Review

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Emerging magic bullet: subcellular organelle-targeted cancer therapy

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Abstract: The therapeutic efficacy of anticancer drugs heavily relies on their concentration and retention at the corresponding target site. Hence, merely increasing the cellular concentration of drugs is insufficient to achieve satisfactory therapeutic outcomes, especially for the drugs that target specific intracellular sites. This necessitates the implementation of more precise targeting strategies to overcome the limitations posed by diffusion distribution and nonspecific interactions within cells. Consequently, subcellular organelle-targeted cancer therapy, characterized by its exceptional precision, have emerged as a promising approach to eradicate cancer cells through the specific disruption of subcellular organelles. Owing to several advantages including minimized dosage and side effect, optimized efficacy, and reversal of multidrug resistance, subcellular organelle-targeted therapies have garnered significant research interest in recent years. In this review, we comprehensively summarize the distribution of drug targets, targeted delivery strategies at various levels, and sophisticated strategies for targeting specific subcellular organelles. Additionally, we highlight the significance of subcellular targeting in cancer therapy and present essential considerations for its clinical translation.

Keywords: cancer; drug targets; subcellular targeting; drug delivery; organelle

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Introduction

Currently, malignant tumors remain the leading cause of premature death [1]. However, the therapeutic outcomes of conventional cancer treatments, such as surgery, chemotherapy, and radiotherapy, still fall short of expectations due to inherent limitations [2]. Surgery is exclusively indicated for nonmetastatic tumors, whereas radiotherapy indiscriminately damages adjacent normal tissues. As for chemotherapy, systemic toxicity and multidrug resistance pose significant challenges that restrict its clinical application [3]. The ultimate objective of cancer therapy is to selectively and efficiently eliminate tumor cells while preserving normal cells [4]. Targeted therapy has emerged as a promising strategy to achieve this goal [5, 6]. At present, targeted drug delivery strategies primarily focus on three levels: tissue-level targeting, cell-level targeting, and subcellular-level targeting. Among these approaches, subcellular organelle targeting represents the most precise technique and has been referred to as a “magic bullet” by some experts in the field [7]. While tissue-level and cell-level targeted drug delivery can enhance intratumoral or intracellular drug concentrations, respectively, which may benefit drugs acting on plasma membrane or cytoplasm, they are insufficient for drugs with specific subcellular targets. For instance, chemotherapeutic agents like doxorubicin (DOX) and cisplatin require nuclear localization [8], indicating that simply reinforcing their cellular internalization is not sufficient to achieve the desired therapeutic outcomes. The dispersed subcellular distribution and nonspecific interactions with subcellular targets significantly compromise their therapeutic efficacy. These concerns underscore the importance of subcellular targeting in improving the therapeutic index in cancer treatment.

In recent years, with the rapid progress of nanotechnology, numerous subcellular targeting strategies have been developed for various organelles [9]. Leveraging the unique and essential biological functions of organelles such as lysosomes, mitochondria, nuclei, and endoplasmic reticulum (ER), subcellular targeting therapy offers new opportunities for cancer treatment [10]. It enables precise attacks on specific organelles and facilitates controlled damage to cancer cells, surpassing conventional cancer therapies in several aspects [11]. Firstly, therapeutic agents concentrated within organelles exhibit superior efficacy compared to randomly distributed agents in the cytoplasm. Secondly, overcoming multidrug resistance becomes feasible due to limited efflux capability within organelles. Thirdly, excessive dosages resulting from premature leakage and off-target side effects can be effectively avoided. Moreover, subcellular targeting strategies also play a significant role in enhancing cancer immunogenicity and accelerating new drug development. Given these advantages of subcellular targeting therapy, significant efforts have been dedicated to developing drug delivery systems that selectively transport anticancer drugs to vital and vulnerable organelles, enabling highly efficient and minimized toxicity cancer treatment [12, 13]. This review summarizes a panorama of the recent advances in targeting strategies for various organelles while delving into the mechanisms behind their specificity (Figure 1). The significances and challenges of subcellular targeting therapy in revolutionizing cancer treatments are also discussed. We anticipate that this review will engender heightened research interest in subcellular targeting therapies and facilitate their clinical translation.

Distribution of drug targets

Drug targets are defined as proteins or other biomolecules (such as DNA, RNA, etc.) that directly bind to drugs, thereby influencing the therapeutic outcome. The identification of drug targets is a complex process [14]. In 2017, Santos et al. presented an extensive map illustrating the molecular targets of approved drugs, highlighting the significant importance of protein targets such as G protein-coupled receptors

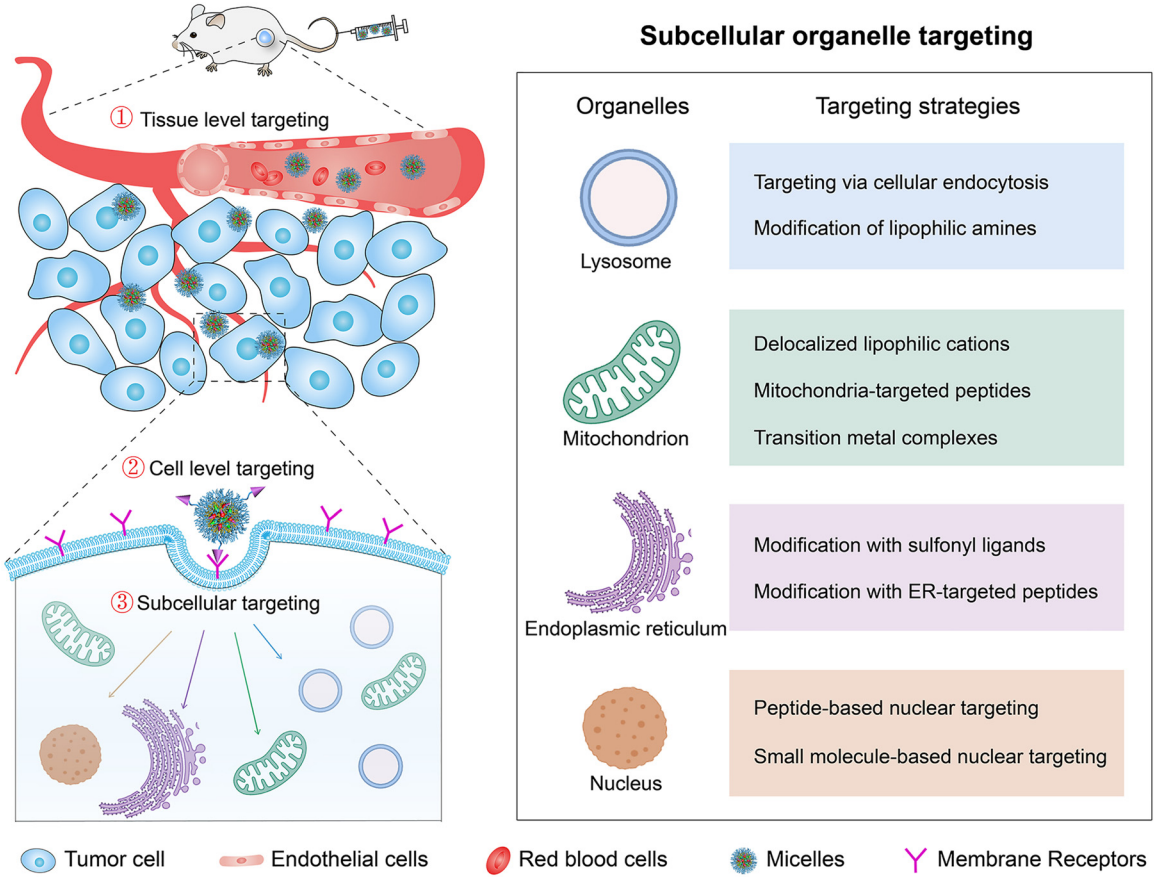

Figure 1: Schematic representation of targeted drug delivery at various levels, along with the corresponding strategies for subcellular targeting.

(GPCRs) and ion channel families [15]. With advancements in super resolution microscopy and fluorescence labeling technology, the distribution of drug targets as well as the drug-target interactions can also be well investigated. The distribution of drug targets for cancer therapy can be categorized into extracellular, membranal, and intracellular domains.

Extracellular targets

The intricate interplay between the extracellular components and the cells within the tumor microenvironment is critical for cancer progression and the efficacy of therapeutic interventions [16]. This complex network encompasses a variety of elements, including the extracellular matrix (ECM), immune cells, and endothelial cells, each playing distinct yet interconnected roles [17]. ECM-related components, including hyaluronan, collagens, tenascin C, fibronectin, and ECM-degrading enzymes, represent the primary targets within the extracellular environment [18]. For instance, high expression of tenascin C in tumor tissues is associated with tumor cell adhesion, migration, invasion, and immune evasion [19]. The human monoclonal antibody F16 has been developed to specifically target tenascin C and selectively accumulate at neovascular tumor sites [20]. To further enhance its therapeutic potential, a fusion protein called F16-IL2 was engineered for targeted delivery of interleukin-2 (IL-2) to vascular tumor sites. When combined with chemotherapeutic agents such as DOX or paclitaxel, this approach exhibited significantly superior inhibition of tumor growth compared to mono-chemotherapy. Moreover, Li et al. demonstrated that suppression of tenascin C in autophagy-impaired triple-negative breast cancer (TNBC) promoted T cell-mediated killing and enhanced the therapeutic effects of anti-PD1/PD-L1 therapy [21]. These studies indicate the therapeutic potential of targeting ECM and provide a comprehensive treatment strategy that integrates ECM regulation with other modalities such as chemotherapy and immunotherapy.

