


Subcellular Performance of Nanoparticles in Cancer Therapy


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Abstract: With the advent of nanotechnology, various modes of traditional treatment strategies have been transformed extensively owing to the advantageous morphological, physiochemical, and functional attributes of nano-sized materials, which are of particular interest in diverse biomedical applications, such as diagnostics, sensing, imaging, and drug delivery. Despite their success in delivering therapeutic agents, several traditional nanocarriers often end up with deprived selectivity and undesired therapeutic outcome, which significantly limit their clinical applicability. Further advancements in terms of improved selectivity to exhibit desired therapeutic outcome toward ablating cancer cells have been predominantly made focusing on the precise entry of nanoparticles into tumor cells via targeting ligands, and subsequent delivery of therapeutic cargo in response to specific biological or external stimuli. However, there is enough room intracellularly, where diverse small-sized nanomaterials can accumulate and significantly exert potentially specific mechanisms of antitumor effects toward activation of precise cancer cell death pathways that can be explored. In this review, we aim to summarize the intracellular pathways of nanoparticles, highlighting the principles and state of their destructive effects in the subcellular structures as well as the current limitations of conventional therapeutic approaches. Next, we give an overview of subcellular performances and the fate of internalized nanoparticles under various organelle circumstances, particularly endosome or lysosome, mitochondria, nucleus, endoplasmic reticulum, and Golgi apparatus, by comprehensively emphasizing the unique mechanisms with a series of interesting reports. Moreover, intracellular transformation of the internalized nanoparticles, prominent outcome and potential affluence of these interdependent subcellular components in cancer therapy are emphasized. Finally, we conclude with perspectives with a focus on the contemporary challenges in their clinical applicability.

Keywords: organelle, proton sponge effect, intracellular pathways, cancer therapy, nanocomposites

Introduction

Despite the significant advancements in understanding the origination, development, and maturation of cancer on one end, and the development of numerous therapeutic strategies in its eradication on the other end, the research is still under progress for the development of highly advanced therapeutic strategies for efficient ablation of cancer.¹ In this framework, enormous efforts over the past few decades have been dedicated to the tremendous development of a series of several therapeutic strategies, including chemotherapy,^{2,3} radiotherapy,⁴ surgical therapy,⁴ or even palliative therapy,^{3,5} which have been under practice to fight against this fatal disease. Nevertheless, these traditional strategies suffer from several shortcomings of each method. Along this line, although there has been a significant decline in the overall mortality rate and an increase in the life span of patients,

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however, the traditional chemotherapy utilizing several chemotherapeutic molecules either alone or in combination has been facing several hurdles. Predominantly, severe adverse effects are instigated in patients on systemic administration of chemotherapeutic drugs due to the undesired accumulation of drugs and metabolites in the vital organs. Secondly, the efficiency of administered drug dose might not be effective as anticipated due to the acquired multidrug resistance (MDR) by the cancer cells through cell surface efflux pumps for cell defense. These predominant consequences often result in poor therapeutic outcomes, leading to the high recurrence rate and utilization of altered therapeutic regimens at high doses.^{6,7} To a considerable extent, there has been significant progress in the modification of drugs to augment their intracellular bioavailability through various approaches such as chemical functionalization. However, regardless of its success in overcoming the non-specific distribution, this chemical modification approach substantially reduces the efficacy of drugs and can be pragmatic to certain drugs with limited chemical functionalities.

Broadly speaking, various therapeutic approaches known since antiquity are predominantly based on the palliative care, i.e., which has been merely focused on the delivered responses of the sensual systems of a person, by attempting to achieve the temporary respite from the alleviated pain or just to ease their mind as humanistic care. However, eradicating the growth of tumors has remained the predominant goal of therapy at those times.⁸ In comparison, it is increasingly recognized that the progressively emerged experimental therapeutics possessed the near-standard trials to save patients enduring with miserable diseases,⁹ which significantly fascinated by dealing with the primary symptoms in the late 19th century. Subsequently, researchers have been more concentrated on targeting the tumor site specifically, by conducting the stereotactic therapy for patients, who were in the early stage with the infeasible surgery practices.¹⁰ With the progression of modern society, the technological advancements over several decades have garnered great potential in overturning the aforementioned conventional strategies of discovery, diagnosis, and therapy, to precisely achieve clinical goals relevant to early diagnosis and effective treatment of diseases including cancer.

In this modern era, nanotechnology has garnered significant interest from researchers in various fields for the generation of materials with diverse compositions and morphological attributes.^{11–14} In the past two decades, these interesting features have significantly influenced the researchers to explore

enormous varieties of innovative nanobiomaterials with engineering characteristics and ideal functions through involving supramolecular, nanocrystal growth, and sol-gel chemistries.^{15,16} The application of nanotechnology to medicine has sparked enormous interest in cancer treatment and diagnosis due to its given special attributes in generating materials with typically controlled multi-dimensional (1–3 D) structures on the nanoscale range (approximately 1–100 nm in one of the measurable dimensions) for drug/gene delivery, diagnostic probes for radioactive or other advanced therapeutic strategies.¹⁷ This technology offers enormous advantages in fabricating materials through fine-tuning of physicochemical properties by altering the sizes, shapes, and composition, among others.¹¹ Compared to various traditional chemotherapeutic agents, these engineered materials as drug delivery platforms hold a great promise in offering controlled release of drugs/genes/therapeutic peptides,^{18–24} exhibiting cancer microenvironment responsiveness for drug release,^{6,7,25,26} and other sophisticated external stimuli-response delivery including photothermal therapy (PTT),^{25,27–30} photodynamic therapy (PDT),^{30–32} ultrasound-responsive and immunotherapy, among others.^{33–36} Among such delivery systems, a few of them have been approved for cancer therapy, and numerous others are currently active and/or recruiting additionally under clinical trials.³⁷

Compared to their bulk counterparts or precursors, the fabricated nanoparticles of specific composition exhibit two intrinsic advantages of high surface-to-volume ratio and abundant surface chemistry.³⁸ The high surface-to-volume ratio of nanoparticles enables the encapsulation of diverse therapeutics molecules (drugs/genes/contrast agents/therapeutic peptides) either in the interior or over the surface through physical adsorption or electrostatic interactions.³⁹ In addition, the availability of abundant functionalization surfaces facilitates the ease of immobilizing therapeutics guests, and diverse biomacromolecules for altering their behavior in vivo, and feasible targeting ligands for augmenting their bioavailability.⁴⁰ Broadly speaking, all the available nanomaterials used for biomedical applications can be categorized based on the chemical composition into three major divisions of organic, inorganic, and inorganic/organic hybrid systems. The carbon-based organic materials, for instance, liposomes, emulsions, among others, are relatively highly compatible and degradable in the physiological environment, which have driven their earlier translation to clinics, for example, Abraxane, Doxil, DaunoXome, and, Myocet, among others.^{37,41} Despite their success, numerous formulations based on the organic materials have been withdrawn, and many struggling to pass further

clinical translation procedures due to their intrinsic features of poor stability in the physiological fluids and deprived drug encapsulation efficiency.³⁸ Comparatively, the inorganic-based nanobiomaterials offer exceptional advantages like intrinsic thermal and colloidal stabilities, resistance, unique structural and chemical optical features, as well as morphological attributes, ease of tailoring the architectures, but relatively low degradation rates.⁴² Specific examples of well-known inorganic materials include layered materials, magnetic nanoparticles, semiconductor quantum dots, metal nanoparticles (MNPs), mesoporous silica materials, which are of particular interest in diverse biomedical applications.^{6,7,43,44} However, the translation of these stable constructs have been encountering a great challenge due to the safety and degradability concerns in the biological environment.^{38,45–47} Finally, the hybrid systems combined with the intrinsic advantages of both organic as well as inorganic offer great benefits of compatibility, degradability, and stimuli-responsive delivery of therapeutic guests.^{48,49}

In general, the functional nanoparticles for cancer therapy are predominantly administered through the intravenous route, which could circulate systemically throughout the blood circulation due to their tiny sizes providing extended circulation *in vivo*. Appropriate design and specific modifications resulting in augmented physiochemical attributes of nanoparticles facilitate their precise adherence to different proteins in the systemic circulation, which prolongs the circulation time and conveniently facilitates reaching the tumor site.⁵⁰ Further, the cellular internalization could be achieved either by passive (Enhanced Permeation and Retention, EPR) or active targeting modes, which could lead to better therapeutic outcomes over conventional therapeutic strategies. Nevertheless, they still suffer from significant limitations of nonspecific distribution and deprived therapeutic efficiency, which yet to remain unaddressed. Moreover, it should be noted that rate of cellular internalization and final bioavailability of the carrier is affected by the size, shape or surface charge of the nanoparticle. The final size of the materials in the nanoscale range plays a crucial role in creating these complex structures, which accelerate experts in shifting their attention from targeting macro-/or micro-sized pathological tissues or organs to specific cells concerning their promising, safe and effective delivery as well as subsequently understanding the mechanisms of action and degradation pathways. For instance, Tammam et al⁵¹ used chitosan nanoparticles as models, where different-sized nanoparticles showed size-dependent cellular internalization efficiency and subsequent protein delivery. Similarly, the final particle size,

as well as surface charge of the nanoparticles, significantly affected their subcellular localization. Moreover, numerous reports indicated that the ultra-small positively-charged nanoparticles had a higher affinity toward specific organelles, such as mitochondria and nucleus, facilitating their permeability. Similar to the cell surface, these membranes also possess surface charge enabling the electrostatic interactions with the guest species.^{52–54} Furthermore, via triggering phase transformation, free energy release, reorganization, and dissolution, these internalized nanoparticles subsequently respond to the cell membrane proteins, and organelles as well as nucleic acid content to establish various kinds of interfaces that could sustain by the colloidal forces and dynamic biochemical reactions. Substantially, these consequences promote the formation of the protein corona, particle encapsulation, intracellular uptake, and biocatalysis during the course of transportation in the biological microenvironment.⁵⁵

Notably, there exist specific distinctions between organs and microscopic cellular substructures in possessing unique specific morphology and structural as well as functional attributes. In this context, numerous reports have demonstrated targeting the cancer cell surfaces using different ligands on nanoparticles and their subsequent fate in cells by focusing on the basic transport or transfer mechanism during the internalization process. In addition, a step forward, a few of the reports have explored some specific ligands that could target a few of the organelles and reconnoitering their specific pathways that act through.^{38,56–58} In this vein, some of the intriguing reports by Steichen³⁸ and Varkouhi⁵⁶ reported the behavior of nanoparticles concerning their entry into cells. These precise reviews gave a summary of selected information on the formation of vesicles that encapsulate nanocarriers from the early endosome to the late endosome with a pH transformation from 7.4 to 5.0 for degradation and their escape mechanisms for their delivery in the cytosol, contemporary pore formation, pH-buffering effect, and protein fusion.^{38,56} As mentioned earlier, the specific interactions and precise effects between nanoparticles and the organelles play a crucial role in determining the behavior and the fate of nanoparticles intracellularly, which could explicitly provide the evidence in designing the efficient delivery systems.

In general, the intracellular route map of nanoparticles is explicitly discussed hereunder. As shown in [Figure 1](#), nanoparticles are predominantly engulfed by cells through different pathways depending on their size and surface charge, which significantly depend on various modifications over the nanocontainers and intrinsic properties of the core material.

