, allowing preproteins to enter the TOM40 translocation pore more easily.
TIMM23	Inner membrane	Transports peptide-contain proteins across the inner membrane as part of the translocase of inner membrane 23 (TIM23) complex.

B) Cellular localization of mitochondrial diagnostic & prognostic biomarkers

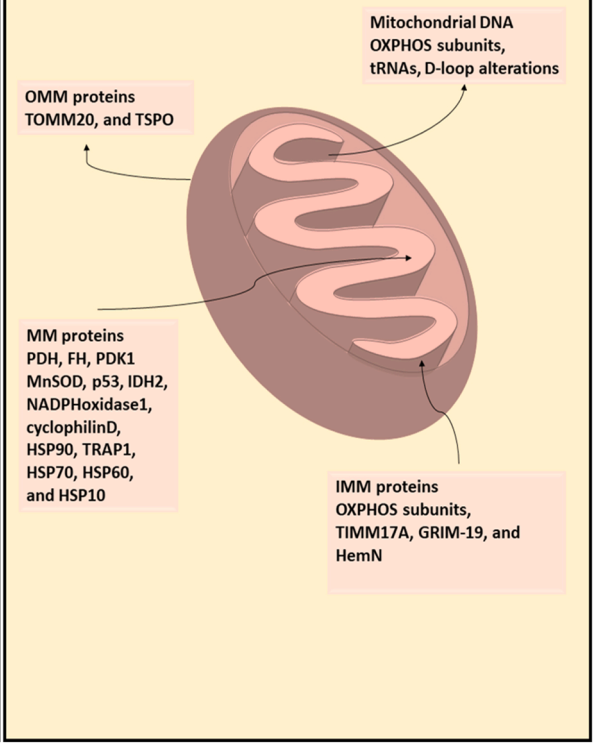

Fig. 8. A) Mitochondrial biomarkers; B) Cellular localization of mitochondrial diagnostic and prognostic biomarkers.

is one type of mitochondrial morphological change related to cancer; (b) hepatocellular carcinoma has decreased numbers and has degraded to mitochondrial fragments; (c) in clear cell cancer and undifferentiated retinoblastoma, enlarged mitochondria; (d) in the aldosterone-producing adrenal cortex, kidney cells with extended tubular mitochondria and (e) osteosarcoma cell lines have fewer cristae, but Warthin's tumor has more densely packed villiform and lamelliform cristae [114].

Using electron microscopy, researchers found that the number and size of mitochondria per cell were significantly reduced in the human gastric cancer cell line as compared to the normal rat stomach mucosa. Eosinophilic clear cell renal cell carcinomas have the most severe mitochondrial abnormalities, with most mitochondria appearing bloated with loose matrix and short attenuated cristae in an ultrastructural examination. In a primary mammary cancer cell culture, 72 % of mitochondria have broken cristae, compared to only 20 % in a normal mammary epithelial cell line [115]. Mitofilin is one of a number of cristae remodeling indicators that have been identified. The integrity of mitochondria is maintained by a variety of mitochondrial fusion/fission factors. The site of tBid's interaction is cardiolipin, an MIM-found phospholipid with a negative charge. For mitochondrial fusion to take place, an ideal potential across the mitochondrial membrane is required. Mitochondrial fission factors are abnormally low in cancers [116]. When tumor tissue from lung cancer patients is compared to nearby healthy tissue, dynamin-related protein 1 (DRP1) levels increase and mitofusin 2 (MFN2) levels drop.

6.3. *Miscellaneous mitochondrial markers*

Mitochondria also perform a variety of other tasks, such as protein translation, preprotein import and export, protein folding and assembly, and cell cycle and growth regulation. Protein folding and assembly are regulated by HSP60, a well-known chaperonin in the mitochondria [117]. An abundantly produced pleiotropic protein called prohibitin, whose dysregulation has been connected to various age-related and neurodegenerative disorders as well as metabolism and metabolism-related diseases.

7. Major drawbacks involved in mitochondrial targeting for advanced cancer therapy

Toxins and antioxidants have been transported into mitochondrial compartments within cells using lipophilic cations. This approach could be improved in a number of ways, including the addition of extra protective chemicals such as iron or calcium chelators etc. Moreover, controlling mitochondrial activity rationally should be possible by attaching substances with known pharmacological or biological functions to the lipophilic cations. While there are many potential applications for such technology, it is clear that repairing or avoiding mitochondrial damage, as well as regulating apoptosis to prevent or trigger cell death, are top priorities. Disrupting insulin production by changing the mitochondrial matrix redox balance is potentially a possibility, as is designing medications that modulate the activity of uncoupling proteins to improve oxidative phosphorylation efficiency [118].