Membranal targets

Cell membrane serves as the gateway for cellular communication with the external environment, making it the primary point of contact for anti-tumor drugs when they interact with tumor cells [22]. Due to its accessibility and recognizability, the cell membrane has emerged as an ideal target for drug development. Primary targets on the cell membrane include receptors, adhesion factors, and ion channels. The signal transduction pathway mediated by the epidermal growth factor receptor (EGFR) family plays a crucial role in various processes such as proliferation, adhesion, invasion, apoptosis, and metastasis of cancer cells [23]. Anti-cancer drugs targeting EGFR can be broadly categorized into monoclonal antibodies or tyrosine kinase inhibitors (TKIs), several of which have been approved by FDA [24, 25]. For example, cetuximab and panitumumab are extensively used in treating head and neck cancer as well as metastatic colon cancer. When combined with DOX or paclitaxel in chemotherapy regimens, they significantly enhanced the therapeutic efficacy [26]. Furthermore, subsequent to the endorsement of first-generation TKIs such as gefitinib, successive generations of EGFR TKIs have been developed for clinical management of small cell lung cancer. These agents have emerged as primary therapeutic options for patients harboring EGFR mutant lung cancer [27].

Intracellular targets

Notably, intracellular targets also play a pivotal role in anticancer therapy due to their involvement in essential physiological processes such as protein synthesis, energy production, and signal transduction within cells. Subcellular organelles are particularly investigated as drug targets [28], benefiting from their crucial biological effects. Targeting specific organelles provides novel opportunities for effectively delivering therapeutic drugs [29]. Specifically, lysosomes are involved in the digestion of macromolecules, autophagy, and cellular defense mechanisms. Mitochondria are responsible for synthesizing adenosine triphosphate (ATP), regulating calcium ion cycles, and controlling apoptosis. The ER and Golgi apparatus play essential roles in protein synthesis and transport. Additionally, the nucleus regulates gene expression and cell proliferation [30]. For drugs that target intracellular components such as antitumor cytotoxic drugs, nucleic acids (siRNA, mRNA), and protein drugs, their efficacy is dependent on specific subcellular localization. Qi et al. developed an ultra-pH-sensitive micelle with dual-targeting capacity for early endosomes and mitochondria [31]. This micelle consists of pH-activatable micelles encapsulating photosensitizers modified with mitochondrial targeting groups. Upon endocytosis, the micelle rapidly disassembles in response to the acidic environment of early endosomes, leading to the release of triphenylphosphine-modified photosensitizer molecules. Complete mitochondrial colocalization was observed within 30 min, and the targeted photosensitizer induced more efficient mitochondria dysfunction and remarkable tumor

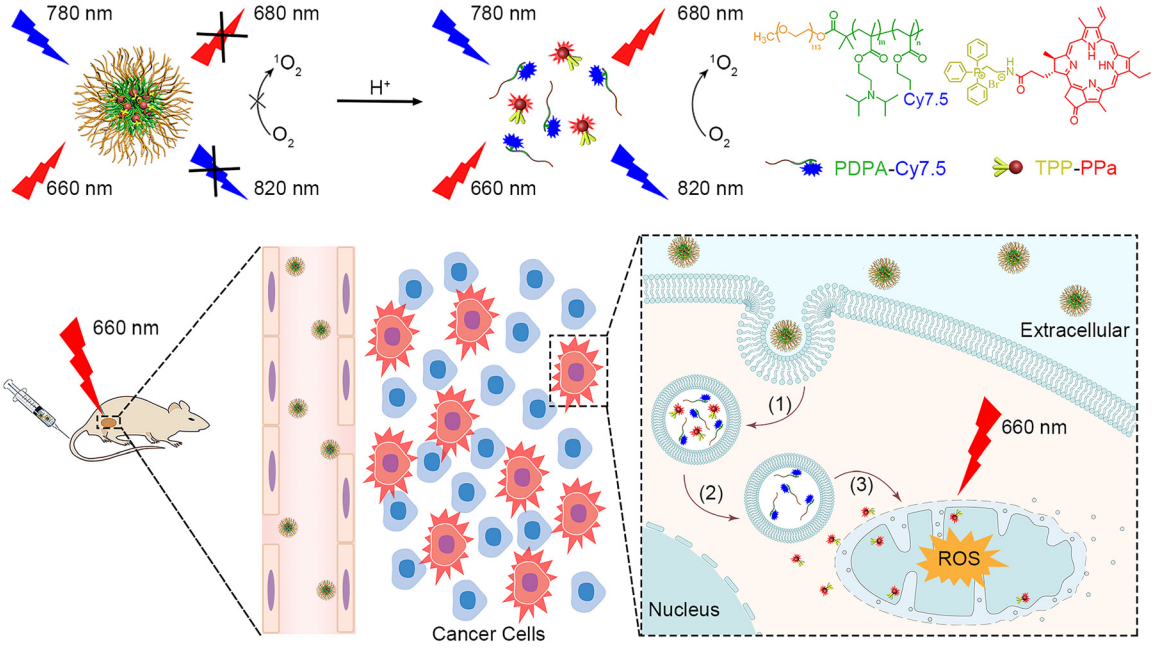

Figure 2: Schematic illustration of the ultra-pH-sensitive micelle with dual-targeting capacity towards early endosome and mitochondria. After cell endocytosis, the micelle quickly disassembled in early endosome and released TPP-modified photosensitizer for precise mitochondria-targeted photodynamic therapy [31]. Copyright © 2019 Elsevier Ltd. All rights reserved. TPP, triphenylphosphonium.

growth inhibition than untargeted group, indicating the superiority of precise sub-organelle targeted drug delivery (Figure 2).

Furthermore, a drug-target residence time model proposed by Copeland et al. has gained widespread adoption in the fields of biotechnology, pharmaceuticals, and chemical biology [32, 33]. This model emphasizes the critical role of prolonging the residence time of drug-target complexes in achieving pharmacological effects and target selectivity, while highlighting that the efficacy of a drug is determined by its concentration at the target site. Therefore, increasing drug concentration at the intended target has emerged as an important strategy to enhance efficacy, reduce toxicity, and reverse drug resistance.

Targeted drug delivery

Targeted drug delivery holds great promise in augmenting the localized drug concentration. Due to the lack of target-specific affinity towards tumor sites, systemic administration of drugs may lead to off-target systemic toxicity and significant side effects. Moreover, non-specific therapeutic agents often exhibit insufficient bioavailability under standard dosing regimens, necessitating higher dosages for efficacy. Additionally, small molecule drugs face challenges related to rapid metabolism and clearance *in vivo* [34], limiting their transient efficacy in meeting clinical needs. Given these major obstacles, it is imperative to explore targeted delivery nanotechnology that can selectively enrich drugs within the tumor site for improved efficacy while minimizing toxicity [6]. Consequently, researchers have developed various targeted drug delivery systems operating at different levels of targeting.

Tissue level

Tumor tissue targeting relies on exploiting physiological and morphological disparities between normal tissues and tumors [35]. The enhanced permeability and retention (EPR) effect has been extensively utilized in the design of nanocarriers. However, its efficacy in patients has become controversial following clinical studies [36]. Recent research has highlighted considerable heterogeneity in the clinical effectiveness of the EPR effect in tumors, suggesting that it may not be as pronounced as observed in animal models [37, 38]. Additionally, studies have begun to explore mechanisms beyond the EPR effect, such as the lymphatic elimination of nanoparticles and the promotion of tumor penetration through activated transcellular transport pathways [39–41]. These insights imply that the accumulation of nanoparticles within tumor tissues could be governed by a more intricate array of biological processes, rather than

solely relying on the EPR effect. Notably, although targeted drug delivery at the tissue level has improved *in vivo* drug distribution to some extent [42], there remains a lack of specificity. Increasing evidence suggests that solely delivering nano-carriers to tumor tissues is insufficient as poor accumulation and penetration significantly restrict the therapeutic effects of drugs [43]. Furthermore, merely determining the targeted accumulation of a drug at the tumor tissue level often falls short in accurately predicting its therapeutic efficacy [44, 45]. Doubts persist regarding the effective binding ability between free drugs delivered by nano-carriers and their intended targets, posing a major limitation to tissue targeting.

Cell level

To achieve higher precision in drug delivery, certain specific markers overexpressed on tumor cell membranes can be exploited for distinguishing them from normal cells [46]. These markers include tumor-specific antigens (CD44 [47], CD47 [48], etc.), folate receptors (FRs) [49], and various protein receptors (such as transferrin receptors [TfRs] [50]). Surface modification of nanoparticles with targeting moieties enables their directed binding to these specific receptors overexpressed on tumor cells, thereby enhancing cellular uptake and promoting drug delivery efficiency. Among these targets, FRs are commonly employed due to their high prevalence on tumor cell surfaces. Folic acid (FA), serving as its ligand, exhibits non-toxicity, good stability, weak immunogenicity, and high affinity towards FRs, making it widely applicable in targeted drug delivery systems [51]. Liu et al. designed folate-PROTACs (proteolysis-targeting chimeras) for cancer-selective target degradation [52]. This strategy enables the selective delivery of PROTACs into cancer cells, thereby facilitating controllable targeted protein degradation while minimizing potential toxicity towards normal cells, ultimately improving the therapeutic windows of PROTACs.

Additionally, since the FDA approval of Adcetris® in 2011, antibody-drug conjugates (ADCs) have emerged as a prominent modality for cancer treatment [53]. ADCs possess a modular chemical structure comprising monoclonal antibodies (mAbs) covalently linked to cytotoxic agents through changeable linkers, enabling them to address the intricate tumor microenvironment [54]. Enabled by specific antigen-antibody binding, ADCs selectively target tumor cells and undergo internalization via endocytosis to release free drugs, thereby exerting potent anti-tumor effects [55].

Subcellular level

Both tissue-level targeting and cell-level targeting share a common objective of enhancing cellular uptake. While these delivery methods may improve drug efficacy when targeting plasma membrane or cytoplasmic drug targets, such as siRNA or small molecule drugs [56], they pose limitations for chemotherapy agents, such as DOX and cisplatin, that require nuclear localization [57], as well as singlet oxygen generated during photodynamic therapy (PDT). Given the short half-life and limited diffusion distance of singlet oxygen, PDT necessitates close proximity between bioactive macromolecules and singlet oxygen for effective destruction and therapeutic outcomes [58]. In these cases, simply increasing intracellular drug concentration is insufficient to achieve satisfactory therapeutic effects. Diffuse distribution of drugs and nonspecific interactions within cells greatly compromise therapeutic effectiveness [59]. Considering these concerns, subcellular level targeting cancer therapy with higher precision has been developed, attracting substantial research interest.