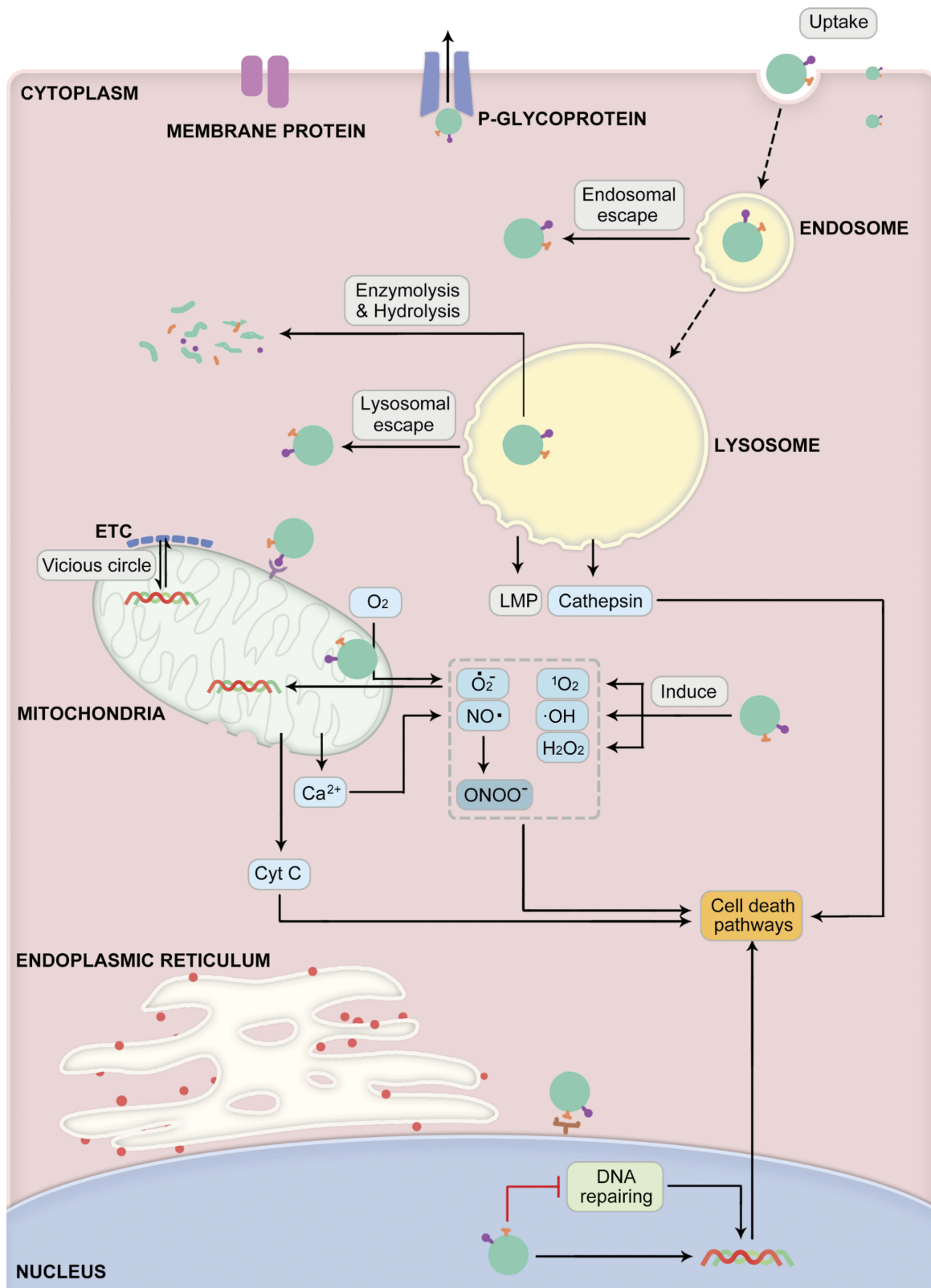


Figure 1 Schematic illustration showing the intracellular performance and fate of nanoparticles in cancer therapy that explicitly determining their internalization, interaction with different subcellular organelles, delivery of drugs, and mechanisms of action. **Abbreviation:** ETC, electron transport chain.

Broadly speaking, the particles with larger size ($>1 \mu\text{m}$) can be generally entered into the cells by micropinocytosis. Contrarily, the nanoscale products are most commonly internalized through the endocytosis pathway, which is dynamic, convenient, and well-regulated internalization route for any particle.⁵⁹ However, the type of endocytosis for the entry of nanoparticles is different, which significantly depends on the eventual size of the particles. Small-sized particles ($\sim 60 \text{ nm}$) could conveniently enter the cell by the caveolin-dependent endocytosis, in which the nanoparticles could be exposed to neutral pH in a caveosome. On the other hand, the large-sized nanoparticles ($\sim 120 \text{ nm}$) could be internalized through clathrin-dependent/independent endocytosis, which predominantly depends on the modifications over the nanoparticle surface by ligands and their types as well as the shape of the particles.⁶⁰ The nanoconstructs that can pass through the cell membrane via endocytosis is exposed to the acidic micro-environments in the endosome and subsequent lysosome.⁶¹ More often, the internalized nanoparticles are encapsulated in the endosome-based liposome packets, which further pass them to the lysosomes with a highly acidic microenvironment, where they undergo enzymolysis and hydrolysis or discharged the inert nanocontainers unmodified into the cytoplasm through lysosomal escape pathways.⁵⁹ Further, these liberated nanoparticles into the cytoplasm continue to interact intracellularly with other specific organelles, for example, reacting to the components in the acidic environment of endocytosis/lysosome, activating electron transport chain or the production of reactive oxygen species (ROS) in mitochondria, or Golgi apparatus and regulating DNA-control in nucleus or mitochondria. In some instances, after interacting with the specific organelles and performing their predetermined specific tasks, such as delivering the therapeutic agents, and photoactivation, the nanoparticles, such as polymer-based soft composites that are sensitive to these micro-environments, can be degraded along with the cell deterioration after death, whose remnants can be then dealt with by macrophages like all other components of cells. In the case of non-degradable materials, they may continue to internalize into the adjacent cells.^{45,62} To this end, the non-biodegradable inorganic-based nanoparticles could be taken up by the lymphatic drainage and eliminated subsequently after prolonged circulation in the physiological environment. Hence, specific structural attributes, biodegradability behavior, and the appropriate acting mechanisms of specific organelles reconnoitering the facts behind the subcellular performance and the fate of nanoparticles should be explored for better insights to exploring new innovative technologies.

In the further sections, we first give emphasis on recent advancements concerning the escape pathways of internalized nanoparticles through endosome/lysosome, highlighting the advantages and limitations. We then focus on the intracellular performance as well as the fate of nanoparticles with the essence showcasing the precise interactions between nanoparticles and different key organelles, such as mitochondria, nucleus, endoplasmic reticulum, and Golgi apparatus. Finally, we conclude the prominent outcome, contemporary challenges, and the potential influence of these interdependent subcellular components in cancer therapeutics.

Subcellular Performance and Intracellular Fate of Nanoparticles

A cell is a typical unit of the body possessing several organelles, which are much like organs in the human body as every organelle has its specific duties to perform in providing all needs to the cell, such as supplying nutrients, removal of waste, cell repair, growth, and reproduction, among others. In fact, the word organelle refers to a mini-organ. Along this line, various subcellular key organelles play a crucial role in handling nanoparticles-based cancer therapeutics after their successful internalization such as endosomes as well as lysosomes for digestion of the internalized foreign bodies, autophagy, and first line of cell defense,⁶³ mitochondria for monitoring the apoptotic cascades, calcium cycle, and ATP synthesis,⁶⁴ nuclei for DNA alteration, gene expression, and cell proliferation,⁶⁵ and endoplasmic reticulum, as well as Golgi complex apparatus, facilitate the protein synthesis and their transportation to other organelles.^{66,67} Meanwhile, they also play different and necessary roles in relevant to cancer ablation and diverse metastasis inhibition pathways, such as apoptosis, autophagy, necrosis, and Ferroptosis, among others.⁶⁸ Considering these aspects, it could be beneficial in driving these prominent biological effects of various organelles towards the therapeutic opportunities in eradicating cancer. For instance, some of the drugs, such as cisplatin and doxorubicin (DOX), precisely exhibit their effects in the nucleus.^{69,70} Thus, it is highly required to augment the availability of such drugs in the respective organelles and activate the specific cell death signal. In addition to therapeutic efficiency, these organelle-targeting strategies of conveying therapeutic species could address a significant limitation of high dosage necessity due to premature leakage and drug resistance. These consequences result in achieving high therapeutic index by overcoming numerous biological barriers to reduce the adverse effects of such cytotoxic drugs. Herein, we emphasize the discussions on all these aspects, highlighting the

Table 1 Examples Presenting Different Organelle-Targeted Nanoformulations for Cancer Therapy

Organelle	Composition	Size/nm	Responsiveness	Pathway	Outcome	Reference
Endosome/ lysosome	LDH-hydrazone-Dox	104.33±14.15	pH	Proton sponge effect	Enhanced ROS generation	[68]
	ORMOSIL NPs	<100	Autophagy	Caveolae-mediated endocytosis	Endocytosis and subcellular localization	[217]
	CALNN-capped gold nanoparticles	5	–	Peptide cleavage by the protease cathepsin L	Applied for Biosensing, fluorescence quenching	[78]
	pRNA-3WJ	5–20	Immune	Internalized through endocytosis	Endosomal escape for siRNA delivery	[218]
	FA-SLICs	–	pH	Internalized through the receptor-mediated endosomal pathway	Controlling the release of loaded genes by the endosomal microenvironment	[85]
	L-histidine-based polymeric micelles	–	pH	Endosomolytic activity	Ablating drug-sensitive ovarian cancer as well as drug-resistant counterpart cells	[84]
	V-ATPase inhibition	–	Cisplatin	ERK/MEK	Regulating autophagy that assisting in chemoresistance in ovarian cancer	[70]
	YPSMA-1-PEOz-PLA	–	pH	Degradative lysosomes pathway	Efficient delivery of anticancer drugs for treating PSMA-positive prostate cancers	[81]
	Nanosomes	–	Carbohydrate–lectin	LMP pathway	Facilitating targeted molecular delivery and intracellular traffic through an endocytic route without the influence of protein corona	[219]
	T-UPSM	37.6±1.9	pH	Lysosomal catabolism inhibition	KRAS mutant pancreatic cancer treatment through simultaneous lysosomal pH buffering and rapid drug release	[86]
F-Gly-MTX NPs	46.82±5.03	Enzymatic, pH	Acid lysosomal compartment and protease	Release of the MTX via peptide bond cleavage in the presence of proteinase K	[220]	
Mitochondria	CdTe QDs	5.5	–	Inhibition of mitochondrial respiration and induce mitochondrial MPT	Augmented cell apoptosis and demonstration of QDs toxicity at subcellular (mitochondrial) level	[52]
	TPP-CeO ₂	2.2	–	Scavenge mitochondrial ROS efficiently to reduce oxidative stress	Suppression of neuronal death	[94]
	PDA-PEG-TPP-DOX	28	pH	Enormous levels of ROS generation	Overcoming drug resistance in cells	[93]
	TL-CPT-PEG ₁₀₀₀ -TPP with DSPE-PEG	77.1~149.4	ROS	Elevate PDT efficacy and interfering with DNA replication as a topoisomerase inhibitor	Augmented chemotherapy and PDT	[156]
	AuNPs@CP/SA	20	–	Efficient dynamin-dependent and caveolae-mediated endocytosis pathway	Highly specific localization in mitochondria	[152]

(Continued)

Table 1 (Continued).

Organelle	Composition	Size/nm	Responsiveness	Pathway	Outcome	Reference
Nucleus	DOX@MSN@TAT/RGD	38.6~47.2	–	Vasculature-/membrane-to-nucleus sequential drug-delivery strategy exploiting RGD and TAT dual-peptides	Enhanced in vivo chemotherapeutic efficacy in clinics	[190]
	IONPs-TAT/PEG	20	–	Enhancing the therapeutic effect by nuclear targeting	Simultaneous cancer targeting, imaging, and PTT	[191]
	UCNPs@TiO ₂ -Ce6-TAT	25	NIR light (980 nm)	Generation of multiple ROS ($\cdot\text{OH}$, O_2^- , and $^1\text{O}_2$) using the dual-photosensitizer	Enhancing PDT can be achieved against MDR cancer	[192]
	Chitosan nanoparticles-NLS	25, 150	–	Study on impaired nuclear import and aberrant nanoparticle intracellular trafficking in glioma	Effect of nanoparticle size and NLS density on nuclear targeting in cancer and normal cells	[189]
	AuNPs@PEG@RGD/NLS	35	–	Mechanical stiffness of the nucleus and stimulate the overexpression of lamin A/C located around the nuclear membrane	Increasing nuclear stiffness and slowing cancer cell migration and invasion	[186]
	NLS/RGD-AgNPs	35	–	ROS generation and effect on cell cycle and cell division	DNA damage and apoptosis	[187]
	Acridin-9-Methanol nanoparticles	60	Visible light (≥ 410 nm)	Nucleus targeted intracellular controlled drug release	Fluorescent imagine and killing the cancer cells	[194]
Golgi apparatus	PTX-CS-RA	192.7 \pm 1.8	pH	Loss of cystic structure	Enhancement of antimetastasis combined with chemotherapy	[83]
	M-COPA	–	–	EGFR-TKIs	Overcoming the vicious cycle of TKI resistance in EGFR-mutated NSCLC cells via downregulating cell surface RTK expression	[67]
	Alb-GNPs	49	–	Caspase-3	PTT of Alb-conjugated GNPs in liver cancer-initiating Golgi apparatus-endoplasmic reticulum dysfunction with consequent caspase-3 apoptotic pathway activation and cellular apoptosis	[66]
	ANQ-IMC-6	–	Fluorophore	Cyclooxygenase-2	Visualizing dynamic changes of the Golgi apparatus during cancer cell apoptosis and possible application in early diagnosis	[210]
	Amicoumacin A	0.31	–	Translation elongation inhibition	Translation inhibition in yeast and mammalian systems by affecting the translation elongation	[221]

(Continued)

Table I (Continued).

