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REVIEW

Novel Approaches of Dysregulating Lysosome Functions in Cancer Cells by Specific Drugs and Its Nanoformulations: A Smart Approach of Modern Therapeutics

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Abstract: The smart strategy of cancer cells to bypass the caspase-dependent apoptotic pathway has led to the discovery of novel anti-cancer approaches including the targeting of lysosomes. Recent discoveries observed that lysosomes perform far beyond just recycling of cellular waste, as these organelles are metabolically very active and mediate several signalling pathways to sense the cellular metabolic status. These organelles also play a significant role in mediating the immune system functions. Thus, direct or indirect lysosome-targeting with different drugs can be considered a novel therapeutic approach in different disease including cancer. Recently, some anticancer lysosomotropic drugs (eg, nortriptyline, siramesine, desipramine) and their nanoformulations have been engineered to specifically accumulate within these organelles. These drugs can enhance lysosome membrane permeabilization (LMP) or disrupt the activity of resident enzymes and protein complexes, like v-ATPase and mTORC1. Other anticancer drugs like doxorubicin, quinacrine, chloroquine and DQ661 have also been used which act through multi-target points. In addition, autophagy inhibitors, ferroptosis inducers and fluorescent probes have also been used as novel theranostic agents. Several lysosome-specific drug nanoformulations like mixed charge and peptide conjugated gold nanoparticles (AuNPs), Au-ZnO hybrid NPs, TPP-PEG-biotin NPs, octadecyl-rhodamine-B and cationic liposomes, etc. have been synthesized by diverse methods. These nanoformulations can target cathepsins, glucose-regulated protein 78, or other lysosome specific proteins in different cancers. The specific targeting of cancer cell lysosomes with drug nanoformulations is quite recent and faces tremendous challenges like toxicity concerns to normal tissues, which may be resolved in future research. The anticancer applications of these nanoformulations have led them up to various stages of clinical trials. Here in this review article, we present the recent updates about the lysosome ultrastructure, its cross-talk with other organelles, and the novel strategies of targeting this organelle in tumor cells as a recent innovative approach of cancer management.

Keywords: lysosome, cancer, lysosomotropic agents, nanoparticles, lysosome drug targeting

Introduction

Previously, the lysosomes were just thought to be static, enzyme-loaded, membrane-enclosed organelles, mainly involved in the degradation and recycling of different macromolecules.[1] This limited view of lysosomes has been dramatically overturned by some recent discoveries. Lysosomes are dynamic organelles which participate in vast number of cellular activities in addition to the degradation of

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different biomaterials. The different activities of lysosomes include roles in metabolic signalling, gene regulation, plasma membrane repair, cell migration and adhesion.[2] The lysosomes also contribute in the regulation of environmental stimuli and immune response, especially the contribution to macrophages in both innate and adaptive immune responses. These organelles are the final destination of engulfed pathogens by macrophages, which also contribute in processing and secretion of inflammatory signals, and produce peptides which bind with major histocompatibility complex (MHC) molecules.[3]

Multitude of innate and adaptive mechanisms are deployed by the immune system to prevent malignant transformation as well as ward off pathogens (immune surveillance). An apparent tumor represents malignant cell clones having the capability to evade immune recognition (immune evasion).[4] The treatment of cancer since last decade has been revolutionized by promoting antitumor immune response in patients with different tumor types. The important therapeutics that target T cell inhibitory checkpoint proteins like PD(L)1 and CTLA-4, are effective in different cancers, leading to tumor burden reduction and increased long-term survival of patients. The significant effects of these immunotherapies have encouraged for additional measures that modulate antitumor immunity through effects on T cell, myeloid cells and other cell types within tumor microenvironment.[5]

A typical mammalian cell can have between 50 to 1,000 lysosomes, depending upon its role in the different cellular activities. The numbers, activity and internal composition of lysosomes vary continuously in response to the environmental cues. The lysosomes significantly communicate with other organelles and cellular structures by constantly exchanging their information and contents. The position of lysosomes within the cell is dynamically regulated, so any dysregulation of these activities may result in different diseases. Lysosomal dysfunctions could result in rare lysosome storage diseases (LSD), almost (1:5,000 cases). These LSD include common metabolic and neurodegenerative diseases, in addition to cancer.[6]

Lysosomes receive cargoes which are destined for degradation or recycling through endocytosis or autophagy. In cancer cells, the routine signalling pathways are dysregulated, which leads to the variation in lysosomal structure and functions.[7] So, these activities make the cancer cells more susceptible to lysosome membrane permeabilization (LMP) by different endogenous (oxidative stress, p53 activation) and exogenous (cationic amphiphilic drugs) triggers.