Yet, subcellular organelle targeting represents the most precise delivery technology, often referred to as the “magic bullet” in scientific research. It selectively targets specific organelles and effectively damages cancer cells with enhanced control, thereby improving therapeutic efficacy of drugs while minimizing side effects from a pharmaceutical perspective [60]. Concurrently, advancements in emerging therapeutic strategies like gene therapy and protein degradation therapy (e.g., PROTAC) have led to an increasing number of drug targets located within specific organelles (e.g., nucleus or mitochondrion), consequently driving the demand for subcellular delivery technologies [61]. Notably, complex cellular environments may impose restrictions on nanoparticle transport towards subcellular targets. Therefore, there is an urgent need for subtler designs of nano-delivery systems based on the unique characteristics of each organelle to meet diverse demands in cancer treatment and maximize therapeutic outcomes of subcellular organelle-targeted therapy.

Subcellular targeting strategies

Organelles are undoubtedly the most crucial subcellular targets due to their essential functions and distinctive structural features. Gaining a comprehensive understanding of the biochemical processes and unique characteristics associated with each organelle holds immense potential for the rational design of targeted delivery systems. The

subsequent section will present several pivotal strategies for organelle targeting.

Lysosome

Lysosomes serve as the dominating sites for sorting exogenous and a portion of endogenous substances, housing numerous hydrolytic enzymes that play crucial roles in degrading, repairing, and recycling various biological macromolecules [62]. In addition, lysosomes are involved in essential physiological processes including energy metabolism, plasma membrane repair, immune response, and cell death [63]. Importantly, tumor cells exhibit an increased number of lysosomes with larger volume and higher cathepsin activity compared to normal cells. These characteristics are closely associated with carcinogenesis and poor cancer prognosis [64]. Consequently, lysosome-targeted cancer therapy has garnered significant research interest over the past decade. Moreover, the distinctive acidic microenvironment (pH≈5.0) of lysosomes can be exploited for controlled activation or release of therapeutic drugs. This feature makes it an attractive subcellular target for anticancer treatments [65].

Generally speaking, strategies targeting lysosomes can be broadly classified into two categories. One involves delivering therapeutic agents into lysosomes through cellular endocytosis such as receptor-mediated endocytosis, while the other focuses on localizing nanocarriers or small molecule drugs within lysosomes via modification of lipophilic amines.

Lysosome-targeted delivery via cellular endocytosis

Currently, various strategies based on cellular endocytosis have been developed for targeted drug delivery to lysosomes [66]. The most commonly used approach is receptor-mediated endocytosis [67], which involves targeting folate receptor, TfR, low density lipoprotein receptor (LDLR), and mannose-6-phosphate receptor (M6PR) on the cell membrane using antibodies or small molecular ligands. Li et al. fabricated a multifunctional nanoparticle (PPCNP-Ce6/FA) for PDT, enabling lysosomal homing and enhanced cellular uptake by incorporating FA that selectively targets overexpressed folate receptors on cancer cell surfaces [68]. These nanoparticles generated abundant reactive oxygen species under near-infrared irradiation, resulting in significant tumor inhibition efficacy against drug-resistant MCF-7/ADR tumors. Tian et al. designed a pH-responsive photosensitizer (NEt_2Br_2BDP) loaded into cRGD-functionalized nanomicelles to achieve lysosome-targeted delivery through αvβ3 receptor-mediated endocytosis [69]. The photoactivity of the photosensitizer can be activated by the physiologically acidic pH of lysosomes, enabling efficient and low-toxicity photodynamic treatment of tumors. Furthermore, several studies have also achieved lysosome targeting through other receptor-mediated endocytosis pathways [70].

Notably, the stability of proteins or small molecular drugs in lysosomes is challenging due to the strong acidic environment and degradation function of lysosomes. To address this issue, Banik et al. proposed the lysosome-targeting chimaeras (LYTAC) technology, which enables the degradation of therapeutically relevant extracellular protein and cell membrane protein through the lysosomal degradation pathway [71]. LYTACs consist of glycopeptide ligands that selectively bind to M6PR and specific antibodies targeting substrate proteins for degradation. Initially, the antibody binds to the target protein, followed by LYTAC entering the lysosome via M6PR-mediated endocytosis, resulting in targeted protein degradation. This study utilized EGFR, PD-L1, and CD71 as examples to demonstrate both feasibility and effectiveness of LYTACs. Unlike traditional drug delivery technologies, LYTAC technology leverages a robust degradative environment where delivered agents are target proteins requiring degradation, thereby reflecting diverse perspectives on cancer treatment [72, 73].

Lysosome-targeted delivery via modification of lipophilic amines

Morpholine and most tertiary amine groups, such as diethylamino, diisopropylamino, and piperidine, possess lysosomal targeting ability. This targeting mechanism is attributed to their weak alkalinity, which facilitates aggregation and protonation within the acidic environment of lysosomes [74]. Consequently, these targeted groups exhibit significantly enhanced hydrophilicity and reduced transmembrane capacity, impeding their escape from lysosomes and leading to lysosome-specific accumulation. Kand et al. successfully targeted BODIPY photocage into lysosomes through the modification of morpholine group [75]. The bioactive molecules can be selectively photo-released *in situ* under visible light irradiation and exert their therapeutic effects. Additionally, Xiao et al. reported a series of pyridine-embedded phenothiazinium (pyridophenothiazinium) dyes [76], utilizing their N, N–diethylaminophenyl moiety for localization within lysosomes. Upon light irradiation, the pyridophenothiazinium derivatives generated 40-fold higher levels of reactive oxygen species (ROS) compared to commercial methylene blue while exhibiting efficient photodynamic antitumor effects.

Notably, in addition to small molecules, certain macromolecular polymers containing tertiary amine structures

also demonstrate lysosome-targeting capability. Wang et al. developed a series of ultra-pH-sensitive (UPS) polymers that enable precise imaging of various stages of endocytic organelle maturation by regulating the alkalinity of their tertiary amine groups [77, 78]. This system also exhibits significant potential for drug delivery. Xia and colleagues achieved accurate lysosomal delivery of TLR7/8 agonist (imidazoquinoline, IMDQ) using this platform [79]. The UPS polymer was covalently conjugated with IMDQ through an enzyme-responsive linkage, which could self-assemble into nanoparticles. Upon endocytosis into the lysosome, these nanoparticles rapidly disassemble in response to the acidic environment. Subsequently, the enzyme-responsive linkage is cleaved by cathepsin B within the lysosome leading to *in situ* release of free IMDQ. Ultimately, IMDQ activates its TLR7/8 receptors located on the inner membrane of the lysosomes thereby promoting dendritic cell maturation and inducing potent immunotherapeutic effects (Figure 3).

Mitochondria

Mitochondria are unique organelles enclosed by bilayer membranes, often referred to as the powerhouses of the cell due to their ability to generate energy through aerobic respiration [80]. Additionally, mitochondria play a crucial role in various essential cellular biochemical processes including calcium storage, amino acid biosynthesis, regulation of apoptosis, and fatty acid oxidation [81]. Consequently, mitochondrial dysfunction is implicated in numerous diseases, such as cancer, cardiovascular diseases, and neurodegenerative disorders [82]. Alterations in mitochondrial metabolic status and signal transduction closely contribute to tumor initiation and progression. Tumor cells exploit metabolic reprogramming to induce enhanced mitochondrial anabolic metabolism while impairing apoptotic pathways for uncontrolled growth [83]. Therefore, targeting mitochondria holds immense potential for manipulating cancer cell death and represents a promising avenue for cancer therapy.

In recent decades, mitochondria-based anticancer strategies have prominently emerged by leveraging the key cellular functions of mitochondria. These strategies encompass modification with delocalized lipophilic cations (DLCs), utilization of mitochondria-targeted peptides, and employment of transition-metal-based complexes. A compilation of representative mitochondria-targeting approaches is presented in Table 1.

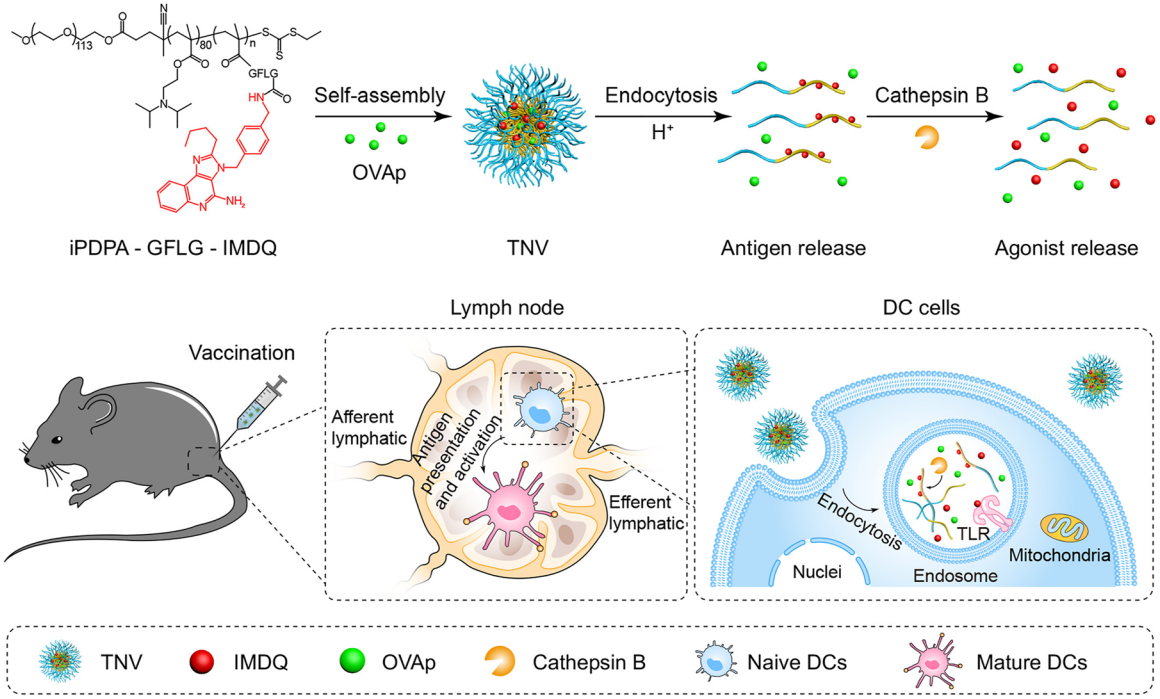

Figure 3: The pH-/enzyme-responsive micelles enable precise delivery of TLR7/8 agonist to the lysosome. Upon exposure to the acidic environment and cathepsin B in the lysosome, these micelles exhibit accurate release of IMDQ, thereby activating TLR7/8 and subsequently inducing an immune response [79]. Copyright © 2022 American Chemical Society.