Organelle	Composition	Size/nm	Responsiveness	Pathway	Outcome	Reference
Ribosome	LUCAT1 lncRNA	–	–	L40-MDM2-p53	Suppression of LUCAT1 inducing CRC cell cycle arrest and apoptosis by binding UBAS2 and activating the RPL40-MDM2-p53 pathway	[222]
	Dd-mrp4	–	–	p53- or RB-like functions	Ectopically expressed Dd-mrp4 suppressing the cell proliferation through inducing apoptotic cell death	[223]

Abbreviations: Alb-GNPs, Albumin-gold nanoparticles; AgNPs, Silver nanoparticles; ANQ-IMC-6, Acenaphtho[1,2-b]quinoxaline-indomethacin-6; AuNPs@CP/SA, Gold nanoparticles coated by biotin-NNLACCALNN-COOH/tetrameric streptavidin molecules; CdTe QDs, Cadmium telluride quantum dots; Dd-mrp4, Dictyostelium mitochondrial ribosomal protein S4; DSPE-PEG, 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)]; EGFR-TKIs, Epidermal growth factor receptor-tyrosine kinase inhibitors; FA-SLICS, Folic acid functionalized Schiff-base linked imidazole chitosan; F-Gly-MTX NPs, Methotrexate-conjugated glycine-coated magnetic nanoparticles; PDA, Polydopamine; IONP-TAT, Iron oxide nanoparticles-YGRKKRRQRRR peptide; LDHs, Layered double hydroxides; M-COPA, 2-methylcoprophilnamide; MDR, Multidrug resistance; MSN, Mesoporous silica nanoparticles; NLS, Nuclear localization sequence; ORMOSIL NPs, Organically-modified silica nanoparticles; PDT, Photodynamic therapy; pRNA-3WJ, Three-way junction motif of packaging RNA molecules; PTT, Photothermal therapy; PTX-CS-RA, Retinoic acid-conjugated chondroitin sulfate loading with paclitaxel; RGD, RGDRGDRGDRGDPGC peptide; RTK, receptor tyrosine kinases; TAT, a HIV peptide; TL-CPT, thioketal linker-modified camptothecin; TPP, triphenylphosphonium; T-UPSM, Triptolide prodrug-loaded ultra-pH-sensitive micelles; UCNPs@TiO₂-Ce6-TAT, NaFY₄:Yb,Er,Tm@TiO₂-Chlorin e6-TAT; YPSMA-1-PEOz-PLA, Anti-(prostate specific membrane antigen) antibody-poly(2-ethyl-2-oxazoline)-poly(D, L-lactide).

critical behavior of the nanoparticles and the treatment received from the organelles (Table 1).

Endosome/Lysosome

Typically, the endosomes are membrane-bound organelle compartments of endocytic transmembrane that represent the primary sorting endomembrane system in cells, involving the transportation of different substituents through major sorting pathways. These resultant endomembrane pockets predominantly act as primary doorsteps for nanoparticles toward their cellular internalization and selective conveyance to the interior of the cell through the inner compartments called lysosomes. Distinguished by three different phases of early, late, and recycling compartments, the endosomes significantly provide the acidic environment for endocytosed materials to be sorted before reaching the degradative lysosomes, which is the terminal point of the endocytic pathway, that also stemmed from the trans-Golgi membrane system.^{71,72} While on their way toward the target site intracellularly, the endocytosed nanoparticles face an extreme shift in the pH of their surrounding medium from an environmental pH of 7.4 in the extracellular medium to approximately 6.0 in the early endosomes, and further to 4.5 in the lysosomes due to excess proton supply in the endocytic medium.⁷³ Such a concomitant shift in pH has become an efficient biological trigger in delivering the active therapeutic guest molecules from the bulk of the containers. These consequences involve renowned changes through the spatiotemporally controlled way by utilizing the stimuli-responsive molecular switches that contain ionizable weakly acidic/

alkaline groups, e.g., disulfide linkage, hydrazone, and dinitroimidazole, among others.^{74,75}

In addition to the conveyance of the guest species, the endosomes facilitate the recycling of the guest molecules via fusion, where they are transported from the plasma membrane to early endosomes or delivered through the vesicles back to the plasma membrane.⁷⁶ This unidirectional transport from the late endosomes to lysosomal compartments is the unique characteristic feature of the entire internalization process, as a result of consumed fusion with lysosomes. However, in some of the peculiar cases of extremely tiny constructs (size approximately less than 1 nm) that can pass directly through the phospholipid bimolecular layer, the cellular internalization process of most of such nanoparticles, no matter the passage through which pathway they select such as clathrin-dependent/independent endocytosis, or micropinocytosis and phagocytosis driven by specialized actin, would come in early endosomes and subsequently toward the late endosomes.^{76,77} In addition, it should be noted that there exist some specific instances, where if no effective or specific modifications were made on the surface of the nanoparticles to increase the surface charge or enable proton sponge effect, the nanoparticles would stay in the late endosome till it adjoins with the lysosomal compartment.^{73,78} More often, the nanocarriers that are modified with specific modification with ligands can escape through various pathways resulting in further events of ablation, while the particles without any surface modification that are prone to the lysosomal environment get subsequently degraded in this compartment. However, the degradation behaviour of

unmodified particles stringently depends on the intrinsic stability of nanocarriers. Herein, the intracellular behavior and fate of nanoparticles that correlating with the several functions of these organelles are discussed in relevance to the ablation of cancer cells, highlighting the degradation and escape mechanisms.

Degradability

Since their discovery, lysosomes have been considered as the “recycling bins” of the cells, due to their capability of degradation by high amounts of destructive enzymes like hydrolases in their lumen.⁷⁹ Notably, lysosomes act as degradative compartments for most of the resultant products of biosynthesis that often participate in various cellular processes, such as membrane repair, pathogen defense, cell autophagy, and signal transduction, as well as foreign bodies internalized through endocytosis.⁷⁹ From this perspective, Grant and Donaldson classified the roles of caveolar endocytosis containing the dynamin-dependent caveolin coat for the internalization of glycosphingolipids and some specific viruses, or independent modes like cell division control (CDC) protein-42 homolog, ADP-ribosylation factor (ARF)-1 and actin, among others.⁷⁶ In addition, the authors demonstrated that the nanoparticles without any specific surface modification are highly prone to the acidic environment in lysosomes similar to all other intracellular constituents as they are exposed to multiple hydrolases of the lysosomal lumen. Further, the surface of nanoparticles was gradually dissociated, and the intact structure of nanoparticles was eventually broken, resulting in the small compartments as well as highly-charged ions. However, it should be noted that the resultant products significantly depend on the molecular structure and chemical composition of the nanomaterials.⁸⁰ These tiny resultant substituents, such as metal ions or other molecules at a high concentration released into the cytoplasm, can significantly affect the normal biochemical activities of cells and homeostasis, leading to the significant imbalance in the intracellular biochemical components and other cytoplasmic constituents. Moreover, in some instances of highly-charged metal species, these consequences might also lead to changes in the expressions of intracellular proteins and genes, resulting in the ablation of cancer cells through apoptosis.

In general, the nanoparticles predominantly aggregate in the lysosomal compartment, which, however, depends on the physicochemical properties as well as morphological attributes of materials, e.g., particle size, surface charge, shape, and chemical composition.^{78,81} In addition to the aggregation behavior, the highly acidic environment facilitates the degradation of acid-prone architectures, which are discrepant and

predominantly based on the chemical composition. These specific nanoarchitectures are highly attractive as the lysosomal targeted systems eventually reach after their internalization through receptor-mediated endocytosis. In this framework, the organic material-based nanoparticles, usually the so-called biodegradable polymers (Poly-L-lactide, PLA),⁸¹ can be designed by a kind or more of monomers linked by various sensitive ionizable weakly acidic and basic linkages (polymers from natural origin, dextran and chitosan and synthetic origin that possessing acid and amine functional groups),⁸² as well as specific molecular switches, such as disulfide, hydrazone, dinitroimidazole-based linkages.^{81,83–86} In fact, these molecules are highly responsive to the acidic environment and can significantly facilitate the degradation of the construct.^{87,88} To this end, some of the inorganic nanoparticles, such as metal-organic frameworks (MOFs), periodic mesoporous silicas (PMOs), and other stable nanoparticles such as gold and platinum nanoparticles, that are immobilized with acid-responsive linkages can be degradable in the endosomal/lysosomal micro-environment, resulting in the small-sized nanocrystals or even free ions.^{78,89–91} These stimuli-responsive linkers offer enormous advantages in drug delivery over conventional carriers, including the deprived premature release of therapeutic guests in the physiological fluids, improved encapsulation efficiency, and reduced undesirable adverse effects, among others.^{82,92} To the other end, some of the inorganic nanomaterials are acid-prone such as layered double hydroxides (LDHs), which can not only facilitate the precise release of drugs in the lysosomal environment through controlled degradation but also conveniently enable degradation in the physiological fluids.^{49,68}

Escape Pathways

In addition to the specific internalization pathways through active or passive modes, the surface-modified nanoparticles functionally for controlled drug release, and conformational or photothermal conversion are directed out of the lysosomes through various discharge pathways. Numerous efforts over the past decade have been dedicated to designing different versatile nanoparticles-based formulations with several modifications on their surface that precisely guide them to the site of specific intracellular organelles for exhibiting specific functions, namely organelle targeting. Various examples of organelle-targeting ligands include mitochondriotropic moieties like triphenylphosphonium (TPP), dequalinium (DQA), and mitochondrial penetrating peptides (MPPs).^{93–95} However, it should be noted that the modified particles are required to escape from the lysosome and subsequently drive themselves toward the targeted organelle as well as facilitate

the release of therapeutic cargo in the cytoplasm or targeted organelles.⁵⁷ The mechanism of the endosomal/lysosomal escape of nanoparticles happens to be favorable in various ways: 1) Formation of membrane apertures in the endosomes or lysosomes, 2) Proton sponge effect, 3) Fusion with the membrane, and 4) Photochemical disruption of endosomal or lysosomal membranes.

Formation of Membrane Apertures

Lysosomes, the multivesicular bodies (MVBs), are generated from the matured vacuolar elements that pinched-off from the regions of perimeter endosomal membrane bud toward the luminal compartment.⁹⁶ Indeed, these are the inseparable targets of late endosomes as well as the destination of the endocytic pathway. However, the degradation membrane relies on the superficial pore dynamics involving the subsequent opening and closure of the gap, which can be predominantly controlled together by the line tension as well as the membrane tension, enabling the expansion or shrinkage of the pore, respectively. However, the initiation of pore formation happens to be conducive at such circumstances of an upsurge of the membrane tension or reduction of the line tension, and vice versa (Figure 2). In this context, Chen et al demonstrated the fact lying behind the transition of alamethicin from S state (embedded in the head group regions on account of the hydrophobic interaction) to I state (forming transmembrane pores subsequently killing cells), in which the concept of membrane elasticity and the transformations tangled between barrel-stave pores and toroidal pores were involved.^{97,98} Furthermore, utilizing the water-soluble amphipathic antimicrobial peptide, Lee et al confirmed the formation of equilibrium pores that induced by peptides while

monitoring of giant unilamellar vesicles (GUVs),⁹⁹ in which these kinetic pores-implanted single membranes significantly exhibited optimized effects with stable size. Along this line, the superficial modification of nanoparticles are formulated with peptides or the similar components of polypeptides, such as melittin,^{100–102} bovine prion protein (bPrPp),¹⁰³ listeriolysin O,¹⁰⁴ and streptococcal streptolysin O,¹⁰⁵ among others, have shown excellent affinity to the edge of the pores, which significantly reduced the line tension after associating with the edge of the pores. However, the loss of balance between the membrane tension and the line tension could not stabilize the original pore size, facilitating the subsequent expansion of pores to form irreversible apertures in the membrane of lysosome or endosome.¹⁰⁶

Proton Sponge Effect

Although the lysosomal escape pathway of internalized nanoparticles is often debated and elusive, the proton sponge effect hypothesis is generally accepted. Oftentimes, in the high pH conditions, the lumen of lysosomes allows the inflow of excess protons by enabling the membrane pumps, which subsequently drive the intramolecular exchange with the chloride ions and water. Further, these consequences enable the infiltrate swelling, resulting in the burst of lysosome and subsequently release the intraluminal constituents into the cytoplasm. Oftentimes, this phenomenon is significantly achieved through specific modifications, involving the unsaturated amino chelation, in which the continuously opened proton pump (V-ATPase) induces the exchange of chloride ion and a water molecule retention for each proton. Then, the excess inflow of various ions and water molecules triggers the swelling and substantial outburst of the lysosome.⁵⁶ Considering the polyplexes,

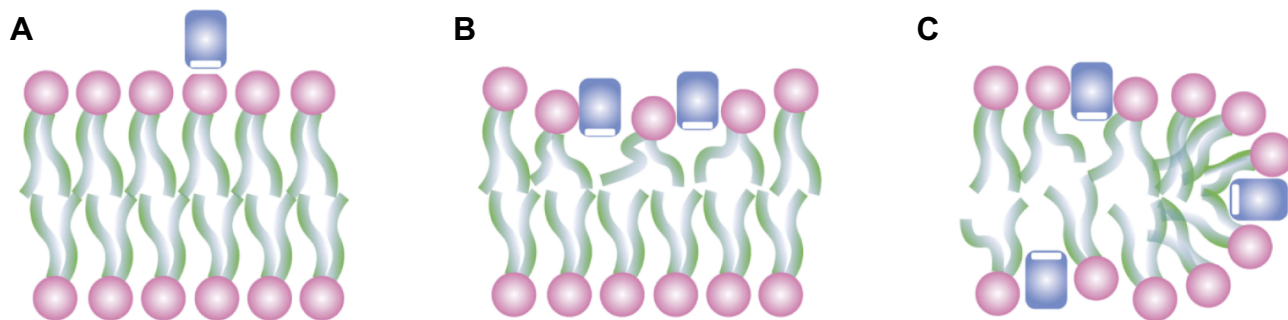


Figure 2 Proposed interactions between peptides (rectangles) and lipid bilayers. **(A)** Peptides are soluble in water but have a high affinity for binding to lipid bilayers. **(B)** Peptides inserting into the head group region stretch the membrane area, cause thinning of the chain region, and thus create an internal membrane tension. **(C)** Peptides preferentially bind to the edges of the pores, which have the consequence of loosening the internal membrane tension. (The depicted pores are called toroidal, or wormhole models. Some peptides make barrel-stave pores. It should be noted that the mechanism is the same for both types of pores.) Reprinted figure with permission from Huey W Huang, Fang-Yu Chen, and Ming-Tao Lee, *Physical Review Letters*, 92(19), 2004.] Copyright 2004 by the American Physical Society,¹⁰⁶