Cancer cells practice a smart strategy of bypassing LMP facilitated lysosomal cell death (LCD) by the caspase-dependent apoptotic pathway. This strategy is believed to be a novel target of apoptosis and drug resistant cancer cells.[8] Some important drugs used include antihistamine, antimalarial and anticancer drugs. Furthermore, drugs used to screen LMP like fluphenazine, thioridazine or toremifene, are under clinical trials, as they are known to achieve lysosomal accumulation, thus leading to LMP.[9]

Drug loaded-nanoparticles/nanoformulations have revolutionized the therapeutic and diagnostic strategies. But these approaches can lead to toxicity concerns, so extra efforts are required to focus on their physicochemical properties and targeting strategies.[10] To understand comprehensively the novel direct or indirect lysosome dysregulation target sites within the cancer cells, it is important to understand the recent advances in lysosomal ultrastructure and its role within the cells. In addition, the mechanism of action of lysosome-targeted anticancer drugs and their formulations is important to understand to engineer more specific antitumor drugs and their nanoformulation in future. Here, in this review, we describe in detail the recent updates regarding the lysosome ultrastructure, its signalling and crosstalk with other organelles, the lysosomal dysregulation during cancerous state, and the novel strategies of direct or indirect lysosome targeting in cancer cells with drugs and their nanoformulations.

Lysosome as Eukaryotic Cell Organelle

Lysosomes are housekeeping organelles that perform the enzymatic degradative functions in coordination with cellular metabolism. These organelles are usually 0.2–0.3 μm in diameter and they originate from the Golgi apparatus. Recently, multiple models have been proposed for the biogenesis of lysosomes.[11] These organelles can move around the cytoplasm, change their shape and size, and/or can undergo fission and fusion. The lysosome's lumen is acidic with pH between 4.5–5.0 which is maintained by the action of proton pumps in their membrane.[12] Different types of enzymes which can digest proteins, carbohydrates, nucleic acids and lipids are present in lysosomes. Among those are the cathepsin proteases which represent a diverse enzyme family.[13]

The lysosome contains lipid bilayer which forms the external limiting membrane containing vast numbers of embedded proteins and intraluminal vesicles (Figure 1). Like other cellular membranes, the lysosomal membrane composition is properly regulated.[14] However, unlike other cellular membranes, the proportion of sphingolipids and glycerophospholipids is richer in the lysosomal membrane, besides having a rich amount of bis (monoacylglycerol) phosphate (BMP).[15]

The lysosomes are covered by a lipid bilayer embedded with different types of proteins among which lysosome associated membrane protein 1 and 2 (LAMP-1 and -2) are most abundant. LAMP (-1 and -2) constitute almost half of the lysosomal transmembrane proteins. They are highly glycosylated at luminal N-terminal domain, and are essential for the lysosomal structural integrity.[16] The glycosylated tails of these proteins form a sugar coat (glycocalyx), to protect the lysosomal membrane from hydrolase degradation. The LAMPs also serve in lysosomal trafficking, chaperone-facilitated autophagy, exocytosis, autophagosome-lysosome fusion and cholesterol transport.[17]

Besides the lysosomal integral membrane protein 2 (also identified as SCARB2) and v-ATPase, the lysosome lipid bilayer also contains some other proteins like lysosome integral membrane protein 1 (LIMP1) also known as