Table 1: Overview of representative mitochondria-targeting strategies in cancer therapy.

Mitochondria-targeting unit	Agent	Application	Function	Ref.
Delocalized lipophilic cations	TPP-AsDox TPP-AsPt	Cancer therapy	Mito-targetable chemotherapy drugs	[84]
	PEG-PLA@TPP-cabazitaxel	Cancer therapy	Enhance the antitumor effect of drugs	[85]
	TPP-atovaquone	Cancer therapy	Improve the tumor immunosuppressive microenvironment	[86]
	Nd^{3+}-sensitized upconversion MOFs	Mito-targeted photosensitizers	Photodynamic therapy with minimized overheating effect	[87]
	Rho-mito	Mito-targeted fluorescent probes	Real-time monitoring of platinum accumulation in the mitochondria	[88]
	TPP@(DCAx-UiO-66)	Mito-targetable drug delivery systems	Greatly improved the drug delivery to mitochondria	[89]
	Mito-targeted magnetic nanospinners	Cancer therapy	Use mechanical forces to treat deep tumors	[90]
	MAP-I	Mito-targeted photosensitizers	Achieve cancer-efficient ICDs	[91]
	aB2MG-TPP@CSNRs	Eliminate senescent cells	Selectively cause mitochondrial damage and apoptosis of senescent cells	[92]
	IR808@MnO	Mito-targeted photosensitizers	Enhanced phototherapy by synergistic ROS and hyperthermia	[93]
	TPP-PDMA-b-PCPTSM	Cancer therapy	Endogenously activated mtROS enhance chemokinetic therapy	[94]
	TPP-lonidamine	Cancer therapy	Alleviates lung cancer development and brain metastasis	[95]
Mitochondria-targeting peptide	α-CD-DOX-NO-DA	Cancer therapy	Drug resistance reversal and metastasis inhibition	[96]
	SS-31 loaded nanopolyplexes	Acute kidney injury	pH-responsive and AKI-kidney targeted nanopolyplexes	[97]
	Dendritic lipopeptide (DLP) liposome	Mito-targetable drug delivery systems	Efficient targeted mitochondrial delivery of drugs	[98]
	Ru-MPP	Mitochondrial DNA-targeted fluorescent probes	Specific staining and phototoxic binding of mitochondrial DNA	[99]
	CAMP-hMT1A	Reduce mitochondrial damage	Rescue movement disorders and dopaminergic neuronal degeneration	[100]
Mitochondria targeting sequence	SWNT-PM-CytKH9	Gene delivery systems	Delivering DNA to intact plant mitochondria	[101]
	HPMA-DOX-R8MTS	Cancer therapy	Inhibition of breast cancer metastasis	[102]
Transition metal complexes	BT-Ir	Mitochondria-to-nucleus cascade organelle targeted photosensitizer	Induce nucleic acid damage and cell death	[103]
	$Re_2(CO)_6$ $(dip)_2L]$ $(PF_6)_2$	Cancer therapy	Target mitochondria and influence redox homeostasis	[104]
	fac-Ir-CHO	Mito-targeted photosensitizer	Strong DNA binding affinity and apoptosis induction	[105]
	Hf-DBB-Ru	Mito-targeted photosensitizer	Use low doses of deep penetrating X-rays to enhance cancer therapy	[106]

TPP, triphenylphosphonium; MOF, metal-organic framework; ICD, immunogenic cell death.

Delocalized lipophilic cations

Mitochondria maintain a negative internal and positive external membrane potential, which is approximately 3–5 folds higher (150–180 mV) than the plasma membrane potentials (30–40 mV) [107]. Driven by strong negative membrane potential, lipophilic cations can selectively cross the inner membrane and accumulate in the mitochondrial matrix in a reverse concentration gradient. Therefore, DLCs including triphenylphosphonium (TPP) [108], rhodamine, dequalinium (DQA), are widely utilized in mitochondria targeting therapy [109]. Various small biologically active molecules have been conjugated with DLCs to achieve mitochondrial targeting.

For example, Reddy et al. conjugated TPP cation with curcumin for selective mitochondrial delivery, resulting

in significantly enhanced mitochondrial accumulation and improved efficacy [110]. Zhang et al. designed a mitochondria-targeted photothermal agent called Mito-BWQ by incorporating a thiazole orange unit and the targeted TPP group [111]. Under NIR irradiation, it exhibited high photothermal conversion efficiency and excellent antitumor effects due to precise heat generation within thermally susceptible mitochondria. Nanocarriers can also be modified with DLCs for targeted drug delivery to mitochondria [112], as demonstrated by David's team who covalently linked metal-organic framework (MOF) encapsulating cancer drug dichloroacetate (DCA) with TPP for mitochondria-targeted drug delivery [89]. The results showed that the targeted MOF system significantly enhances DCA's drug efficacy while reducing the required dosage to less than 1 % compared to free drugs or approximately 10 % compared to non-targeted MOFs. Furthermore, Ren et al. developed mitochondria-targeted nanotaxanes with pseudo-stealth properties by encapsulating TPP-conjugated cabazitaxel (pro-taxane) into a polymeric nanoparticle decorated with low-density TPP [85]. This nanoparticle exhibited prolonged systemic circulation time and its positive surface charge facilitated mitochondrial accumulation, enabling selective delivery of protaxane molecules to the mitochondria (Figure 4). Moreover, mitochondria play a crucial role in immune response activation. Huang et al. modified atovaquone with TPP to create Mito-ATO. *In situ* injection of Mito-ATO triggered potent T cell immune responses and significantly decreased regulatory T cells (Tregs) and myeloid-derived suppressor cells within the tumor microenvironment, thereby enhancing the antitumor efficacy of PD-1 blockade immunotherapy [86]. However, it should be noted that high concentrations of DLCs may exhibit cytotoxicity, which limits their application to some extent.

Mitochondria-targeted peptides

Mitochondria-targeting peptides, such as mitochondrial-targeting sequences (MTSs) and mitochondria-penetrating peptides, have been successfully utilized for the modification of drugs or nanocarriers to achieve targeted drug

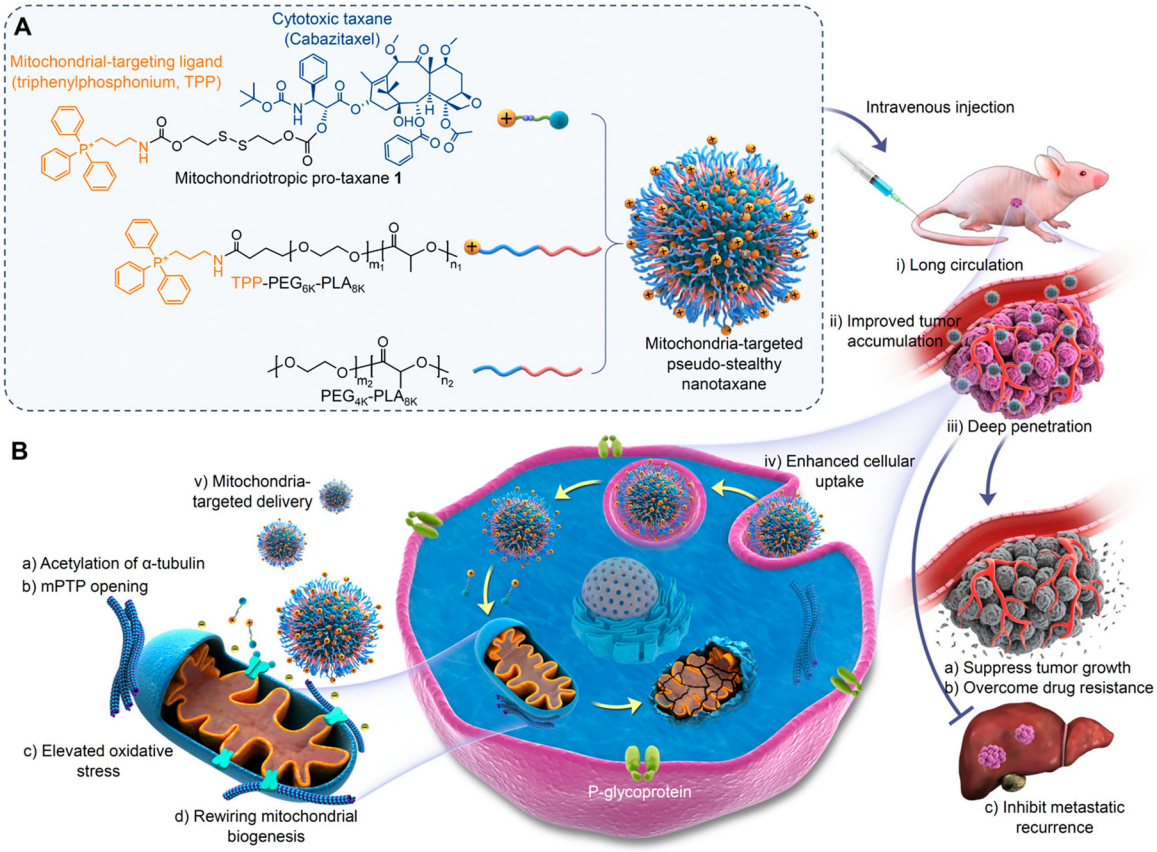

Figure 4: The design and physiological process of pseudo-stealthy mitochondria-targeted nanotaxanes [85]. (a) Chemical structures of the cabazitaxel-based mitochondriotropic pro-drug, PEG-PLA, and TPP-tethered PEG-PLA. (b) The nanoparticles have a long circulation ability and achieve deep penetration into tumors. After internalization, they specifically target the mitochondria, and then cabazitaxel is activated *in situ* to target mitochondrial tubulin, ultimately leading to severe mitochondrial damage and creating durable tumor inhibition. Copyright © 2022 American Chemical Society. TPP, triphenylphosphonium.