Nel et al⁵⁵ supposed that the superficial unsaturated amines could chelate protons and retain the operating pumps, which were significantly responsible for the acidification of lysosomes. For every proton carried by the nanoparticles during the internalization to the lysosomes, an equivalent number of chlorides, as well as water molecules needed to be retained for maintaining the homeostasis (Figure 3). However, the particles could be escaped from the swelled and ruptured lysosomes due to the influx of chloride and water molecules, and eventually deposited in the cytoplasm. Simultaneously, the leakage of lysosomal content and calcium ions (Ca^{2+}) might result in the reduction of ATP and the increment of a cytotoxin, inducing further apoptosis. In one instance, the escape pathways of nanoparticles with similar size and shape, but different charges of positive (MNPs, MIL-101, Zeolitic imidazolate framework (ZIF)-8

and nanoparticles modified with amine functionality), neutral (UiO-67 and nanoparticles coated with polyethylene glycol, PEG), and negative (SiO_2 , MOF-801 and nanoparticles modified with carboxyl functionality) potentials were examined. It was concluded that the nanoparticles with positive charge could only be feasible to escape from the lysosome, and nanoparticles with negative or neutral charge could almost be retained in the lysosome before enzymatic hydrolysis. These consequences happened to be conducive as the positively-charged nanoparticles (for instance, PEI) could be efficiently internalized into the lysosome due to the differences in charge. Moreover, a large number of chlorine ions were pumped-in to neutralize the charge density in the lumen of the lysosome, leading to the expansion and disruption due to a sharp enhancement of osmotic pressure in the lysosome.¹⁰⁷ Although the substantial exchange of ions

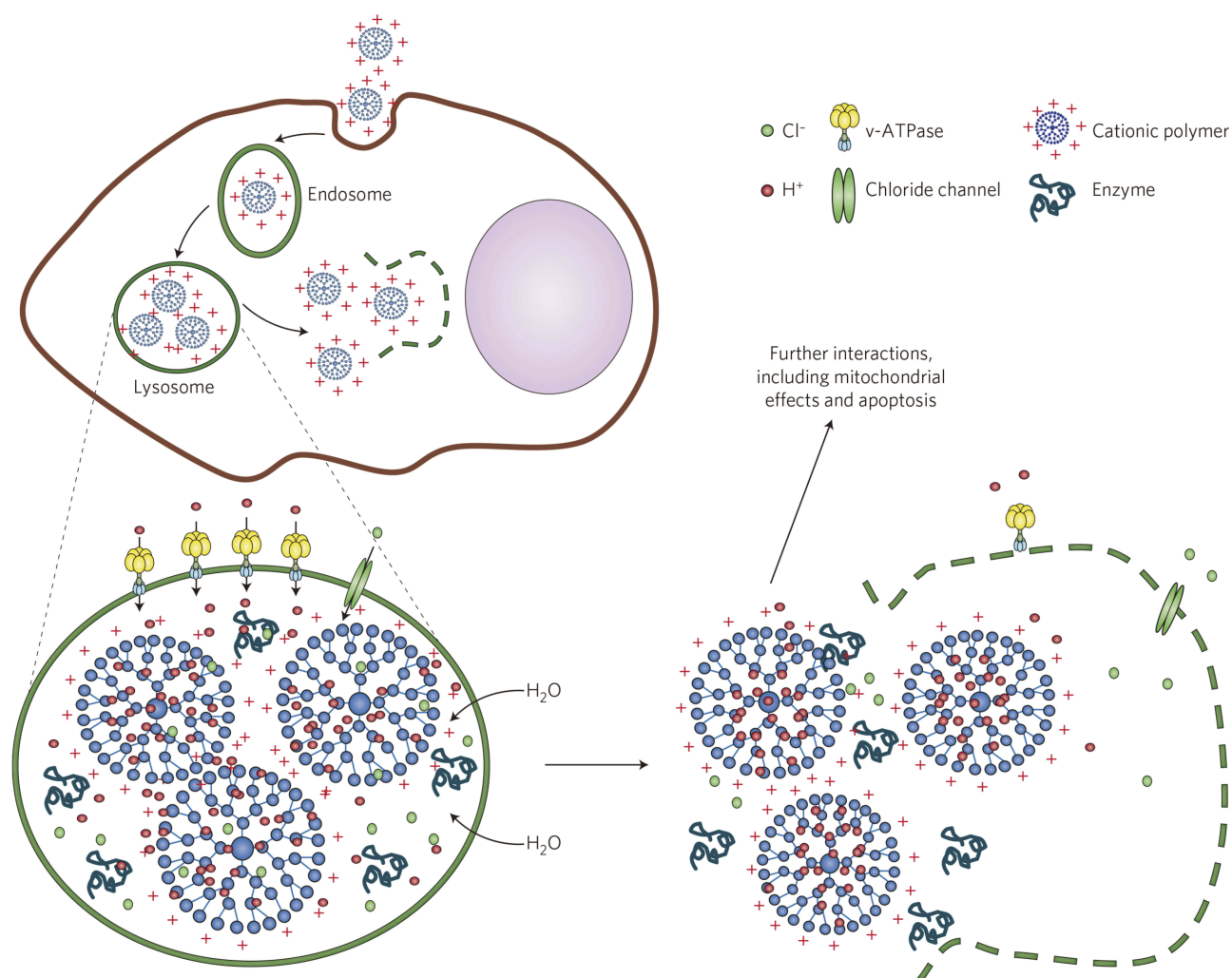


Figure 3 Schematic illustrating the proton sponge effect leading to the lysosomal damage and the induction of cytotoxicity by the cationic nanoparticles. Cationic (for example, PEI-coated) nanoparticles bind with high affinity to lipid groups on the surface membrane and are endocytosed in the tight-fitting vesicles. Reprinted by permission from Springer Nature, Nature Materials, Understanding biophysicochemical interactions at the nano-bio interface, Nel AE, Madler L, Velegol D, et al., Copyright 2009.⁵⁵

happened, it should be noted that these consequences have no significant influence on the change in the lysosomal pH.

Fusion with the Membrane

This critical phenomenon of escape by membrane fusion can be often observed in the cell transport and endocytosis processes, usually involving the participation of fusion molecules with a similar composition like peptides or lipids (liposomes, emulsions, or similar biomimetic molecules) coated over nanoparticles favoring their escape. With respect to the physicochemical properties of subcellular targeted nanoparticles, the intracellular concentration, exposure duration, and the surface reactivity are all indeed the predominant attributes that are responsible for driving the successful interactions between them prior to the escape of the guest species.⁵⁵ Notably, the nanoparticles immobilized with specific fusion peptides (e.g., Green Fluorescent Protein (GFP)-tagged Rabs, Rab4, Rab5, Rab7, Rab11, GTPases) on the surface can interact with the

lysosomal membrane and comprehensively achieve the escape of nanoparticles through facilitating the membrane fusion phenomena.¹⁰⁸ For instance, the confocal microscopic analysis indicated that the green fluorescent assemblies of the perinuclear aggregate in Enhanced Green Fluorescent Protein (EGFP)-tagged Rab7 wt-expressing cells were partly co-labeled with the late endosome marker CI-MPR (Figure 4A–C) but at a high degree of the lysosome markers Lamp-1 (Figure 4D–F), Lamp-2, and cathepsin D (Figure 4G–I). Nevertheless, CI-MPR and Lamp-1 markers presented a certain degree of overlapping. Similarly, Song et al¹⁰⁹ used wheat germ agglutinin-functionalized polymeric nanoparticles (WGA-NP) to explore the mechanisms of transcellular passage in Caco-2 cells, which exhibited that the various molecular weights of surface PEG resulted in altered cellular association, transcellular conveyance, and endocytosis pathway efficiencies. However, the core material, PLGA significantly contributed to a better lysosome escape and higher

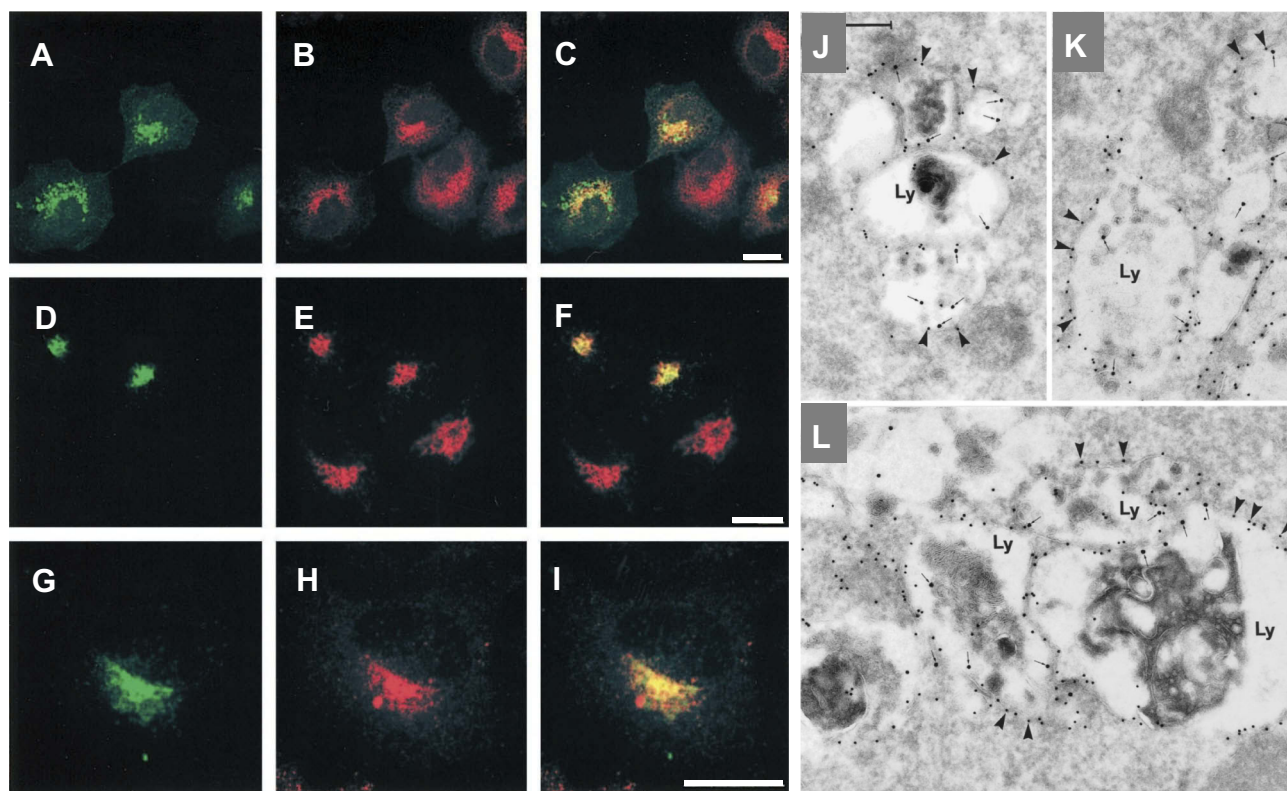


Figure 4 The EGFP-Rab7 wt fusion protein is mainly associated with lysosomes. Green corresponds to the EGFP signal (A, D and G) and red to the immune-detected markers CI-MPR (B), Lamp-1 (E), and cathepsin D (H). (C), (F), and (I) show the merged images, where yellow indicates co-localization. Note that the co-localization of EGFP-Rab7 wt with CI-MPR is only partial (C), whereas there is a more distinct co-localization of EGFP-Rab7 wt with Lamp-1 (F) and cathepsin D (I). Bars, 20 nm. (J, K and L) Immunogold labeling of cells expressing the EGFP-Rab7 wt. The panels show examples of large vesicular structures forming tightly packed aggregates. These structures appear as multi-vesicular bodies with numerous small, internal vesicles, or they have a more typical lysosome-like appearance with a dense content of membranous material. Note that all of these aggregated, late endosome/lysosome-like structures (Ly) are distinctly labeled for EGFP (10-nm gold; arrowheads) on the cytoplasmic surface of their outer membranes, as well as for Lamp-1 internally (15-nm gold, small arrows). Correspondingly, note that minimal cytosolic labeling for EGFP is seen. Bar, 250 nm. Republished with permission of American Society for Cell Biology. Rab7: a key to lysosome biogenesis, Bucci C, Thomsen P, Nicoziani P, McCarthy J, Bo VD, 11(2), 2000; permission conveyed through Copyright Clearance Center, Inc.¹⁰⁸

WGA-NP transcytosis after incubation for 2 h. In addition to the type and varied length of the polymer, the size and shape of the eventual nanoconstructs had shown a significant influence on the phagocytic fusion mechanism. In an attempt to elucidate these attributes, Chithrani and coworkers¹¹⁰ opted for the colloidal gold nanoparticles (sizes from 14 to 100 nm) as the ideal model system for uptake and evaluated their subsequent internalization. In comparison, the authors demonstrated that the spherical-shaped nanostructures showed higher entanglement to the cells and subcellular compartments over the rod-shaped nanoparticles. Further, Rejman and coworkers¹¹¹ revealed that the intracellular routes of nanoparticles were intensively dependent on their particle diameters, where the internalization of particles with diameter less than 200 nm was predominantly favored by clathrin-coated cavities. Together, the authors concluded that the caveolae-mediated internalization was highly conducive owing to the increase of particle diameter. However, the destination of particles with a diameter greater than 500 nm was not unconcealed to the lysosomes.