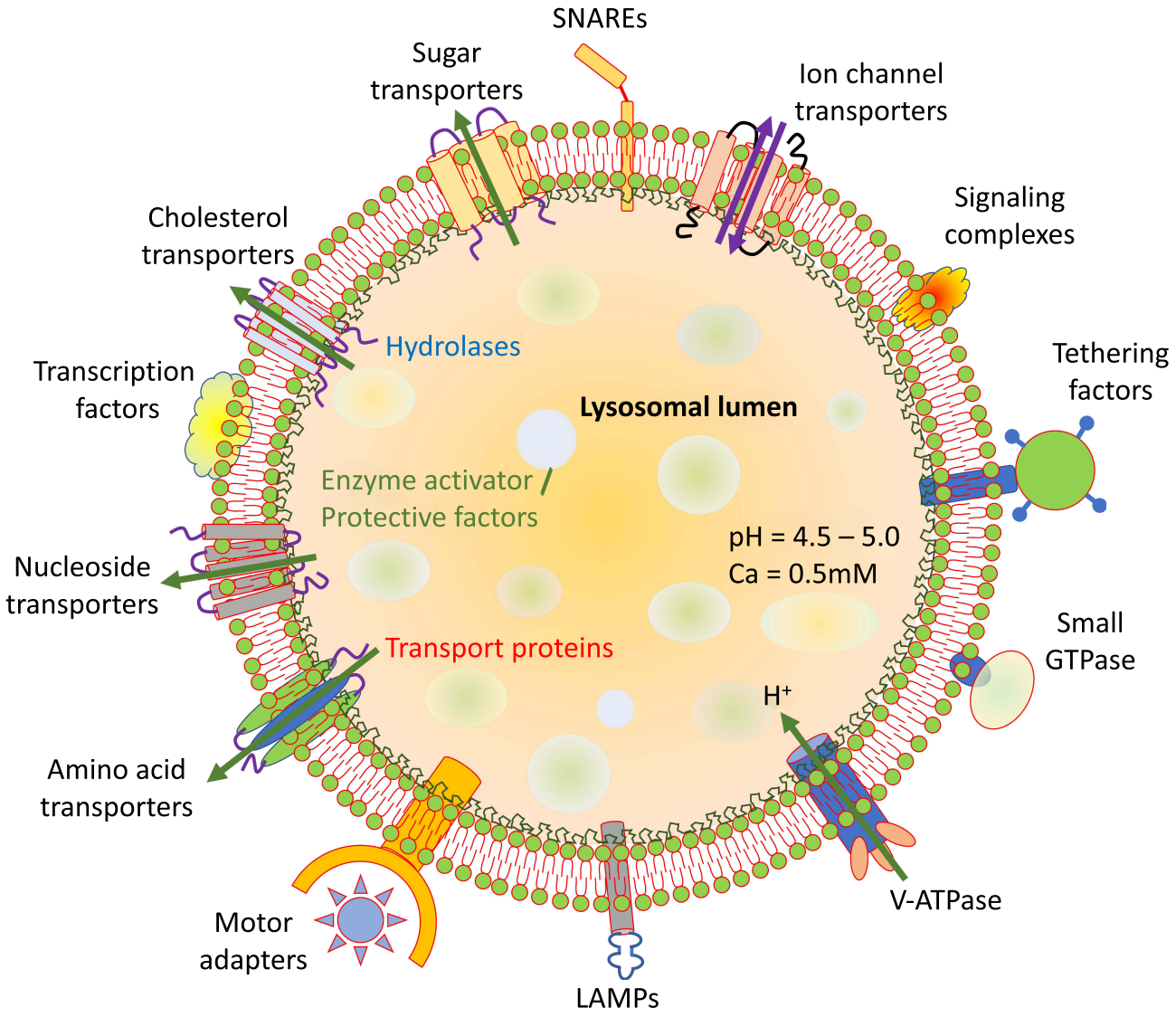

Figure 1 General structure and properties of lysosomes. Lysosome lipid bilayer with peripheral and integral membrane proteins with different functions.

tetraspanin or CD63 in addition to other less characterized transport proteins.[18] The luminal side of these proteins is generally highly glycosylated that gaps the lysosomal membrane from stored digestive enzymes (Figure 1).[19]

The final step during autophagy results in the fusion of autophagosomes and lysosome and this step is facilitated by the soluble NSF (N-ethylmaleimide-sensitive factor) attachment receptor (SNARE) proteins. Among the different SNARE proteins, the lysosome localized SNAREs include vesicle-associated membrane protein 7 or 8 (VAMP7 or VAMP8) and Q-SNAREs are autophagosome-localized which includes STX17 and SNAP29. Recent studies have revealed the role of R-SNARE and YKT6 in lysosome-autophagosome fusion as well.[20]

Multiple fission and fusion events take place along the endo-lysosomal system for the transportation of proteins and lipids. The mechanism of membrane fusion at endosome and lysosomes takes place by two homologous tethering complexes known as homotypic fusion and vacuole protein sorting (HOPS) and class C core vacuole/endosome tethering (CORVET). Both of these two complexes are hetero-hexamers and interact with Rab GTPases and SNAREs and tether the membranes. CORVET is a Rab5 effector complex while HOPS binds efficiently to late endosomes and lysosomes via Rab7.[21]

The lysosomes are constantly engaged in functional and physical interactions with other cell organelles either with signal transduction or by membrane contact sites. The master regulators like transcription factor EB (TFEB) and microphthalmia associated transcription factor (MITF) control the lysosomal biogenesis. These proteins sense the signals from the cytoplasm and are translocated to the nucleus to start the induction of lysosomal biogenesis network gene transcription.[22] In cytoplasm, mammalian target of rapamycin complex 1 (mTORC1) phosphorylates the MITF and TFEB and are retained there by binding to 14-3-3 proteins.[23] During the stressful condition, mTOR pathway is inhibited that leads to the activation of lysosomal biogenesis.