delivery to mitochondria. These peptides possess a sequence rich in positively charged amino acid residues, which contribute to their selective localization within the mitochondria [98]. Peptides offer better biocompatibility compared to lipophilic cations and their mitochondrial targeting mechanisms primarily involve recognition by mitochondrial transmembrane receptors or penetration driven by the mitochondrial electrochemical gradient. Considering the potential impact of covalent attachment to mitochondria-penetrating peptide (MPP) on the interaction between the appended drug and its cellular target, Lei et al. employed a disulfide bond to connect Luminespib, an HSP90 inhibitor, with MPP in order to obtain its deactivated form [113]. Subsequently, the disulfide bond could be cleaved by ubiquitous glutathione present in tumor cells, thereby achieving traceless release of active Luminespib within mitochondria for effective induction of cell apoptosis. In contrast to MPPs, MTSs exhibit larger structures and lower solubility leading to insufficient permeability across membranes [102]. Chan and colleagues developed a sequential targeting drug delivery system based on photostable nanodiamonds (ND) that were dually modified with FA and MTS [114]. The DOX-loaded multifunctional ND could accurately identify cancer cells overexpressing folate receptors, effectively traverse the cell membrane, and selectively deliver DOX into mitochondria. This approach resulted in higher cellular uptake of DOX specifically targeted towards mitochondria compared to lysosome-localized DOX while significantly improving cell cytotoxicity even in DOX-resistant models.

Transition metal complexes

Some transition metal complexes with a positive charge possess inherent mitochondrial targeting ability, which have been exploited by conjugating them to therapeutic agents for enhanced anticancer efficacy. Among these complexes, Rhenium (Re), Ruthenium (Ru), and Iridium (Ir) are commonly employed. Imstepf et al. successfully redirected the cellular distribution of Dox from nuclei to mitochondria through modification with organometallic rhenium complexes [115]. Chao's group developed a mitochondria-targeting magnetothermogenic nanozyme (Ir@$MnFe_2O_4$ NPs) for effective cancer therapy [116]. Leveraging the excellent targeting ability of iridium (III) complex, Ir@$MnFe_2O_4$ NPs can precisely localize in mitochondria where they exert magnetic hyperthermia therapy under an alternating magnetic field. The study also demonstrated that the cell-killing effect of Ir@$MnFe_2O_4$ NPs was significantly superior compared to γ-$Mn_{0.2}Fe_{1.8}O_3$ NPs without Ir complexes, highlighting the advantage of mitochondria targeting. Additionally, transition metal complexes serve as ideal photosensitizers due to their exceptional photost ability and phototoxicity index [117]. Lin et al. constructed Ruthenium based nanoscale metal-organic frameworks (nMOFs) with strong mitochondrial targeting properties [106]. Upon exposure to X-ray irradiation, these nMOFs efficiently generated hydroxyl radicals and singlet oxygen, resulting in significant regression of colorectal tumors. However, due to the potent cytotoxicity commonly observed in transition metal complexes, a rational design approach is imperative for achieving precise mitochondria-targeted anticancer therapy with minimal toxicity.

Nucleus

The nucleus serves as both the storage center for cell genetic materials and the central regulator of cell proliferation, metabolism, and cycle management. It is protected by a double-layered nuclear membrane and nuclear pore complex (NPC) [118]. The nuclear pores act as entry and exit passageways, controlling the exchange of macromolecules between the cytoplasm and nucleoplasm while maintaining nuclear transport stability [119]. DNA-toxin small molecule drugs like cisplatin, DOX, camptothecin, etc., must reach the DNA-enriched nucleus to induce cell death. Similarly, oligonucleotides and nucleic acid drugs used in anticancer gene therapy also require entry into the nucleus for gene editing participation [120]. Therefore, targeting the nucleus is crucial in cancer treatment.

In recent decades, various strategies for achieving nuclear localization have been developed. For instance, biological vectors such as viral vectors or plasmids have proven effective in delivering cargo to host cells with nuclear specificity [121], but safety concerns and limited cargo versatility hinder their clinical applications significantly. Fortunately, nanocarriers have greatly advanced the development of nuclear-targeted systems due to their rapid progress and versatile properties. Some studies demonstrate that nanoparticles with a diameter smaller than 9 nm can passively diffuse into the nucleus through NPC's size limitations [122], whereas larger nanoparticles require active transportation into the nucleus. Compared to passive targeting strategies, active targeting strategies are more efficient and widely employed, encompassing peptide-based nuclear targeting and small molecule-based nuclear targeting.

Peptide-based nuclear targeting

Nuclear localization signal (NLS) modification is a commonly employed strategy for the efficient nuclear transport of

large nanoparticles [123]. NLS (SV40 T antigen, TAT peptide, adenoviral, etc.) have been extensively utilized to actively facilitate nanoparticle entry into the nucleus via the importin α/β pathway, making them valuable tools in nuclear targeting therapy [124]. Du et al. developed biodegradable silica nanocapsules (BSNPs) as an effective means to deliver native proteins/antibodies into the nucleus [125]. It is well-known that protein drugs are susceptible to pH-induced conformational changes upon cellular internalization. In this study, proteins were encapsulated within biodegradable and physically/chemically stable BSNPs to effectively protect them from endogenous degradation. In addition, surface modification of NLS ensures the lysosomal escape and nuclear targeting ability of BSNPs, and fusion with cancer cell membranes achieve homologous targeting effect and reduced immunogenicity of BSNPs. Employing enhanced green fluorescent protein, histone H3 monoclonal antibody, and DNase I as model drugs confirmed successful selective delivery of these agents into the nucleus using BSNPs. Similarly, Cheng and colleagues designed a versatile gene-delivery strategy for efficient and visual delivery of therapeutic genes into tumor nuclei [126]. This involved sequential incorporation of targeted peptides (DGR or RGD), a NLS (KRRRR), a cell-penetrating peptide (RRRR), and an aggregation-induced emission molecule (PyTPE) followed by self-assembly with therapeutic genes through electrostatic interactions to form TNCP nanoparticles for highly effective nuclear-targeted therapy. Additionally, owing to the integration of an AIE fluorescent probe, this platform also possesses the capability of real-time monitoring the delivery process of therapeutic genes, thereby offering a valuable therapeutic avenue for clinical gene interference therapy.

Small molecule-based nuclear targeting

Compared to NLS, small molecules possess the advantages of low molecular weight, facile synthesis, and convenient modification. Tang et al. initially reported the utilization of a nonpeptidic benzyl boronate moiety for nuclear-targeted protein delivery [127]. They observed that the concentration of benzyl boronate-modified protein in the nucleus was three times higher than that in the cytoplasm, while unmodified protein exhibited uniform distribution throughout the cell. Furthermore, small molecules targeting nuclear receptors such as dexamethasone (DEX) and all-trans retinoic acid (ATRA) can also achieve nuclear localization. Xiong et al. functionalized mesoporous silica nanoparticles (MSNs) with DEXTPp and FA for sequential cell-nucleus targeted drug delivery [128]. A model drug, DOX, was loaded into MSNs where DEX ligands facilitated specific localization of DOX within the nucleus. The dual-ligand-modified MSNs delivered DOX induced more efficient cellular apoptosis and fewer side effects compared to other groups. However, DEX and ATRA have high pharmacological activity in clinical application, and their own role cannot be ignored when they are used as targeted molecules for nuclear targeted drug delivery.

Endoplasmic reticulum

ER is an extensive organelle that spans from the nuclear membrane to the cell membrane, playing a crucial role in various physiological processes such as protein folding and processing, lipid biosynthesis, and intracellular calcium storage [129]. Aberrant ER functions resulting from protein misfolding or mutation are implicated in diverse diseases including diabetes mellitus, neurodegenerative disorders, and cancer [130]. Furthermore, ER stress can be triggered by genetic and environmental insults such as hypoxia, nutritional deficiency, low pH levels, and DNA damage [131]. In tumor cells specifically, persistent ER stress occurs with mild stress enhancing cellular tolerance while excessive stress directly induces cell death [132]. Given the essential role of ER in maintaining cellular homeostasis, numerous strategies targeting this organelle have been developed to enhance anticancer efficacy. These approaches primarily involve modification with sulfonyl ligands or ER-targeted peptides.

Modification with sulfonyl ligands

Sulfonyl ligands selectively target the ATP-sensitive K^+ channel (sulfonylurea receptors) on the ER membrane, exhibiting a high affinity towards ER [133]. The hypoglycemic drug glibenclamide is a classic sulfonyl ligand. Commercially available ER labeling probes, such as ER Tracker Green/Red, were designed based on glibenclamide. However, due to its ability to alter K^+ levels in the ER and exert pharmacological effects of its own, as well as its synthesis difficulties and high cost, researchers have further developed sulfonamide-based ligands for targeted delivery to the ER. These ligands possess the same targeting mechanism as glibenclamide but with reduced pharmacological effects and improved availability. Du et al. successfully delivered photoactivatable raman probes to the ER for imaging and tracking of dynamic migration by utilizing methyl sulphonamide as a targeting group [134]. Gilbert et al. constructed an ER-targeted hydrogen sulfide (H_2S) donor by conjugating a p-toluenesulfonamide group to a caged thiocarbamate scaffold [135]. Caged thiocarbamates first release carbonyl sulfide which is then hydrolyzed by ubiquitous carbonic anhydrase to produce H_2S for regulating ER function and stress response.