Photochemical Disruption of the Endosomal/Lysosomal Membrane

In regard to the utilization of nucleosomes and lysosomes, the photochemical internalization (PCI) approach is reckoned as a localized hydrophilic process. Notably, the photosensitizer-mediated chemiluminescence reaction happens to be favorable upon its stimulation, resulting in the generation of ROS that significantly destroys the membrane structure. Moreover, it leads to the evacuation of nanoparticles from the lysosomes or other membrane-bound organelles such as the nucleus.¹¹² Upon conducting the experiments on the xenotransplantation models of over 80 different cells and 10 different tumor cells, Selbo et al¹¹³ demonstrated these implications of PCI for the first time that the nanocarriers could escape and release the therapeutic guests into the cytoplasm.^{114,115} Further advancements on the PCI approach resulted in driving them to Phase I/II clinical trials in 2010.¹¹³ Irrespective of the mechanism, either active or passive mode of disruption, the integrity of the endosomal/lysosomal membrane is either partially or completely destroyed due to the deadly ROS. In one of the recent studies, it was observed that the disruption of lysosomal membrane often led to the release of cathepsins through a process of lysosome membrane permeabilization (LMP) pathway due to the toxic ROS, resulting in the activation of endogenous apoptotic stimuli.¹¹⁶ These consequences could be more evident in cancer cells due to the higher availability of cathepsins compared to the normal cells.

Correlating to these aforementioned facts, we recently demonstrated that the 2D layered materials, LDHs with excess hydroxyl groups on their surfaces, could also induce the enrichment of cathepsins release, which might have initiated the cell death through activating apoptotic cascades.⁶⁸ In another case, See et al demonstrated that the CALNN-capped gold nanoparticles were digested by the protease cathepsin L, and could be used for biosensing application.⁷⁸ Similar to the fusion-like escape, the particle diameter also significantly influences the PCI-based approach. However, contrarily, the interference of the nanoparticles with the lysosomal membrane is inversely correlated to the particle diameter, indicating that the impact of diameter (>500 nm) is insignificant.¹¹¹ Regardless of their mechanism of lysosomal escape, it also results in evacuating the nanoparticles from lysosome and substantial disruption of lysosomal membrane and ablation of the cancer cell. In addition, specific mechanical abrasion modes such as alternating magnetic fields can also be applied for improving the LMP. Owing to their non-invasive and excellent controllability attributes, this strategy can effectively break down the lysosomal membrane, which not only drives the nanoparticles but also augment the release of lysosomal enzymatic cascades and substantial lysis of the organelle.¹¹⁷ Further, the improved accumulation of nanoparticles through various lysosomal targeted systems facilitating the lysosomal disruption mechanisms enhance their delivery efficiency and substantial antitumor efficacy.

Lysosomal Cell Death Mechanisms

In addition to recycling cellular constituents through the degradation of intracellular and internalized foreign bodies for maintaining homeostasis, lysosomes act as crucial sites in initiating and accomplishing various cell death pathways such as apoptosis and autophagy or necrosis.^{118,119} Amongst various cell death mechanisms, autophagy is the lysosome-dependent self-destructing approach, which often degrades or recycles biomacromolecules and undesired organelles.¹²⁰ Lysosomal cell death pathways are often rapidly executed while altering the LMP-mediated pathways and substantial discharging of their hydrolytic contents into the cytoplasm.^{73,121} LMP can be significantly induced in lysosomes through various mechanisms, involving ROS generation, and utilization of lysosomotropic agents, and some cell-specific mortality effectors, such as Bcl-2 family proteins and microtubule toxins.¹²² In this framework, inhibition of various enzymes can also induce the stimulation of LMP in the lysosomal lumen. Contrary to the normal cells, lysosomes possess significantly larger

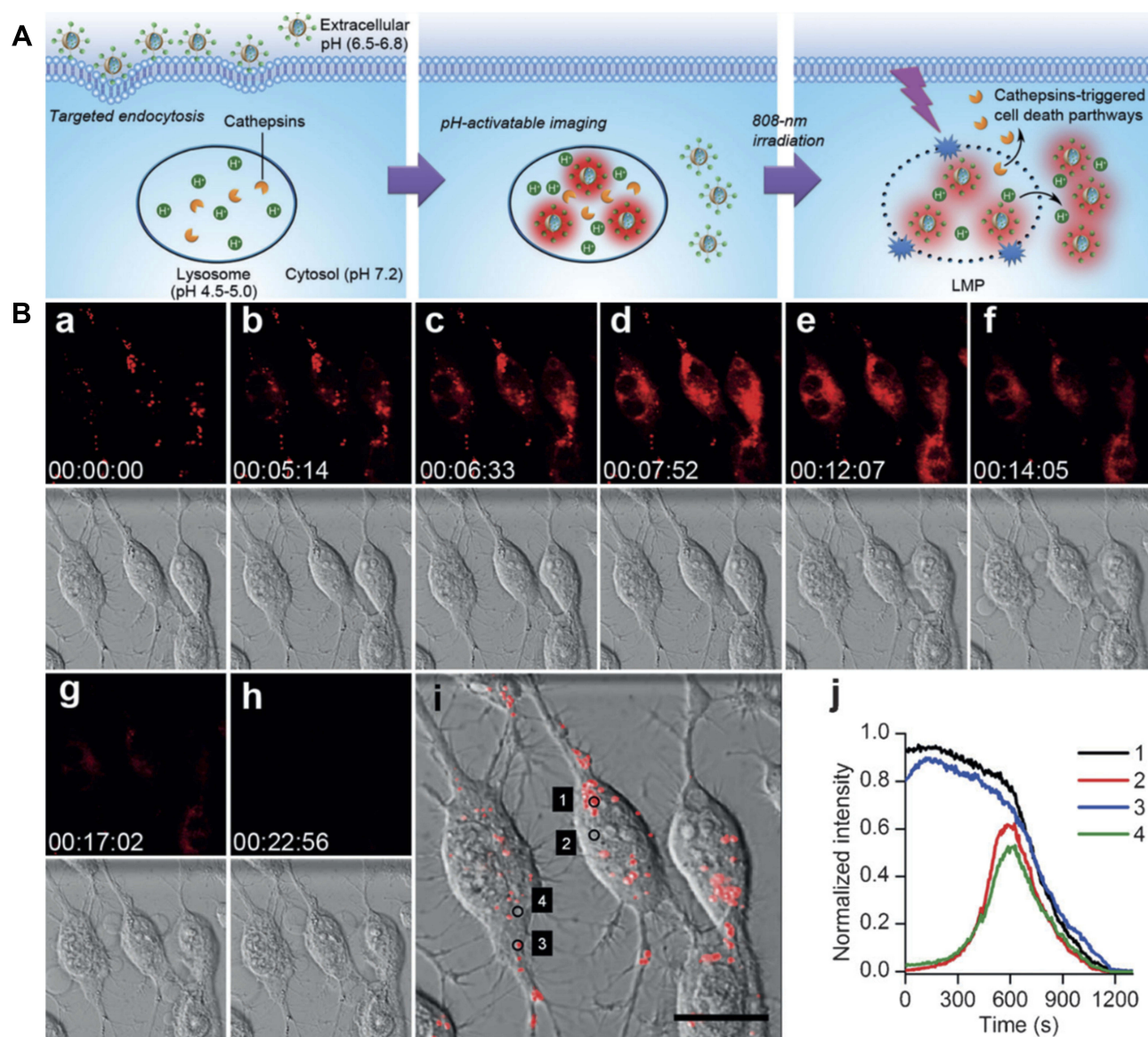


Figure 5 (A) Lysosome-aimed strategy for simultaneously targeted delivery, lysosomal imaging and destruction, and real-time self-feedback of therapeutic efficacy. Real-time monitoring of fluorescence and morphology during PDT. (B) a–h) Real-time fluorescence images at λ_{Exc}/Em of 633/660–720 nm (top) and bright-field images (bottom) of APT-TNP-loaded MDA-MB-231 cells under 808 nm irradiation at 100 mW cm^{-2} . i) Merged fluorescence and bright field image at the starting time of PDT. Scale bar, 20 μm . j) Time course of fluorescence intensity collected at dots 1–4 shown in (i), corresponding to the lysosomes (1, 3) and cytosol (2, 4). Tian J, Ding L, Ju H, et al. A multifunctional nanomicelle for real-time targeted imaging and precise near-infrared cancer therapy. *Angew Chem Int Ed*. 2014;53(36):9544–9549. With permission from Copyright 2014 John Wiley and Sons.⁶³

volumes of proteases in the tumor cells. For instance, acid sphingomyelinases (ASM) that catalyze the hydrolysis of sphingomyelin can be inhibited by utilizing the cationic amphiphilic drugs (CADs) by interacting with the lysosomal membrane, accompanying the integrity of lysosomal membrane to stimulate LMP (Figure 5). Contrarily, the release of cathepsins into the extracellular space that can engage in the degradation of basement membranes and exterior matrix sequentially promotes the development of the tumors.^{123–126}

Apart from these tumor-accelerating functions, the cathepsins also show a strong ability toward suppressing the growth of the tumor, which could be potential targets in ablating tumors.^{63,127,128} Specific consequences of lysosomal escape that can alter LMP or destruct the lysosomal membrane lead to the release of cathepsins significantly. Further, the destruction of the lysosomal membrane leads to the discharge of hydrolytic enzymes, initiating the unselective damage to the cell constituents, and further triggers the process of necrosis through cytosolic acidification.¹²²

Kashkar et al demonstrated that the UV irradiation-triggered ASM activation played a crucial role in inhibiting tumor metastasis and an essential prerequisite for Bax conformational change.¹²⁹ Contrarily, in the ASM-deficient cells, the discharge of enzymes was highly concentration-dependent, which in turn activated the caspase-independent necrosis. It was also observed that the matured lysosomes were more anticipated to be induced by activated LMP, while the lysosomes in the close proximity to the mitochondria suffered from slight membrane injury.¹²² In another case, Xue et al demonstrated that the cells with antiapoptotic oncoproteins (26-kDa Bcl-2 and 24- to 33-kDa Bcl-xL protein) loss due to the light-induced damage could be more sensitive to apoptosis.¹³⁰ Although there have been some pieces of evidence on the potential of nanoparticles in overcoming MDR, it was highly anticipated that this light-induced lysosomal death pathway could be one of the targets of action for exploring the therapeutic strategies in tumor ablation, especially in PDT.^{63,131,132} However, in-depth analyses on developing specific markers for tracing the cathepsin moieties are required to explore their release into cytoplasm. Despite their promising effects involved in cancer therapy, lysosomes have not received much attention compared to other organelles such as mitochondria and nucleus. Further efforts are required to explore the mechanistic insights involved in the endosomal or lysosomal-targeted systems.

Mitochondria

Mitochondrion, referred to as the powerhouses of the cell, is a bilayered film-coated semi-autonomous organelle, reserving its own genetic material,^{133–135} including pivotal oxidative phosphorylation (OXPHOS) genes.^{136,137} This semi-autonomous organelle significantly participates in the differentiation, signaling, and apoptotic events of cells. Notably, the outer membrane, with ultra-small Poron enclosing the entire organelle, contains a wide variety of enzymes engaged in diverse physiological activities. Cytochrome C, one of such electron transport proteins situated at the intermembrane localization, is involved in the bio-oxidation process. The inner space of mitochondrion contains an extensive impermeable membrane under the contribution of numerous cristae. Over the decades, several advancements have been made and realized that numerous essential functions involving the cell regulation systems take place in the mitochondria including the generation of energy (primary source of ATP synthesis), regulation of redox reactions and resultant ROS generation, immunity regulation, integration of reticulum, transduction of signals, differentiation of cells, balance of intracellular calcium

ions, and the biological precondition of the synthesis of partial cell plasma, e.g., acetyl coenzyme A and pyridine, to adjust the metabolism and control the cell cycle.¹³⁸ In addition to these normal physiological and biochemical activities, mitochondria are also involved in some specific cell death mechanisms such as apoptosis and antiviral responses of the cells relevant to diseases, such as chronic lung infections.^{139–141} These critical biological effects of this organelle offer enormous potential towards effective cancer treatment by precisely targeting the mitochondria over conventionally disposing the drugs in the cytoplasm.