Lysosomal Signalling and Cross-Talk with Other Organelles

Previously the lysosomes were thought to be in relative isolation, but recent years have witnessed that these organelles are fully engaged with all other organelles within the cell (Figure 2). Lysosomes possess a unique position to get information about various biomolecular degradation and recycling events as this organelle can sense the nutritional status of a particular cell. A key factor in this nutrient-regulation mechanistic process is performed by mTORC1, known as a chief regulator of cellular biosynthetic pathways.[24] This complex is dynamically associated with lysosomes, which supports the cell growth and anabolism by sensing the growth factors and nutrients. This complex also inhibits the catabolic pathways like autophagy by mediating the phosphorylation of Unc-51-like kinase 1 (ULK1).[25] mTORC1 can also regulate the lysosome reformation at the time of autophagy, and helps to restore the organization of mature lysosomes during prolonged starvation.[26] The recruitment of mTORC1 on lysosomal surface initiates its activation, which is mediated by resistance associated gene (RAG)-GTPase.[27] The lysosomal surface recruitment of mTORC1 is also mediated by cholesterol-binding Niemann-Pick type C1 protein (NPC1). The RAG GTPase also modulates the recruitment of some more nutrient-responsive elements like tuberous sclerosis complex (TSC) and folliculin-interacting protein 1 (FNIP) complex and TFEB. The TFEB is a master modulator for lysosome biogenesis and autophagy (Figure 2).[22]

The lysosome regulates some of its important functions through the release of Ca^{2+}, which includes its fusion with other organelles and structures like plasma membrane, endosomes and autophagosomes (Figure 2).[28] There are three main kinds of Ca^{2+} channels on lysosomal membrane which includes trimeric Ca^{2+} two transmembrane channel (P2X4), two-pore channels (TPC) and trimeric transient receptor potential cation channel of the mucolipin family (TRPML).[28] The Ca^{2+} mediated interaction results in repair of membrane damage, endocytic membrane trafficking and autophagy. In addition, the Ca^{2+} release from lysosomes also results in the formation of contact sites with endoplasmic reticulum (ER), which in turn refills back the lysosome with Ca^{2+}.[29] The Ca^{2+} homeostasis is also significant for the proper functioning of other organelles and the acidification within lysosomal lumen, an important requirement for the activity of lysosomal hydrolases (Figure 2).[30]

The lysosomal Ca^{2+} channels can respond to different stimuli like pH, stress, nutrient level as well as the level of phospholipids, sphingosine, ATP and NADP. The stimuli of all these molecules with the Ca^{2+} channels suggests that their activities are differentially modulated, which depends on the overall cell conditions.[31] The TRPML1 also called as mucolipin 1, lysosomal Ca^{2+} channel is the most