While the mitochondrial protein import system and lipophilic cations appear to be the most suitable pathways for mitochondria targeting of drugs, additional approaches may be feasible. One option is to make use of the particular lipid composition of the MIM, which includes the majority of the cardiolipin of the cells. So, this property of cardiolipin helps to target nonyl acridine orange to the mitochondria [119,120]. It was recently revealed that the amount of benzodiazepine receptor binding by particular porphyrin derivatives used in photodynamic treatment was linked to their efficiency. When combined with other mitochondrial targeting techniques, the use of these binding sites may be helpful. Compounds that target mitochondria should, ideally, also target the corresponding cell or organ type. So, lipophilic cations, which migrate quickly across the plasma membrane and then spread to other organs in the absence of perfusion of a single organ, can be a concern. Whereas modular delivery systems based on protein import machinery may become viable. In combination with a mitochondrial delivery system, a cell targeting system is employed. A modular system could deliver a mitochondrially tailored design to cells via targeted liposomes designed with antibodies to the surface antigens of the cell, which is one approach. The formulation would subsequently be directed to the cell's mitochondria. The use of membrane-permeable peptides to transport polar compounds to cells and mitochondria might be a valuable complement [121]. Some peptides like penetratin (from Kaposi fibroblast) and herpes virus protein help polypeptides and peptide nucleic acids to cross the lipid layer. As a result, large polar molecules can pass through the lipid bilayer by speeding the production of inverted micelles in the lipid layer [122]. This approach might be used to transport molecules to the cytoplasm, where they would be directed to the mitochondrion through mitochondrial targeting. In the future, it will be interesting to combine technologies that can target specific cells and organs with technologies that can target specific cells and organs with drugs.

The development of transgenic or inbred mice models of mitochondrial diseases is a critical tool for evaluating and developing mitochondrial-focused therapies. Transgenic mice deficient in mitochondrial superoxide dismutase (Mo-SOD) are ideal animals model for mitochondrial antioxidants [123]. Similar scenarios involving mice that are heterozygous for Mo-SOD (-+) and because these mice live longer than homozygous Mo-SOD knockout mice and hence have a higher risk of acquiring chronic oxidative stress, they could be employed as better models for the research of chronic oxidative stress. More and more neurodegenerative disease models in mice are now readily available [124]. With rapid ageing, inbred senescence-accelerated mouse breeds exhibit increased oxidative damage. These methods should be reliable for identifying the role of mitochondrial defense against oxidative damage in age-related illnesses. Another fascinating animal model lacks the adenine nucleotide translocator-1 (ANT-1) isoform of the ANT, which is predominantly present in muscles [125,126]. As a result, mitochondria in ANT 1 (-/-) mice function properly. However, since the muscle mitochondria are unable to transmit the ATP that's why they enter the cytoplasm and these mice have abnormalities muscles comparable to those found in individuals with mtDNA disorders. While these animal exhibits many of the symptoms associated with mtDNA disorders and which may be useful in the evaluation of therapeutic agents but these are not appropriate for mtDNA gene therapeutics testing. In the near future, transgenic and knock out mouse models of mitochondrial illnesses caused by specific mutations in mitochondrial genes encoded within a nucleus, such as Wilson's disease and Friedreich's ataxia, will be available [127].

8. Challenges and future direction in mitochondrial targeted cancer treatment

Because of their complicated structure and numerous cellular obstacles, directly targeting mitochondria is extremely challenging. The MOM, MIM, inter-membranous area, and matrix form a complicated structure. So, the focus is entirely on the MIM barrier whether it can be passed or not. As a consequence, the molecule may move across the environment through passive diffusion [128]. The lack of structural components that allow drugs to permeate the mitochondrial matrix is the major hurdle to targeting mitochondria for treatment. The presence of distinct phospholipid cardiolipin across the membrane, as well as a high electronegative potential of 160 to − 180 mV, is the principal hurdle for microscopic molecules moving through membranes. Aside from the mitochondrial inner and outer membrane, the main hurdles to treating mitochondria are the cytoplasm and cytomembrane. Multiple macromolecular species (DNA, RNA, and so on) are firmly packed in the cytoplasmic aqueous phase, as are ions and tiny molecules, and the highly viscous cytosol inhibits targeted substances from migrating into

cells [129].