It is increasingly recognized that apart from the ability of endoplasmic reticulum in regulating apoptosis independently, and many acquaintances need to be coupled with the other organelles. However, the most crucial organelle involved among them is mitochondria, from which BH3 (Bcl-2 homology domain protein) is the only activated protein domain that induces the apoptosis by stimulating the pro-apoptotic effector protein, such as BAX or BAK, after which the triggered oligomerization leads to the mitochondrial outer membrane permeabilization (MOMP).¹⁴² It should be noted that the mitochondrial permeability transition (MPT) is the crucial factor in initiating the apoptotic pathway under the circumstances of oxidative stress with the loss of potential and impairment of the membrane integrity.¹⁴³ The mitochondrial membrane permeability transition pore (mtPTP), known as the polyprotein transmembrane channel permitting the transit of <1.5-kDa solutes,¹⁴⁴ is often regulated by specific conduits, cyclophilin (Cyp) D, voltage-dependent anion channel (VDAC), as well as adenine nucleotide translocator (ANT). Responding to the oxidative stress, mtPTP is activated by the change of membrane osmotic pressure or opened by p53, a central stress sensor (Figure 6).¹⁴⁵ Further, the apoptosis protein signal is excited by the cytochrome C release into the cytosol, in which the apoptosome is formed by the incorporation between cytochrome C and APRF-1, activating downstream cysteine proteases, for example, caspase-3, eventually resulting in the disassembly of cell cluster leading to phagocytosis.^{64,142,146} Consequently, the release of cytochrome C also induces mitochondrial DNA damage, resulting in cell necrosis.⁵⁸ Moreover, the mitochondria in cells show an enormous impact on diverse life activities, especially the occurrence and development of tumors. In the late 1950s, Warburg demonstrated that the cancer cells, even in the aerobic environment, could obtain energy and produce excess lactic acid through low productivity and

Ischemia, oxidative stress

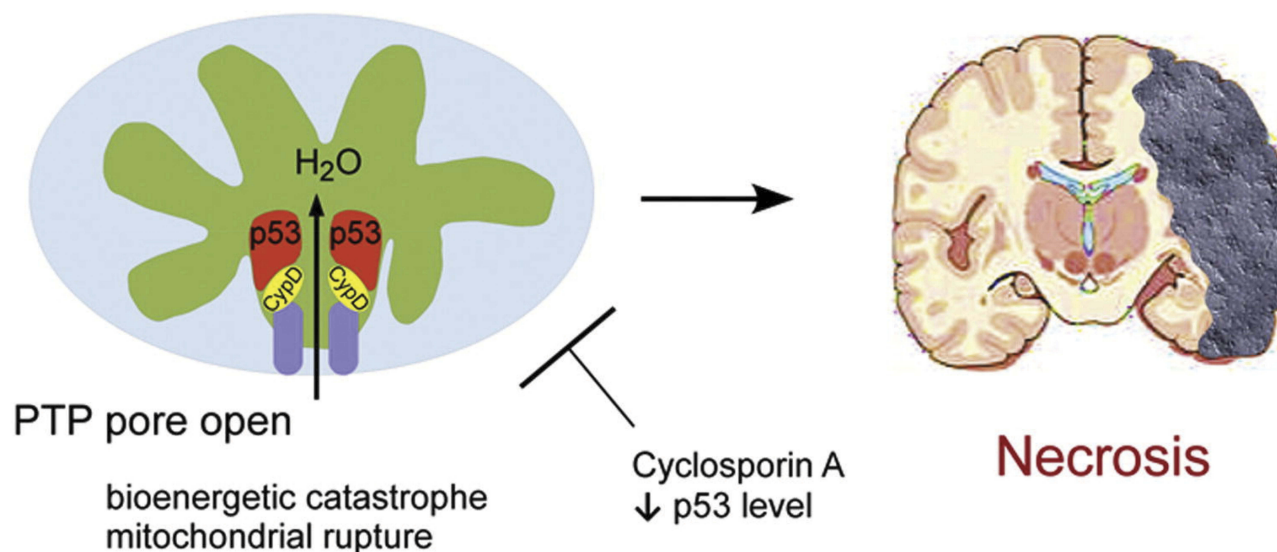


Figure 6 Schematic illustrating the p53 accumulation in the mitochondrial matrix and triggering the opening of mtPTP and substantial necrosis by the physical interaction with the PTP regulator Cyp-D. Reprinted from *Cell*, 149(7), Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM, p53 opens the mitochondrial permeability transition pore to trigger necrosis, 1536–1548, Copyright 2012, with permission from Elsevier.¹⁴⁵

oxygen-free glycolysis pathway called “aerobic glycolysis or Warburg effect”. Meanwhile, Warburg and coworkers also demonstrated that the metabolic changes in the mitochondria are one of the predominant sources for the origin of cancer.¹⁴⁷ In this context, the strategy of using nanoparticles toward targeting at mitochondria in stimulating apoptotic events toward its ablation has been recognized as the momentous finding in cancer therapy.

Targeting Strategies

Based on the above-mentioned facts and considerations relevant to the initiation of apoptosis, several efforts have been dedicated to the advancements of nanoparticles-based therapeutic designs by choosing mitochondria as a potential target toward inhibiting tumor metastasis. In this framework, one of the potential approaches includes the targeting of membrane minimal constituents, including ANT, VDAC, and Cyp-D, that are associated with the entry of nanoparticles into mitochondria. Various other promising strategies include the conveyance of antioxidants, activation of MPT, reduction of ROS, up-regulated expression of pro-apoptotic genes.^{52,93,94,148} However, the targeting approaches utilized for directing various nanoparticles intracellularly, and factors influencing their internalization are predominantly discussed in this section.

Targeting Minimal Particle

In general, the particles with a diameter lesser than 500 nm and molecules larger than 40 KDa could be feasibly accumulated in the tumor microenvironment and limiting their penetration into healthy cells due to the EPR effect. Although numerous innovative supramolecular designs for the delivery of therapeutic guest molecules are available at arbitrary size ranges, it is highly challenging to internalize such composites into the mitochondria.¹⁴⁸ Recently, it was concluded that only a few of tiny metal nanoparticles (gold,⁵³ silver,¹⁴⁹ and some certain metal oxides as well as quantum dots⁵² whose size smaller than 10 nm) could permeate through the mitochondrial membrane into the matrix through the VDAC, while the nanoparticles at a larger size were being obstructed.¹⁵⁰ In an attempt to demonstrate this fact, Salnikov and coworkers⁵³ utilized calibrated 3-nm-sized gold nanoparticles to stride cardiac mitochondria through VDAC pores ranging from 3 to 6 nm, substantiating the breach caused by osmotic swelling. Meanwhile, these particles could affect the transport of respiratory chain and mitochondrial permeability, and their internalization was accompanied by the synergy between permeability alteration and mitochondria expansion, enhancing the mitochondria-targeted ability significantly. Although the entry of particles is restricted due to the size limitations, it is feasible to deliver the therapeutic

cargo at the proximity of mitochondria using the large-sized constructs. Further, these designs could be reprogrammed by attaching several mitochondrial membrane targeting ligands, such that they could be well retained in the proximity of mitochondria, successfully enabling the delivery of drugs.

Modification of Polypeptides

Mitochondria possesses a very precise membrane along with the internal structure, which provides a basis for ascertaining specific sites of some polypeptide sequences.¹⁵¹ Along this line, Ma and coworkers¹⁵² constructed a gold core (20 nm) stabilized with a layer of biotinylated CALNN (Cys-Ala-Leu-Asn-Asn)-based peptide, which was further immobilized with tetrameric streptavidin for effective functionalization of biotinylated molecules, (i.e., a cytotoxic peptide, KLA: (KLAKLAK)₂) toward the specific identification of mitochondria. In another case, Yamada and Harashima^{153,154} synthesized a lipid derivative immobilized with mitochondrial targeting signal peptide, which was then used to fabricate peptide-modified liposomes. They confirmed that these innovative vesicles efficiently delivered therapeutic cargo via targeting endogenous mitochondrial proteins. Although there exist some strategies for targeting the membrane proteins of mitochondria for efficient therapy, it should be noted that the surface charge of the mitochondrial membrane significantly influences the convenient delivery of therapeutic cargo. Lemeshko and coworkers¹⁵⁵ demonstrated that the cationic fluorescent probe safranin O prominently reduced the swelling percentage of mitochondria initiated by BTM-RP1 or by tryptophan derivatives, indicating that the superficial electrical charge has shown a critical impact on the membrane permeabilization and selectivity via cationic peptides. These consequences indicate that the cytotoxicity and selectivity of the appropriate peptide moieties can rely on the agents that are specifically responsible for the superficial charge modulation.

Delocalization of Lipophilic Cations

Indeed, the superficial charge of the mitochondrial membrane specifically controls the circulation of nanoparticles in the cytosol during drug delivery. Accordingly, it is highly feasible to achieve mitochondrial targeting by lipophilic architectures with delocalized cations.¹⁴⁸ Owing to the high-density phospholipids yielding high membrane potential (constant negative charge), mitochondria are

highly selective for the entry of some of the common lipophilic structures, including TPP,^{93,156} rhodamine 123 (Rh123),^{157,158} flupirtine,¹⁵⁹ and MKT-077,^{160–162} which could be selectively retained in the mitochondria. Amongst them, TPP holds a better balance of lipophilicity and charge with a large area of three benzene moieties in its chemical structure, which can be efficiently used for mitochondrial targeting of nanoparticles by immobilizing it over various inorganic nanoparticles⁹⁴ such as cerium and ferromagnetic particles or polymer, drug-polymer block system.^{93,156} In this vein, Biswas and coworkers¹⁶³ fabricated a mitochondrial targeting design by conjugating the dye Rh123 to the amphiphilic PEG-phosphatidylethanolamine (PEG-PE) for paclitaxel (PTX) delivery. They demonstrated that the targeted design resulted in larger amounts of dye in the mitochondria and exhibited better efficacy compared to those of non-targeted ones.

Intrinsic Generation of ROS

In recent times, there has been a growing interest of researchers towards the utilization of ROS science for cancer ablation as most of the drugs and trace amounts of metal substrates participate catalytically in the molecular pathways, resulting in the deadly free radical species through Fenton- and Fenton-like chemistries. Broadly speaking, ROS, highly oxidizable, and metabolically active species, are categorized into two major divisions of oxidants, ie, non-free radicals, and free radicals. Various non-free radicals include hydrogen peroxide (H₂O₂) and single state oxygen (¹O₂), which could be catalytically converted to uncharged highly reactive short-lived, free radical molecules having an unpaired valence electron mediated by superoxide dismutase pathway, such as superoxide anion radical (O₂⁻), and hydroxyl radical (·OH), among others. In addition to these conventional types of ROS, various other types of free radicals include nitric oxide (NO·), lipid peroxide (LOOH), alkoxy free radical (RO·), peroxy radical (·OOH), and metal-oxygen complexes, among others, which can gather to complete the redox reaction in the body and participate in various metabolic processes of normal cells. In 1961, Jensen¹⁶⁴ observed that partial oxygen was involved in the mitochondria redox reaction of NADH or succinic acid, which then resulted in H₂O₂, indicating the generation of ROS. Subsequently, they extended their studies on mitochondria and the generation of various types of ROS and established various complex relationships between them.

However, it should be noted that the formation and subsequent release of ROS are closely related to mitochondrial complex II, mitochondrial complex I, and mitochondrial complex III, which can regulate the mitochondrial oxidative respiration (electron transport) chain. Simultaneously, the redox state of NAD(P)H/NAD(P)⁺, NADPH oxidase, monoamine oxidase (MAO), p66, and α -glycerophosphate dehydrogenase also play crucial roles in the formation of ROS in mitochondria.¹⁶⁵ Xing and coworkers,¹⁶⁶ designed an ROS-responsive nanoparticle system for the delivery of a cellular respiration inhibitor, camptothecin (CPT). The CPT species significantly induced the mitochondrial ROS (mtROS) upregulation, therefore accomplishing successive self-circulation of CPT discharge and mtROS burst, endowing the long-term high oxidative stress and substantial apoptosis of cancer cells.