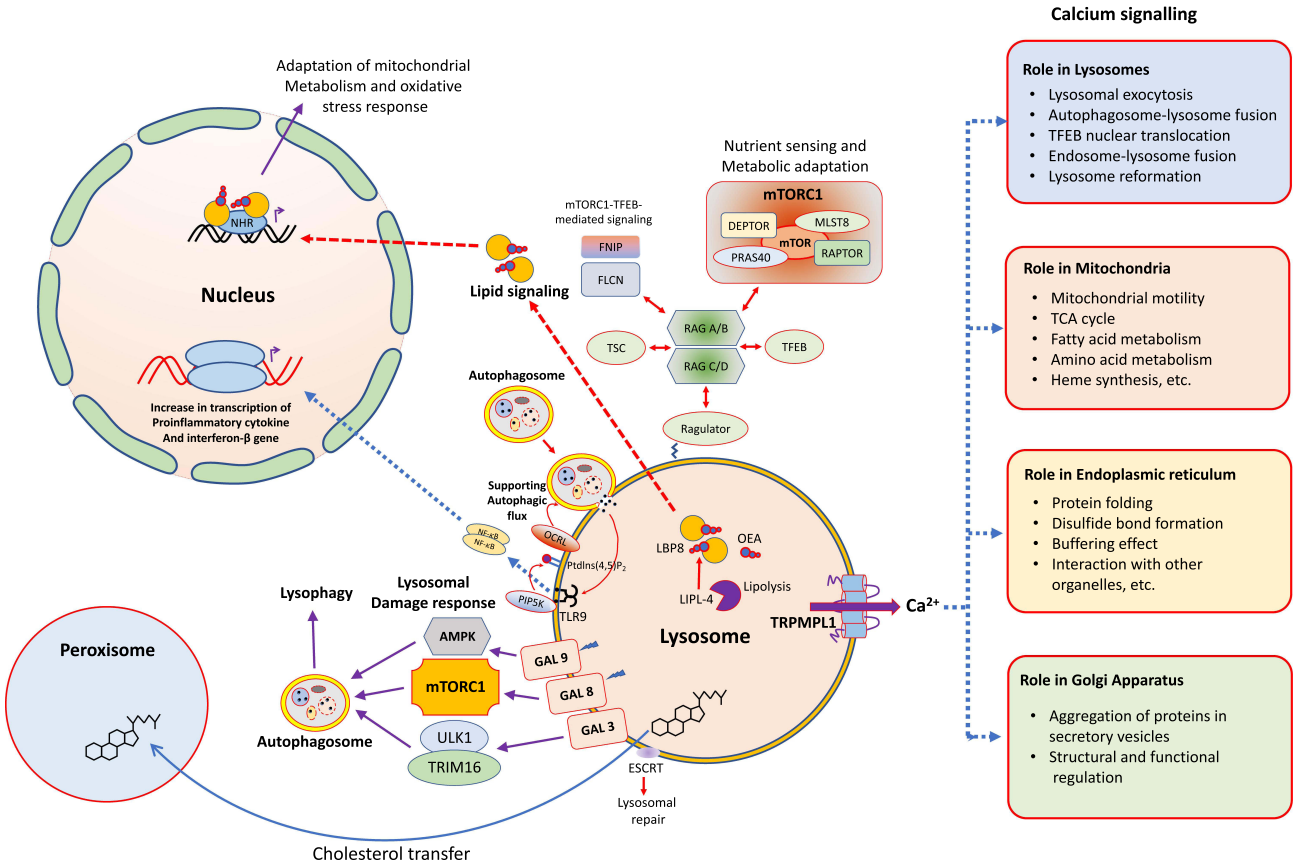

Figure 2 The lysosome as an intracellular signaling core. Different types of cellular processes being controlled by signaling pathways and commenced from the surface of lysosomes.

characterized which mediates the release of Ca^{2+} from the lysosomes and gets activated by several stimuli like reactive oxygen species (ROS)[32] and starvation.[33] This channel is also activated by phosphatidyl inositol-3,5-bisphosphate (PI-3,5-bisP), which associates the lysosomal Ca^{2+} signaling to some intracellular trafficking (Figure 2).[34]

The TRPML1 also makes a positive-feedback loop with TFEB, where TRPML1 regulates the phosphorylation and subcellular localization of TFEB. In back, TFEB regulates the gene expression of TRPML1.[35] The activity of TRPML1 is also linked with some cellular processes in immune cells, which includes large particle phagocytosis.[36] In addition, TRPML1 mediates TFEB for the promotion of intracellular clearance of accumulated substrates in LSDs.[35] Collectively, all these characteristics of TRPML1 makes it a novel target for some pharmacological modulations in variety of diseases, including cancer. Lysosomes are damaged by its rupture or membrane permeabilization during several circumstances like the severe infection or by the use of lysosomotropic drugs. This damage leads to cathepsin leakage, which promotes to the progression of programmed cell death.[37]

The lysosomes can fuse with other lysosomes (homotypic fusion) in addition to plasma membrane, autophagosomes, late endosomes, macropinosomes and phagosomes (heterotypic fusion). These fusion activities are mediated by the assembly of trans-SNARE complex, which is composed of one R-SNARE and multiple Q-SNAREs. These fusions are promoted by the Ca^{2+} release from the lysosomes. Similarly, each organelle can fuse with lysosome through a specific trans-SNARE complex and some precise regulators (Figure 2). Some recent studies have shown that the lysosome and autophagosome fusion is regulated negatively by mTORC1, which promotes the tumor suppressor protein, ultra violet radiation resistance associated gene (UVRAG) phosphorylation. This leads to enhanced Rubicon interaction and diminished HOPS interaction, so inhibiting the fusion events.[38]