Additionally, Zhang and coworkers designed a cascade targeting nanoparticle ($NP^{ER/BO-PDT}$) that sequentially targets bone tumors and the ER for photodynamic-immunotherapy [136]. $NP^{ER/BO-PDT}$ self-assembles through an ER-targeting polymer modified with N-Tosylethylenediamine and a bone-targeting polymer modified with alendronic acid respectively, enabling specific targeting abilities. Under NIR irradiation, $NP^{ER/BO-PDT}$ generates abundant ROS in the ER to damage tumor cells while triggering severe ER stress and strong immunogenic cell death (ICD) effect for effective immunotherapy.

Modification with ER-targeted peptides

Currently, the most commonly used ER-targetable peptides include pardaxin, KDEL, KKXX (where X represents any amino acid), and Eriss. Acharya et al. successfully delivered NADPH Oxidase isoform 4 (Nox4) siRNA to the ER by conjugating gold nanocarriers with cysteine-terminated KDEL peptide [137]. Since the ER is a primary site where antigenic peptides assemble with major histocompatibility complex class I (MHC I), targeted delivery of exogenous antigenic peptides to the ER is advantageous for enhancing cross-presentation efficiency and improving therapeutic outcomes in immunotherapy. For instance, Stepensky et al. encapsulated antigenic peptide into PLGA nanoparticles modified with KKXX peptide for targeted delivery to the ER [138], resulting in efficient cellular uptake and robust immune responses in antigen-presenting cells (APCs). Additionally, Li et al. developed dual "ER missiles" consisting of an indocyanine green (ICG)-conjugated hollow gold nanosphere and an oxygen-carrying hemoglobin (Hb) liposome, both of which achieved ER targeting through pardaxin (FAL) peptide modification [58]. Compared with non-targeting nanosystems, the constructed ER-targeting PDT-PTT nanosystem caused severe ER stress by ER-localized ROS generation, thereby leading to considerably promoted ICD-associated immunotherapy, and exhibited prominent inhibition of tumor growth (Figure 5).

The significance of subcellular targeting

Improved therapeutic efficacy

Subcellular organelles play a crucial role in the survival and proliferation of cancer cells [139]. Targeting these organelles for therapy offers significant advantages over traditional chemotherapy, including enhanced therapeutic efficacy, reversal of multidrug resistance, as well as reduced toxicity and side effects. Firstly, precise delivery of therapeutic agents to specific organelles allows for concentrated damage to vital or vulnerable sites within tumor cells, resulting in improved treatment outcomes compared to randomly distributed agents in the cytoplasm. Secondly, cancer multidrug resistance is a widespread crisis in chemotherapy, which is also one of the important reasons for the clinical failure of tumor chemotherapy [140]. However, it may be reversed through subcellular targeted therapy, since concentrated chemotherapeutic drugs in narrow subcellular organelles are more difficult to efflux in comparison with those dispersed distributed in the entire tumor cells. Thirdly, subcellular targeted therapy addresses issues related to excessive dosage due to premature leakage and drug resistance. Moreover, by accurately delivering drugs to their intended subcellular targets, off-target effects can be effectively avoided while ensuring controlled drug action and significantly reducing toxicities and side effects on normal tissues.

Numerous researchers have reported achieving enhanced therapeutic efficacy through subcellular targeting technology. Cheng et al. developed a mitochondria-targeting compound called mito-lonidamine (Mito-LND) to selectively inhibit the mitochondrial oxidative phosphorylation of tumor cells, which exhibited 100-fold higher potency compared to non-targeted lonidamine (LND) [95]. Furthermore, by modifying LND with a mitochondria-targeted ligand (TPP), the inhibitory effects on lung cancer growth and brain metastases were significantly improved as well (Figure 6). Additionally, Ma et al. designed peptide-coated platinum nanoparticles (TPP-Pt) that preferentially localized to mitochondria for precise NIR-II photothermal therapy [141]. Due to the thermal susceptibility of mitochondria, *in situ* hyperthermia triggered by TPP-Pt induces more severe mitochondrial damage and significantly enhances tumor ablation compared to the untargeted Lys-Pt group. Moreover, Pan et al. developed nuclear-targeted mesoporous silica nanoparticles (MSNs-TAT) and encapsulated the anticancer drug DOX within them [142]. Their study demonstrated that MSNs-TAT with a diameter below 50 nm achieved efficient intranuclear penetration and successfully delivered DOX to nuclear targets, leading to significantly improved anticancer activity. These studies underscore the indispensability of subcellular targeting technology for enhancing the therapeutic efficacy of anticancer drugs.

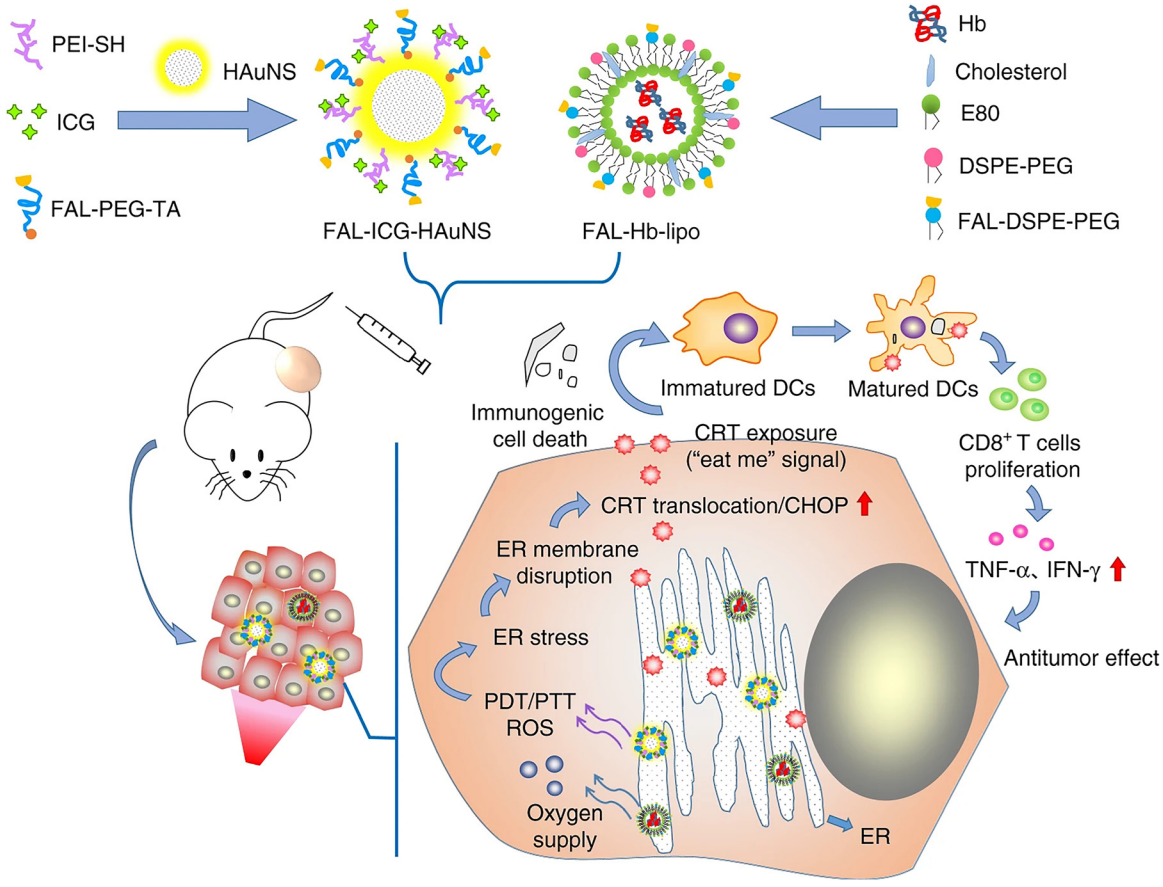

Figure 5: The antitumor mechanism of dual "ER missiles". The "ER missiles" cause severe ER stress by ER-localized ROS generation, leading to enhanced ICD and tumor suppression [58]. Copyright © 2019, the author(s). ER, endoplasmic reticulum; ROS, reactive oxygen species; ICD, immunogenic cell death.

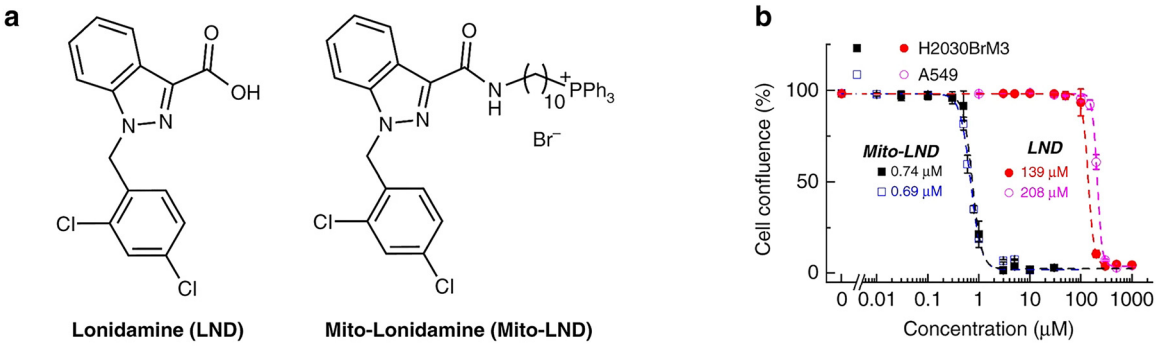

Figure 6: The structures and anti-proliferation effects of LND and Mito-LND. (a) Chemical structures of LND and Mito-LND. (b) Anti-proliferation effects of LND and Mito-LND on human lung cancer cells [95]. Copyright © 2019, the author(s). LND, lonidamine.