In addition, some of the chemotherapeutic drugs (for example, Dox), and trace metal species (copper and iron), and combination of both catalyzing the non-free radical oxidant species in the normal aerobic environment generate free radicals efficiently. These chemotherapeutic drugs delivered from the nanoparticles ultimately augment the apparent intracellular levels of non-free radical oxidants, H₂O₂, through a series of events: i) Reductive metabolic activation; ii) Conversion to its semiquinone derivative; iii) Transformation to superoxide through the one-electron reduction; and iv) Dismutation reaction catalyzed by the superoxide dismutase (SOD) enzyme, resulting in the eventual deadly hydroxyl ions.^{7,90,91} Similarly, the trace amounts of first-row transitional metal ions, such as iron and copper, intrinsically participate in the catalysis of H₂O₂, resulting in the formation of deadly free radicals through Fenton- and Fenton-like reactions, respectively.⁴³ Due to their apparent availability, these H₂O₂ species can be used as effective targets for the ablation of tumors through ROS science. However, specific limitations of ROS, such as poor stability and aggregation over the surface of the organelles and specificity, are remained to be addressed. Furthermore, ROS produced by mitochondria, in return, also contributes toward the enhancement of LMP. The consequence of the 15 min contact with H₂O₂ manifested that the lysosomal rupture can almost instantly even if restricted, results in the significant discharge of lysosomal constituents, leading to the cell damage. Meanwhile, during oxidative stress, phospholipase A2 can be activated to undermine the stability of phospholipid

organelle membranes, causing lysosomal fracture laterally.^{167–169}

Effects of Internalized Nanoparticles in Mitochondria

Indeed, the actively-targeted nanoparticles using specific ligands that with high affinity to the membrane can efficiently infiltrate mitochondria and exhibit specifically defined tasks of a delivery system toward cancer ablation effects. However, the structural damage of the mitochondria is often affected by the particle's physicochemical properties and externally applied triggers (light-induced hyperthermia and ultrasound) that significantly influence the inner microenvironment of mitochondria. Zhang et al¹⁷⁰ fabricated a near-infrared (NIR) fluorescence/photothermal double response probe based on the methylene blue, which could enter the mitochondria of cancer cells. Moreover, the weak alkaline microenvironment maximized the photothermal efficacy of the probe. Subsequent changes in the permeability of mitochondria membrane and enhanced light-induced hyperthermia-induced the apoptosis/necrosis of cancer cells. In another study, Lv et al¹⁷¹ demonstrated the effects of low-intensity (0.6–0.8 W/cm²) ultrasound dynamic therapy on the human oral squamous carcinoma cell line using 5-aminolevulinic acid (ALA). The significant rise of ROS levels in the treatment groups resulted in the morphological changes of swollen mitochondria, indicating the inception of mitochondrial signaling pathways toward the ablation of cells. Similarly, indocyanine green (ICG), as a fluorescent agent, is one of the United States Food and Drug Administration (US-FDA)-approved NIR dyes possess both photothermal and photodynamic effects, which are effective after when the nanoparticles carrying the dye being phagocytosed by the cells.⁹² Although the advanced strategies, such as ultrasound- and light-based therapies have resulted in significant progress, there existed specific problems associated with such therapies of low penetration depth and limited localization of irradiated light, which yet remained to be unresolved.¹⁷²

Disruption of Homeostasis

Engulfment of certain foreign bodies, for instance, nanoparticles carrying chemotherapeutic agents, and their resultant deadly ROS stimulates the cytochrome C release from mitochondria, which sequentially activates the downregulation of Caspase-3/9 precursor, and initiates the mitochondrial permeability transition pores, resulting in the membrane permeability to exchange the

mitochondrial and cytoplasmic constituents. These consequences eventually result in the organelle edema and cause substantial damage. For instance, in response to the internalization of nanoparticles into the cells, some of the organelles such as endoplasmic reticulum (ER), respond to defend such foreign bodies and release large amounts of calcium ions into the cytoplasm in response to the stress. These large amounts of calcium ions are taken up due to the membrane potential gradient, and calcium overload in the mitochondria can be recognized as an apoptosis factor. Moreover, the nanoparticles can also trigger the change of membrane potential, the downtrend of matrix pH, and the formation of cereal cystine peptide, which lead to the initiation of exogenous mitochondrial death pathway, inducing the elevation of apoptotic events in the cell.¹⁷³ In addition, the translocated cytochrome C from the mitochondria to the endoplasmic reticulum interacts with the 3-phosphoinositide receptor, forming positive feedback to apoptosis. In an attempt to demonstrate these issues, Fang and coworkers¹⁷⁴ demonstrated that the natural cyclopeptide RA-V inhibited the coaction of 3-phosphoinositide-dependent protein kinase 1 and AKT, due to the loss of membrane potential, the liberty of cytochrome C and the excitation of the caspase cascade.

ROS Generation

Apoptotic stimulators, such as TNF- α and lipopolysaccharide, are capable of inducing the generation of ROS in mitochondria, which can significantly change the arrangement of mitochondrial membranes, instead of lysosomal membranes. Indeed, the lysosomal enzymes promote the productivity of H₂O₂ in mitochondria, which strengthens the cytoplasmic acidification and then initiates Bax mitochondrial translocation, involving the recruitment of cellular apoptosis.¹⁷⁵ Previous reports indicated that the Ferroptosis, related to the ROS gathering and glutathione depletion, resulted in the shrinkage of mitochondria, increase of membrane density, disappearance of the mitochondrial ridge, and eventual breaching of the mitochondrial outer membrane. Numerous such reports based on the utilization of specific metal ions released from the nanoparticles, such as copper, iron, and manganese, demonstrated that these ions generally induced the Fenton and Fenton-like reactions, which showed enormous potential in the cancer therapy based on Ferroptosis.^{43,44} In addition, the mitochondrial DNA is responsible for the structural protein-encoding of the crucial electron transfer chain and 2 ATP synthase subunits, such that the nanoparticles

may also cause disturbances or mutation of mitochondrial DNA, its associated electronic transmission, and oxidative phosphorylation. These consequences further enhance the ROS levels, which contribute to the endogenous apoptosis. Finally, the interactions between the internal and external factors form a vicious circle, causing the apoptotic cell death.^{176,177}

Nucleus

As the characteristic feature of eukaryotic cells, the nucleus is the central organelle, predominantly containing most of the cell's genetic material, which plays a significant role in specific functions such as cell growth, proliferation, differentiation, and apoptosis. Notably, the nucleus is considered as the control center of cells as it regulates various cell activities through managing the gene expressions. Once the nuclear DNA is damaged, both endogenous and exogenous conditions may cause severe diseases to cells and tissues. For instance, during autophagy, the transcription factor EB that regulating the lysosomal expression network is translocated to the nucleus to trigger this protective mechanism.

Typically, the genetic content of the nucleus is enclosed in a bilayer phospholipid membrane made of different protein compositions, including the speckle domains, the early promyelocytic nucleosome (PMLNB), and Cajal bodies, among others.^{178,179} The subcellular localization of the genetic material, chromosomes, and nucleosomes in the nucleus is not random, and the specific loci or chromosomes are in a specific region, known as the chromosome territories (CTs).¹⁸⁰ These proteins act as objective receptors for various diseases (proteins, nuclear receptors, and DNA) in the nucleus, which can provide great potential toward targeting the nanoparticles to the nucleus. Thus, tremendous progress in the past few decades has been evidenced by the development of various targeting strategies in guiding the nanoparticles toward the nucleus, specifically gene-based therapies. These strategies often favored the conveyance and delivery of therapeutic cargo in the nucleus.^{51,181,182} In general, the internalization of nanoparticles toward the nucleus is facilitated by two predominant pathways. One of these practical ways includes the interaction of the composite structure of phospholipids or similar particles with the nuclear membrane, facilitating the fusion of the outer layer of phospholipids with the nuclear membrane, which consequently enables the intervention through the membranous barriers, and release of the internalized nanoparticles into the

nucleus.¹⁸³ To substantially meet the requirements of membranous fusion and nuclear uptake, the nanosystem is usually modified with specific ligands that can activate nuclear receptors and enable their internalization.¹⁸⁴ In an attempt to address this issue, Qiu and coworkers¹⁸² developed a codelivery system based on drug-silver-gold nanorods, in which one of the ends carried a specific aptamer for specifically internalizing these composites into the nucleus, achieving the responsive aggregation-induced cell death. Another way of nuclear internalization includes the passing of nanoconstructs through the nuclear pores or occupy nucleoplasm that for transferring nucleoprotein. However, the extremely small-sized functional diameter of the nanoparticles (9–40 nm)¹⁸² limit their nuclear diffusion. In the beginning, it was motivated to utilize nuclear-targeted nanoparticles to carry DNA, mediated by unique endocytic pathway and fossa protein, facilitating the bypass the acid environment in the lysosome and reaching the nucleus directly. Nevertheless, over the decades, there has been increasing interest in the fabrication of various advanced strategies toward targeting the nucleus and are explicitly discussed hereunder.

Active Targeting Using Peptides

The substantial entry of internalized nanoparticles into the nucleus through the nuclear membrane predominantly depends on the average size of nanoparticles and the surface-modified ligands, such as protein and peptides. However, the peptide sequences immobilized over the nanoparticles significantly instruct their entry as well as fate intracellularly. Paunesku et al modified the TiO₂-DNA nanoparticles on the surface of isopropyl dehydrated glycerol and grafted with 1–5 dopamine-modified DNA molecules. This nano-sized particulate system specifically identified the mitochondrial DNA sequence of NADH dehydrogenase II (ND2) and the ubiquitous R18S ribose in mammals. The body RNA gene-matched peptide molecule not only possessed nuclear targeting ability but also resulted in the mitochondrial targeting effect.¹⁸⁵ Among all the peptides, nuclear localization sequence (NLS) has been widely applied for targeting the nucleus. In fact, the conventional nuclear import pathway is predominantly facilitated by importin α and β . Amongst them, the importin- α binds to the importin β through NLS utilizing its NLS binding domain. The NLS and the intracellular factor interact with each other, resulting in the formation of a stable complex and impede at the nuclear pore complex. Further, the nanoparticles are then diffused into the

nucleus through an active transport process once the sequence is recognized by the particular protein receptor. The nuclear input pathway results in the entry of nanoparticles to the nucleus. For instance, the NLS/RGD-modified gold nanolet¹⁸⁶ with silver nanoparticles,¹⁸⁷ in which the number of nuclear location sequences is not proportional to the difficulty of nanoparticle entering the nucleus. In another case, the Ebola virus VP24 protein has bounded the untypical NLS binding site of karyopherin alpha 5 (KPNA5) with high affinity, which interrupted the transport of tyrosine-phosphorylated STAT1 and maintained the delivery of other cargos.¹⁸⁸ Salma and coworkers⁵¹ fabricated chitosan nanoparticles in the diameter range of 25 and 150 nm and subsequently modified them with the NLS peptide of multiple density gradient. The experimental results showed that the smaller-sized chitosan nanoparticles could be located in the nucleus in a more significant amount without the nucleation sequence, and the results were contradictory to the intuition for the nuclear location sequence. Moreover, fewer NLS modified nanoparticles have more nuclear localization, and similar results were shown in their further studies.¹⁸⁹ In another instance, Sahin and coworkers⁶⁹ fabricated a nuclear-targeted complex system by immobilizing a 17 amino acid oligopeptide NLS (DRQIKIWFQNRRMKWKK) to DOX-loaded nanoparticles. These nuclear-targeted systems resulted in the substantial localization merely at the nuclear membrane and ablated cancer cells compared to the free DOX molecules, whose internalization was predominantly based in the cytoplasm.

Amongst these peptides contributed by simple modification, TAT (YGRKKRRQRRR) is one of the most widely used NLS peptides. Such NLS peptide, in combination with highly basic functional groups, is predominantly used for nuclear targeting of various organic or inorganic nanoparticles. Several examples include DOX@MSN@TAT/RGD layer structure from Pan et al,¹⁹⁰ TAT/PEG-Mal-grafted iron oxide nanoparticles from Peng et al,¹⁹¹ and TAT-Cerium doping system from Yu and coworkers.¹⁹² Notably, all these studies were proposed using TAT to change the nuclear properties and promote the nuclear penetration of nano-sized particles. In most of the instances, these nucleus-targeted systems efficiently delivered the therapeutic cargo in the proximity of the nucleus for their enhanced theranostic efficacy.¹⁹¹ In another case, Wang and colleagues¹⁹³ fabricated the peptide-modified magnetic nanoparticles, which significantly resulted in synergetic targeting of nuclei and mitochondria. However, it was

evident that the NLS peptide-modified nanoparticles only sieged the nucleus instead of the nuclear internalization. To this end, various specific chemical groups can also be used, which precisely exhibit affinity toward the nucleus. For instance, Jana et al¹⁹⁴ synthesized acridine-benzbutyrate nitrogen mustard nanoparticles, which combined the DNA with the DNA alkylation group to target at nucleus by the acridine molecule.