Cancer treatment has grown increasingly popular as a result of recent developments in biology and technology being combined with more traditional treatments. As a result of nanotechnology, the cancer-targeting qualities of medications can be improved. Using nanomedicines, it is possible to create nanoscale carriers that serve several purposes in the transport of drugs. In addition, it eliminates the problem of cancer therapy's multidrug resistance [130]. In addition, different types of nanometric DDS and their conjunction with other modalities such as phototherapy, immunotherapy, etc., are gaining pace for successful therapy for various malignancies due to the drastically increased anti-tumor effects and protective profiles. To boost the effectiveness of phototherapy against cancer, mitochondria-targeted cancer phototherapy delivers photoresponsive chemicals directly to the mitochondria. Cellular apoptosis is dependent on mitochondria, which play an important role in cancer cell chemoresistance. Hyperthermia and ROS can damage mitochondria, which is why they play such an important role in so many cellular processes. As a result, mitochondria are ideal places for organelle-specific phototherapy [131]. Although nanomedicine and imaging techniques for cancer therapy has made significant progress, the practical use of nanotechnology remains a challenge for a number of reasons. Non-apoptotic cell death is very different between humans and animals, which is a major factor here. Further research is needed in order to close this research gap. Because of this, the creation of superior nanomedicine centered on an anticancer agent for the treatment of mitochondrial dysfunction in the future should take into account a number of factors. Anticancer nanomedicine research for mitochondrial targeting is likely to benefit from an interdisciplinary approach. In our view, the development of more effective and selective nano-drugs and the right combination of treatment with other anti-tumor techniques for maximum synergistic effects are essential to suit various medical demands [132].

Patients typically need therapy, so it's important to use drugs with a good safety profile and avoid taking many medications at the same time that might lead to mitochondrial problems. Because preclinical screening methods may be used, future medicines will be free of mitochondrial liabilities. To further enhance one's mitochondrial function, there must be lifestyle modifications such as weight loss, exercise, and a healthy diet. Molecular homeostatic regulation of mitochondrial integrity or functional capacity, rather than an abrupt depletion of bioenergetic substrates like ATP, appears to be the source of mitochondrial malfunction with age. As a result, bolus supplementation with mitochondrial cofactors has less potential to increase vitality and medication tolerance in older populations than long-term lifestyle adjustments [133].

9. Conclusion

Effective targeted mitochondrial delivery is crucial for the successful treatment of various human diseases, including cancer, metabolic diseases, and neurodegenerative diseases. Because of the lack of an effective delivery system capable of both stabilizing therapeutic nanomaterials and directing them to specific accumulations inside the mitochondria, there is presently no pharmaceutical formulation that is mitochondria-targeted on the market. As part of the cellular metabolism, the mitochondrion aids in the survival or death of cells, and contributes to the development of neoplastic tissues via aerobic glycolysis. So, cancer cells mainly depend on the mitochondria for energy supplementation and growth, which makes it a promising target for the destruction of cancer cells. Among the reported methods of targeting mitochondria, targeting ligand conjugation seems to be the most satisfying by combining the ligand with either a drug or nanocarrier. It may also have some pros and cons like high surface area, small particle size, and conjugation, which may decrease the solubility of the drug and therapeutic effects, respectively. This review has discussed all the possible factors, strategies, and approaches to target mitochondria in cancer cells with their conceptual mechanism. As a result, this review could provide a promising platform for developing theranostic nanomedicine for mitochondria-targeting, imaging, diagnostic, and therapeutic applications.

CRediT authorship contribution statement

Susanta Kumar Rout: Wrote initial draft and prepared images and tables. **Vishnu Priya:** Wrote initial draft and helped in preparing images. **Aseem Setia:** Wrote initial draft and helped in preparing images. **Abhishesh Kumar Mehata:** Wrote initial draft and helped in preparing tables. **Syam Mohan:** Wrote initial draft and helped in preparing images. **Mohammed Albratty:** Contributed in initial draft and helped in preparing images. **Asim Najmi:** Wrote initial draft and helped in preparing tables. **Abdulkarim M. Meraya:** Contributed in initial draft and helped in preparing images. **Hafiz A. Makeen:** Contributed in initial draft and helped in preparing images. **Murtaza M.Tambuwala:** Revised final draft and help in preparing images and tables. **Madaswamy S. Muthu:** Contributed in initial draft, revised final draft and helped in preparing images.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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