Enhanced immunological effect

The subcellular organelle ER is closely associated with the ICD of tumor cells [143]. ICD, characterized by calreticulin (CRT) exposure, high mobility group box 1 (HMGB1) release, and ATP secretion, can significantly enhance the immunogenicity of tumor cells and elicit adaptive immune responses [144, 145]. Remarkably, the induction of ER stress is essential for eliciting ICD, while excessive ER stress can disrupt calcium homeostasis and ultimately trigger the translocation of CRT from the ER lumen to the cell surface, where it serves as an "eat me" signal for APCs [146, 147]. Therefore, targeted therapy aimed at the ER can more efficiently enhance anticancer immune responses through precise induction of

sustained and severe ER stress. Existing ICD inducers are classified into two categories based on whether they directly act on the ER [12]. Type I ICD inducers indirectly induce collateral ER stress by targeting non-ER components. Most identified ICD inducers belong to this category including chemotherapeutic drugs (e.g., DOX, oxaliplatin, cyclophosphamide) and radiotherapy; however, their ability to induce ICD is limited [148, 149]. In contrast, type II ICD inducers selectively target the ER and induce more severe ER stress by directly disrupting its homeostasis, making them the most potent ICD inducers capable of eliciting highly effective immunogenic effects [150]. This highlights the significant importance of precise ER-targeting technology in enhancing immunological therapeutic outcomes.

However, there is still a lack of type II ICD inducers. Currently, only a few have been identified, such as oncolytic viruses and hypericin [151]. Furthermore, the prognosis following oncolytic virus treatment remains poor. Hypericin PDT utilizes visible light excitation with limited penetration depth *in vivo*, which restricts its clinical application [152]. Therefore, it is crucial to explore novel type II ICD inducers with selective ER-targeting ability that can specifically induce ER stress and potentiate the ICD effect for advancing tumor immunotherapy development. In this regard, significant efforts have been made by scientific researchers. For instance, Ma et al. designed a series of thio-pentamethine cyanine (TCy5) dyes [152], which represent the first developed ER-localizable ICD photoinducers. In order to achieve the ER-targeting ability, the TCy5 was further modified with polyfluorophenyl, which also has an additional function of increasing ROS generation ability. The resulting TCy5-Ph-3F effectively triggered ER stress and significantly amplified the ICD effect and immune therapeutic response. Additionally, Deng et al. developed a redox-responsive drug delivery nanosystem comprising Ds-sP nanoparticles loaded with an ER-targeting photosensitizer TCPP-T^{ER} [153]. Through this nanosystem, TCPP-T^{ER} could be selectively transported to the ER where it generated abundant ROS *in situ* to induce potentiated ER stress and ICD response, thereby enhancing immunotherapy (Figure 7). It should be noted that most reported ER-targeting photosensitizers are administered either as free photosensitizers or through physical encapsulation methods. However, these approaches may give rise to significant phototoxicity in normal tissues. Henceforth, there remains a pressing need for highly efficient and low-toxicity drug delivery systems that employ subcellular targeting technology to achieve precise ER-specific delivery of photosensitizers while concurrently enhancing immunotherapy.

In addition to ER, targeting therapies for other organelles, such as lysosomes and mitochondria, can also improve the efficacy of immunotherapy [154]. Li et al. developed a lysosome-targeted NIR type I photoinduced superoxide radical generator (ENBS), which selectively accumulated in lysosomes and disrupted their integrity, ultimately inducing cancer cells' pyroptosis and activating the antitumor immunity response [155]. Moreover, Jin et al. synthesized a mitochondria-targeting polymer micelle (OPDEA-PDCA), which efficiently targeted mitochondria to induce pyroptosis in osteosarcoma cells through activation of mitochondrial oxidative stress. When combined with an anti-PD-L1 monoclonal antibody, it significantly suppressed proliferation of osteosarcoma with prolonged T cell activation. These studies provide valuable guidelines for pyroptosis-enhanced cancer immunotherapy. Therefore, combining organelle-targeting therapies with immunotherapy, such as checkpoint inhibitors or CAR-T cell therapies, can synergistically achieve more effective and personalized cancer treatments [156]. This multi-pronged approach can also potentially overcome resistance mechanisms that cancer cells develop against single treatments. The development of organelle-targeted therapies, while complex, offers a new frontier in the battle against cancer.

Promoting new drug development and mechanism exploration

Currently, the identification of clear targets for certain drugs remains elusive, making it imperative to elucidate their interactions with intracellular structures and advance new drug development through subcellular targeting technology. Furthermore, emerging evidence suggests that distinct subcellular localizations of the same drug can lead to diverse therapeutic outcomes and biological effects. Therefore, subcellular targeting technology plays a pivotal role in identifying optimal drug targets and maximizing drug efficacy. Benhamou et al. demonstrated that when fluconazole, an antifungal agent, is localized within the ER, its potency exceeds 100-fold compared to mitochondrial or dispersed fluconazole, Moreover, it even exhibits effective killing against drug-resistant strains [157]. Thus, they speculated that the drug target of fluconazole is in the ER and revealed that delivering drugs to correct subcellular organelles can significantly enhance their efficacy. Huang lab also confirmed that the cytotoxic effect of the chemotherapeutic drug DOX varies significantly depending on its localization within different cellular compartments, such as the nucleus, mitochondria, and ER. Furthermore, Li et al. developed a single system (DSPE-PEG-Ce6) and conducted a comparative analysis of its photodynamic efficacy in different organelle-targeted therapies [158]. By leveraging the

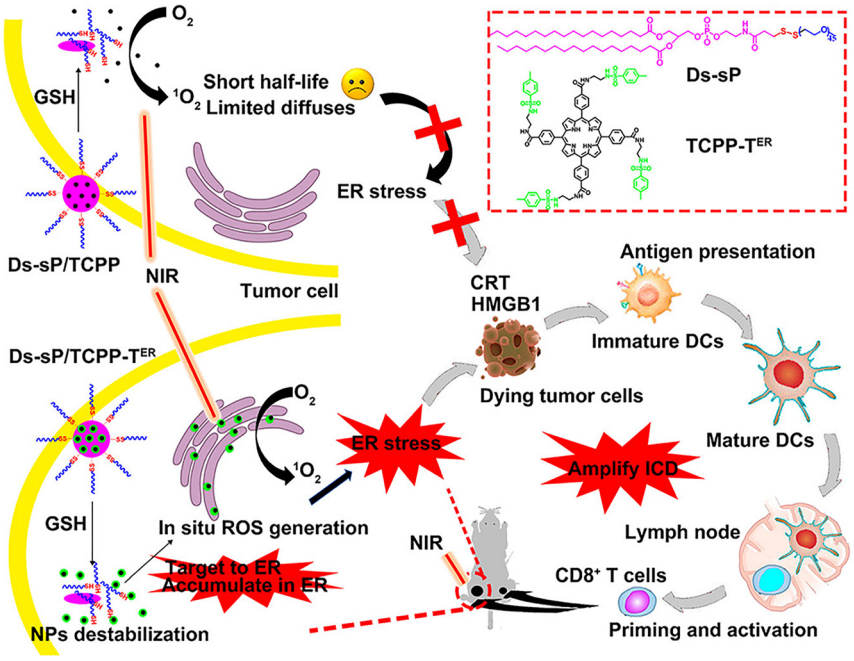

Figure 7: The design of a redox-responsive TCPP-T^{ER} nanoparticle that can selectively deliver photosensitizer to ER. Under NIR irradiation, ER-localized photosensitizer generated abundant ROS *in situ* to induce ER stress and potent ICD [153]. Copyright © 2020, American chemical society. ER, endoplasmic reticulum; ROS, reactive oxygen species; ICD, immunogenic cell death.

dynamic localization of DSPE-PEG-Ce6, which exhibited sequential movement from mitochondria to lysosomes and ultimately to the ER following cellular endocytosis, they accurately determined the order of PDT efficacy for each targeted organelle. Specifically, mitochondrion-targeted PDT demonstrated superior therapeutic effectiveness, followed by ER-targeted PDT as the second most effective approach, while lysosome-targeted PDT exhibited comparatively lower efficacy.

Moreover, our laboratory pioneered to investigate the impact of intracellular drug localization on biological effects [159]. Chen et al. indicated that the endosome maturation and subcellular trafficking can control the cancer cell death model and killing efficacy of nanophotosensitizers. Precisely induction of early endosome stress specifically evoked gasdermin-E-mediated pyroptosis, while the pyroptosis-inducing activity was dramatically reduced after photosensitizers transportation into late endosomes and lysosomes (Figure 8). The endosome maturation-tunable proptosis achieved up to 40-fold and 20-fold increase in anti-tumor efficacy *in vitro* and *in vivo*, respectively. Zeng et al. also designed a series of subcellular-targeting photosensitizers that precisely localize reactive oxygen species to subcellular regions, including mitochondria, lysosomes, and ER, and explored their potential in inducing pyroptosis and ICD [160]. In addition, Shao et al. studied the influence of subcellular localization on the biological efficacy of Hsp70 inhibitors and found that the subcellular distribution of these inhibitors significantly affects their anti-tumor and antiviral effects [161]. Fine-tuning the subcellular targeting of these drugs enhances therapeutic outcomes while minimizing side effects, offering a novel and innovative approach to pharmaceutical development. These findings fully illustrate the complexity and significance of drug-target interactions. Exploring appropriate subcellular targets and directly disrupting subcellular structures through organelle-specific targeted drug delivery can greatly reduce drug dosage, improve therapeutic efficiency, and minimize side effects, which is also vital for new drug development.

Opportunities and challenges of subcellular targeted therapy

Despite significant advancements, subcellular targeted drug delivery still faces numerous unknown factors and untapped potential, presenting immense challenges while offering vast opportunities for development. Enhancing the efficiency of drug delivery to subcellular targets or modulating the interaction between drugs and targets has emerged as a critical scientific concern that requires urgent resolution in the field of subcellular targeted drug delivery.