Effects of Internalized Nanoparticles in the Nucleus

The internalized nanoparticles in the nucleus predominantly interact with the intranuclear constituents, resulting in different types of mutations and severe DNA damage, the block of DNA cycle, and the activation of the apoptosis signaling pathway.¹⁹⁵ In the early 1970s, scientists began to explore the predominant reasons for human cancer, and increasingly recognized that there exists a close relationship between the genetic changes and dysfunction of cancer.^{196–198} Since then, it has been well acknowledged that mutation, whether a gene, a sequence of genes, or even the whole chromosomal transformations, have shown a powerful impact on the origination as well as differentiation of various cancers.^{199–201} It should be noted that the some of the inorganic nanoparticles cause nuclear DNA damage through delivering various therapeutic molecules such as drugs, protein and nucleic acids (DNA and RNA) among others, which can directly interact with DNA by intercalation, leading to the inhibition of replication, transcription and translation-assisted macromolecular biosynthesis. Although the DNA damage in cells can trigger the self-repair mechanism, the damaged large area of DNA subsequently hinders the cell cycle, resulting in the prolonged G1 and G2 phases, and substantial loss of ability to repair by itself prior to next synthesis by mitosis. Henceforth, the apoptotic signal transduction pathway gets activated, driving to eventual cell death.^{202,203}

In addition to diverse therapeutic molecules that precisely interact with the nuclear constituents resulting in cell death, various nanoparticles participate in cell death through various mechanisms similar to their interaction with mitochondria, inducing the generation of enormous levels of ROS. In addition, the enhanced levels of intracellular ROS affect not only various membrane-covered organelles, such as mitochondria and lysosomes, but also show a significant impact on the nuclei. In this context, the ROS scavenger may also substantially purge the nuclear translocation, leading to the activation of an autophagy

mechanism.²⁰⁴ Various inducers in the nucleus can elevate the activation of NADPH oxidases and the generation of NADPH oxidases-derived ROS, triggering the nuclear translocation,²⁰⁵ reducing the ratio between Bcl-2 and Bax afterward, as well as stimulating caspase-3/-9 in mitochondrial approach. Currently, the generation of oxidative lesions caused by stress in the nucleus is widely studied, using specific biomarkers of oxidative stress, for example, 8-hydroxy-2'-deoxyguanosine (8-OHdG). The utilization of this 8-OHdG biomarker is widely considered due to hydroxyl radical and eneyl ring addition, which can be predominantly formed during lipid peroxidation, associated with the formation of mutated electrophilic reagents, such as malondialdehyde, and 4-hydroxyl nonylaldehyde, among others.²⁰⁶ In addition, this biomarker not only acts as a mere biomarker for oxidative stress but also a remedy for oxidative stress-induced gastrointestinal diseases. The oxidative attack on DNA is a predominant reason for DNA damage induced by many nanoparticles and the intracellular toxicity. On nearly all mutations or tumor suppressor genes, the changes, such as deletion or addition of base pairs caused by DNA oxidative damage could be observed.²⁰⁷ In addition, the DNA skeleton (deoxyribonucleic acid chain) under oxidative attack, or DNA in the process of oxidative damage repair, can also detect the single strand damage of DNA in the cell.

ER and Golgi Apparatus

ER is a eukaryotic organelle with cisternae membranous structure that held by the cytoskeleton intracellularly. However, the organelle ER manages its adaptation to the unique tumor microenvironment, depending on the number of folds of transmembrane and secretory proteins, and diverse functionalities such as biosynthesis of lipids, detoxification of drug molecules, and the storage of calcium ions, among others.²⁰⁸ The rough ER that with the embedded ribosomes on its surface participates in the synthesis of various lysosomal enzymes. Further, the transport vesicles carrying lipids and proteins from rough ER deviate such functional substituents to the Golgi apparatus from the smooth ER. For weakly alkaline nature of ER, the acidic feature of lysosomes has been extensively applied for stimulus-triggered cancer therapy. Notably, ER stress has been acknowledged as a crucial pathway toward cancer treatment. Several biocompatible metal complexes can be used to target ER stress for highly efficient cancer therapy.²⁰⁹

To this end, the Golgi apparatus is one of the vital organelles that organizes, processes, and packages various proteins that are produced in the organelle ER. This apparatus is generally consisted of three different compartments such as the cis-Golgi network (CGN), the Golgi stack of cisternae (cis-Golgi, medial-Golgi, and trans-Golgi), and the trans-Golgi network (TGN).²¹⁰ Interesting feature of Golgi complex is that these compartments possess a pH-gradient across the three compartments from CGN (pH 6.7) to TGN (pH 6.0).²¹⁰ Golgi apparatus receives the proteins-contained vesicles synthesized in ER and secretes these cargos through exocytosis, for instance, ANQ-IMC-6 probe.²¹⁰ After combined with specific cyclooxygenase-2 (COX-2) in the Golgi apparatus, the probe performed intense fluorescence by pacifying the photo-induced electron transfer.

More often, the variations in the glycosylation pathway of tumor cells ascend in the significant effects in pH homeostasis as well as function of the organelle, which subsequently leads to the disorganization of the organelle structure and scattering of hypertrophic elements in Golgi apparatus.²¹¹ Henceforth, these consequences could be used as an objective for pH-responsive therapeutic delivery of drugs and imaging probes. In a case, Xue and coworkers²⁵ fabricated a Golgi apparatus-activated pH-triggered asymmetric cyanine dye (pH-PTT in Figure 7), which could be sensitive in the specific pH range (6.2–7.5). In such acidic environment, the pH-PTT dye was transformed into a superior conjugated structure with strong absorption efficiency at 808 nm, which could able to harvest heat energy. Furthermore, this pH-PTT dye could assemble with bovine serum albumin (BSA), resulting in the BSA-pH-PTT nanoparticles, which could favorably accumulate in the Golgi complex of tumor cells due to the hypertrophic morphology.²¹² Therefore, these designed BSA-pH-PTT composites were represented as a smart delivery system as they could be significantly activated in the Golgi apparatus to achieve the PTT effect. It should be noted that these ER and Golgi apparatus-targeted formulations are still in the infant stage as the mechanisms of delivery and targeted pathways are still unclear.

Intracellular Transformation

In addition to the delivery and subsequent therapeutic efficiency, it is a prerequisite to consider several transformations of the internalized nanocarriers, which lead to the degradation pathways of nanoparticles as well as

their excretion from the intracellular environment. Based on the extensive investigations, during the intracellular translocation of nanoparticles, several transformations happen within the cells and the nanoparticles, which result in the change of the generalized pathway of nanoparticles, ie, from endocytosis to main organelles. To this end, the cellular morphology, as well as organelle deformation, notably, nucleus, adapt to the transformable intracellular microenvironment.²¹³ In addition, the intracellular circumstances can also be altered by different surface modification strategies, as is shown in the substitution of endocytosis for transcytosis,²¹⁴ overcoming the barrier for drug absorption. Gu and coworkers,²¹⁵ designed versatile nanostructures with a superior membrane-breaking effect, which could significantly affect after cellular internalization and excluded immediately through the cell membrane. Similarly, Baldini and coworkers²¹⁶ observed that the release of nanoparticles from the cells prevailed for 72–144 h by using fluorescence polymeric nanoparticles. Further, these nanoparticles could strongly permeate tumor tissue through subsequent cell perforation approach.

Conclusions and Outlook

In summary, this review emphasized the subcellular performance and intracellular fate of the internalized nanoparticles in various main organelles such as endosomes, lysosomes, mitochondria, nucleus, ER, and Golgi apparatus. Initially, we gave a brief introduction of nanotechnology and conventional cancer therapeutics, revealing their unexpected limitations due to the adverse effects of resultant end products of diverse nanoparticles. Further, we discussed the fundamental structures, properties, and functions of the organelles referred above, highlighting the escape pathways of nanoparticles assisted with the interactions toward organelles. In addition, we discussed the typical mechanisms and application of these sophisticated approaches, along with possible mechanisms and related applications toward cancer ablation.

Despite the investigations on the intracellular fate of nanoparticles that keep making breakthroughs in recent years, these pathways need more in-depth explorations as the optimal properties of various materials and numerous biological barriers that being faced by nanomaterials, which influence the eventual pharmacokinetic profile of the therapeutic drugs. The integrated depiction

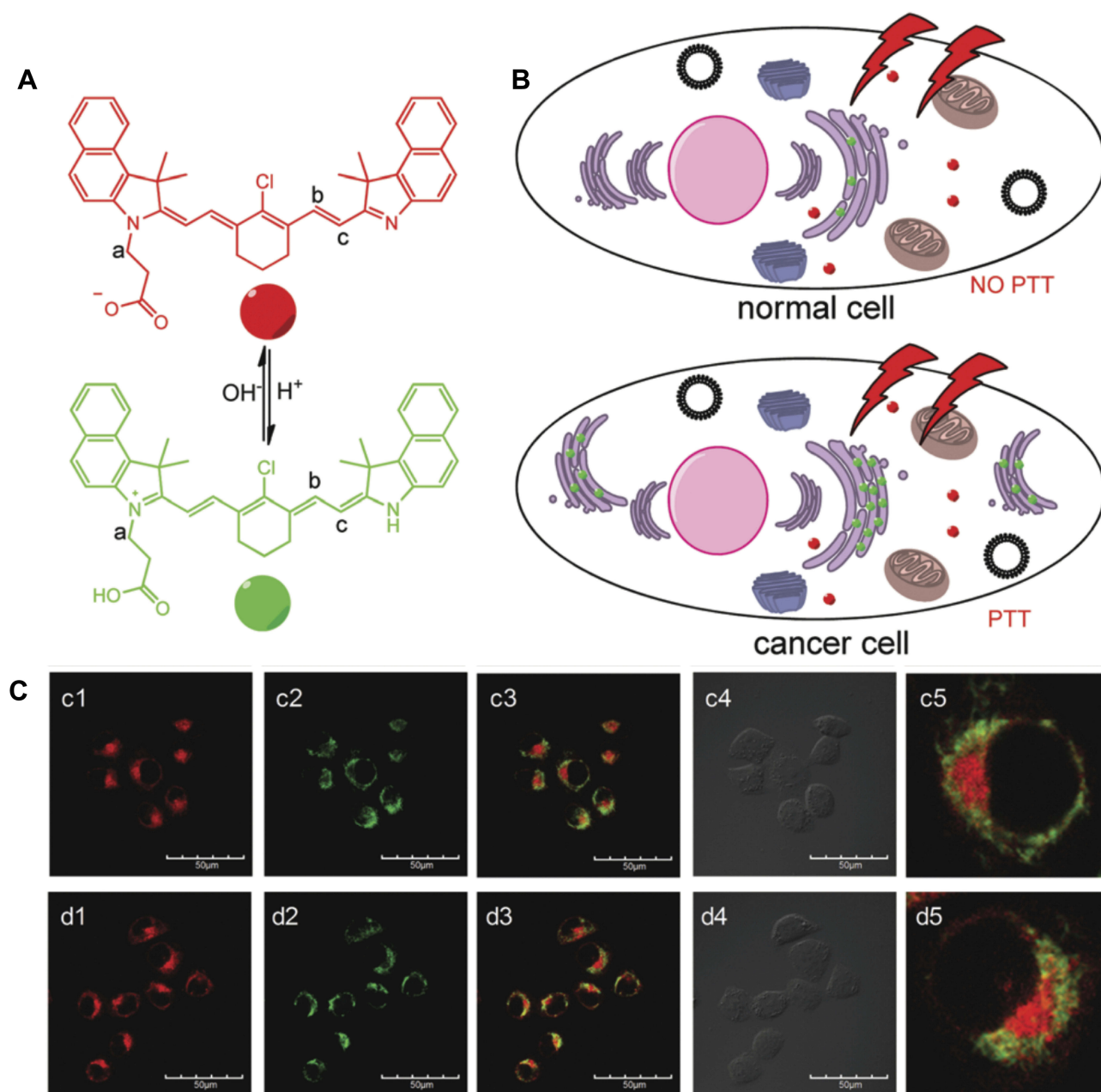


Figure 7 (A, B) Illustration of pH-PTT as a smart drug system for specific Golgi apparatus-activated PTT. **(C)** BSA-pH-PTT/Mito tracker (c and d) and BSA-pH-PTT/Golgi green. (1) Red channel: 650 ± 25 nm for BSA-pH-PTT (25 mM), $\lambda_{\text{ex}} = 543$ nm; (2) green channel: 530 ± 25 nm for LysoTracker (200 nM), Mito tracker (500 nM) or Golgi tracker (200 nM), $\lambda_{\text{ex}} = 488$ nm; (3) merged images; (4) bright field and (5) enlarged images of 3, scale bar: 50 μm . Reproduced with permission of Royal Society of Chemistry from A smart drug: a pH-responsive photothermal ablation agent for Golgi apparatus activated cancer therapy, Xue F, Wen Y, Wei P, et al., 2017.²⁵

from the perspective of specific behavior and physico-chemical properties of nanoparticles, for instance, the effect on size, morphology, potential, and functional bond, still needs to be comprehensively explained. In addition to physicochemical properties, the performance efficacy concerning the controlled delivery and therapeutic mechanisms, as well as compatibility attributes,

could be given paramount importance for their effective translation to clinics. We could able to figure out the overall function of each mechanism inside every organelle with a specific focus on the nucleus as well as mitochondria-targeted systems, but several challenges in terms of classifying the precise effects of every subcellular component still exist. Further, certain assumptions

are also required to study the recycling of cells and cargos, in addition to the clearance pathways of degraded nanoparticle constituents. We are anticipated that the development of organelle-based nanoparticle exertion mechanism will undoubtedly resolve the application of an intracellular drug or gene delivery toward efficient cancer therapy.

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Disclosure

The authors report no conflicts of interest in this work.

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