Currently, subcellular targeted strategies are primarily categorized into two types, small-molecules based targeting strategy and nanomaterial-based targeting strategy [162]. Small-molecules based subcellular targeting is typically accomplished by direct modification of the small-molecule drug with a targeting group. This approach offers significant advantages at the cellular level, including high efficiency in

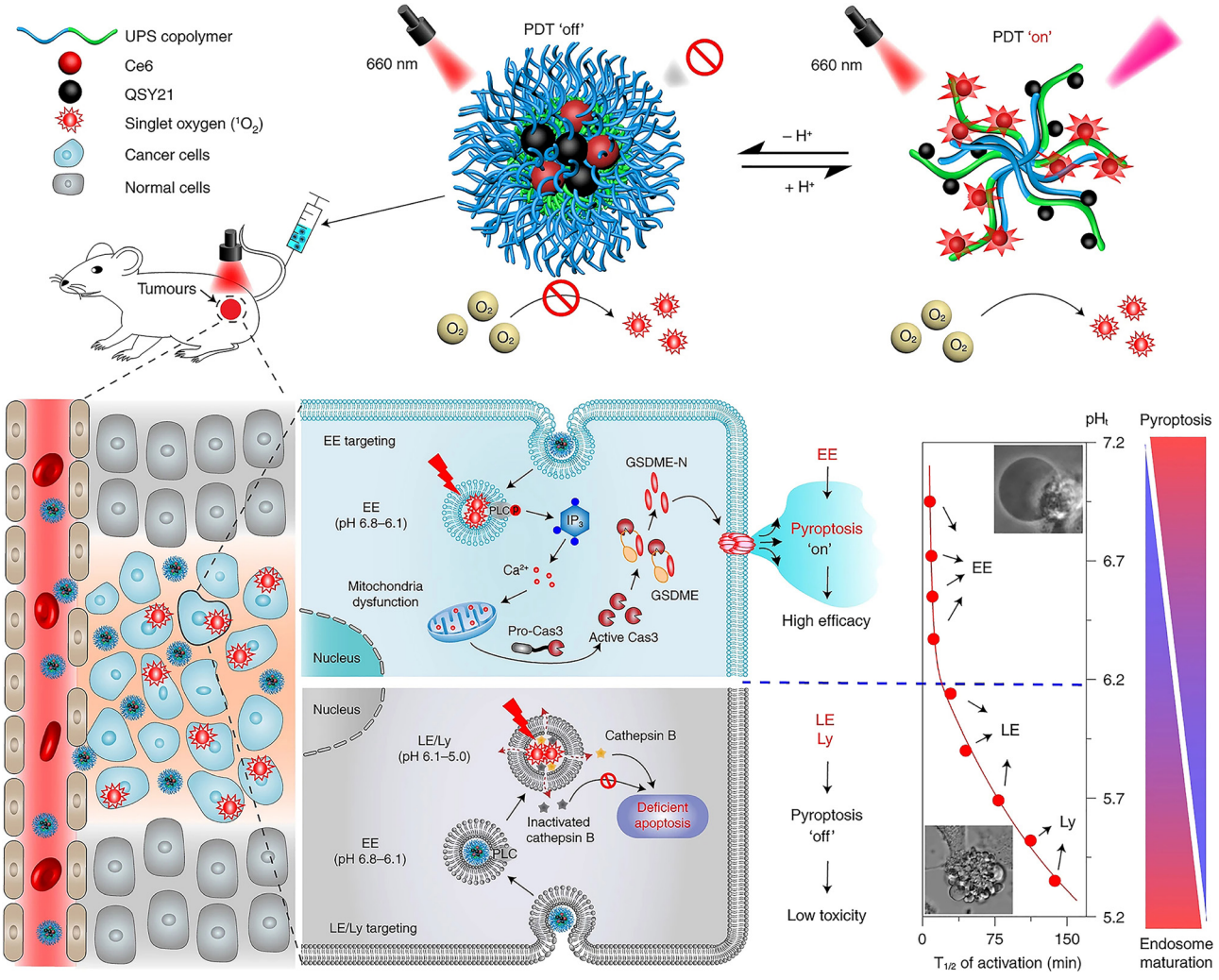

Figure 8: Schematic illustrations of the tunable pyroptosis by delivering to different endocytic organelles (early endosomes or lysosomes) [159]. Copyright © 2022, the author(s).

subcellular targeting, convenient and flexible structure design, and rapid diffusion speed that enables complete subcellular targeting within 10 min [108]. Therefore, numerous commercially available fluorescent trackers of organelles have been designed based on this strategy. In addition, Luo et al. designed a mitochondrial-targeted synergistic anticancer prodrug by conjugating organoarsenic compounds with cisplatin or DOX through reductive responsive bonding [84]. The TPP group's targeting ability enables selective localization of these prodrugs in the mitochondria. Upon reduction, the organoarsenic compounds can disrupt mitochondrial respiratory chain function and enhance ROS production within the mitochondria. Combined with the chemotherapeutic drug's effect on mitochondrial DNA damage, this synergistically induces destruction of mitochondrial structure, release of cytochrome C, and augmented cell cytotoxicity. These findings underscore the promising potential of small-molecule-based targeting strategies for effective cancer therapy. However, this approach also presents certain limitations [163]. For instance, the *in vivo* applications of small molecular drugs may encounter challenges such as rapid clearance, suboptimal pharmacokinetics, and extensive tissue distribution, which can potentially compromise therapeutic efficacy and give rise to adverse effects. Furthermore, the presence of multiple active sites in certain small-molecule drugs poses difficulties for their conjugation with targeting ligands. The targeting group modification may alter the drug's binding affinity for its receptor, affect the drug's selectivity towards its intended target, and change the drug's pharmacokinetic properties, which can have significant implications for the therapeutic efficacy and safety profile. Therefore, it is crucial to consider whether the modification of targeted groups would preserve the pharmaceutical activities of these drugs, limiting the selection of drugs suitable for subcellular

targeted strategies. To address these challenges, several strategies can be employed: (1) Targeting ligand optimization: Careful selection and optimization of targeting ligands can minimize disruptions to the drug's binding affinity and selectivity. This may involve fine-tuning the ligand's structure to ensure no interference with the drug's active sites; (2) Computational modeling: Techniques such as molecular docking and dynamics simulations can predict the impact of modifications on the interaction between drug with its target, helping to design modifications that are less likely to disrupt activity; (3) Prodrug strategies: Designing prodrugs that are selectively activated at the target site can help maintain the therapeutic activity of drug while avoiding premature interactions with non-target sites [164]. This approach can be particularly useful when the drug's active form has multiple active sites that could interfere with targeting ligands.

Nanomaterials, with their unique properties, are revolutionizing cancer therapy [165, 166]. They enable targeted drug delivery, enhance therapeutic efficacy, and overcome drug resistance. Moreover, their multifunctionality and improved pharmacokinetics render them invaluable tools for both diagnosis and treatment [167]. Nanomaterial-based subcellular targeting strategy refers to the transport of the entire nanocarrier to specific subcellular organelles for drug delivery, achieved through surface modification with ligands that specifically target these organelles (e.g., small molecules, peptides) [168]. According to this strategy, Gao et al. designed a reduction-sensitive nanogel based on disulfide bonds [169]. The surface of the nanogel was multivalently modified with triphenyl phosphonium and benzene boronic acid for targeting mitochondria and nuclei, respectively. This work utilizes the programmable "plug to direct" design concept, allowing easy control of subcellular localization by changing the targeting group on the nanogel. Moreover, chemotherapeutic agents (paclitaxel and DOX) can be encapsulated within the nanogel matrix. Upon intracellular reduction-triggered disassembly of the nanogel, these drugs are precisely released at their designated subcellular sites, resulting in targeted drug delivery and enhanced therapeutic efficacy. Compared to the small-molecules based targeting strategy, nanocarrier targeting strategy offers significant optimization for *in vivo* applications. It exhibits advantages such as prolonged circulation within the body and reduced toxicity [170]. Moreover, nanocarriers can safeguard unstable drugs from external factors, thereby enhancing drug stability and pharmacological activity [171]. However, unlike small molecules with high transmembrane ability, the large volumes of nanocarriers pose challenges in crossing organelle lipid bilayers. Furthermore, a fraction of nanocarriers internalized through endocytosis may be sequestered by lysosomes [172], hindering the subcellular targeting role of surface-modified ligands. Additionally, the stability and on-command release of drugs encapsulated in nanocarriers should also be considered, while chemical bonding methods offer excessive stability, physical encapsulation methods are prone to premature leakage.

The aforementioned challenges in subcellular organelle targeting provide us with valuable insights for future development. Firstly, there is immense potential to construct modular smart nanocarriers for targeted drug delivery to specific suborganelles. Smart nanocarriers, which primarily refer to stimuli-responsive nanocarriers [173], can achieve controlled drug release in response to internal (e.g., redox, pH) or external (e.g., ultrasound, light, magnetism) stimuli [174]. This strategy combines the advantages of small molecule and nanocarrier targeting, prolonging circulation time within the body while minimizing premature drug leakage before reaching the organelle, thus ensuring stable drug delivery and efficient subcellular targeting [175, 176]. Secondly, if the nanocarrier enters the cell through endocytic pathways, it is crucial to ensure successful escape of organelle-targeted drugs from lysosomes in order to avoid degradation and preserve their targetability. Lastly, comprehensive consideration of biosafety is essential. Developing safer and more biocompatible organelle-targeted nanocarriers that can be excreted by the body represents an important research direction for future studies and serves as a prerequisite for clinical translation of subcellular organelle targeting therapy.

Conclusions

Subcellular targeting strategy for precise cancer therapy is an emerging technology with immense potential. In this comprehensive review, we elucidate the structural characteristics and diverse targeting strategies employed for four major organelles – lysosomes, mitochondria, nucleus, and ER. We emphasize the significance of subcellular targeting in cancer therapy by highlighting its ability to improve therapeutic efficacy, enhance immunological effects, and facilitate new drug development. Despite significant progress in recent years and remarkable performance in cancer treatment, subcellular targeted therapy still encounters several challenges that require attention prior to clinical translation. Looking ahead, with the rapid advancements in nanotechnology and collaborative efforts from scientists worldwide, subcellular targeted therapy holds great promise for revolutionizing current cancer treatment approaches and has the potential to benefit a larger number of patients.

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Informed consent: Not applicable.
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