The lysosomal exocytosis leads to the fusion of lysosomes with the plasma membrane and this process is mediated by some novel lysosomal functions that includes plasma membrane repair.[39] In addition, the fusion of

lysosomes also leads to the formation of cancer cell invasive protrusions[40] and the lysosome content secretion into the extracellular space, that occurs usually in bone resorption.[41]

The discovery of non-fusogenic contacts of lysosomes with other organelles like Golgi complex, endoplasmic reticulum (ER), peroxisomes and mitochondria is quite recent. These contacts require organelle-specific tethering proteins.[42] These interactions lead to the maturation of early to late endosomes and then to lysosomes, accompanied by increased contacts with the ER.[43] These contacts lead to ER tubular rearrangements and the budding of endosomal tubules.[44] The ER-lysosomal contacts also make non-vesicular transfer of lipids between these two organelles as free cholesterol formed in lysosome is exported out of lysosome by the action of NPC (-1, -2). In contrast STARD3 mediates the cholesterol transfer in opposite direction.[45]

Lysosomes and Cancer Progression

Cancer cells possess relatively fragile and bigger lysosomes as compared to the normal cell counterparts.[46] Many types of cancers have altered sphingolipid metabolism, overexpressing sphingosine kinase and downregulating acidic sphingomyelinase.[47,48] All these regulations affect the lysosomal membrane function and structure. The cancer cells show increased lysosomal biogenesis which also results in lysosomal enlargement.[49] These enlarged lysosomes engulf a significant amount of chemotherapeutic drugs and block it to reach their final destination. In addition, lysosomes arrange a mechanism for the exocytosis of such drugs from cancer cells.[49] All these means render a cancer cell drug-resistant, thus emphasizing the lysosomes as a novel strategy as drug targets for cancer therapy. Furthermore, the enhanced activity of phosphatidyl inositol-3 kinase (PI3K), a characteristic feature of cancer cells, confers stability to the tumor cell lysosomes.[50] Several cellular processes like size, maturation, and lysosome activity are regulated by PI3K.[51] The PI3K inhibition shifts the TNF-facilitated cell death cascade from caspase-dependent mode to cathepsin-dependent way.[52]

Lysosomes play an important role in cancer biology as this organelle commands to fuel the enhanced requirement of energy sources during the tumor progression.[53] Several cancers related to breast, lung, pancreatic, and prostate, etc. have been found to rely mainly on the lysosomal-induced autophagic degradation and the recycling activities, which acts like nutrient-scavenging pathways.[53] The lysosomal-autophagic pathways support the proliferation and cancer cell growth like melanoma, renal cell carcinoma, pancreatic adenocarcinoma, etc., as it is associated with the overexpression of microphthalmia-transcription factor E (MiT-TFE) genes. In these cancer types, the mTORC1 induction by MiT-TFE permits the associated hyperactivation of mTORC1-facilitated and autophagy-reconciled nutrient scavenging biosynthetic pathways.

Thus, the unusual catabolic and biosynthetic activation together supports the energy demanding cancer metabolism within the tumor cells.[54] The acidic medium of tumor cells leads to lysosome redistribution towards the periphery of the cells,[46] and this activity enhances the tissue proliferation by enhanced mTORC1 and mTORC2 signaling.[55] This change also leads to the exocytosis of lysosomal hydrolases, matrix metalloproteinases, and integrins that enhance the invasion and metastasis steps.[56] The cancer cells possess remarkable proteolytic activity, that assists it to digest the extracellular matrix (ECM). The cancer invasion is associated with increased expression of cathepsin B and other cysteine cathepsins.[57] The cathepsins released from the cancer cells degrade the ECM components like laminin, elastin, and fibronectin, which eases the invasion, angiogenesis, and metastasis.[58]

The cancer cells are efficiently successful to develop chemotherapeutic resistance, which depends upon pro-apoptotic pathways modulation and the modifications of lysosome-facilitated cell death pathways. Cancer cells overexpress cytosolic and lysosomal protease inhibitors which inhibit the LMP.[59] In addition, the cytosolic heat shock protein 70 (Hsp70) gets translocated to the lysosomal lumen within the cancer cells. This translocation helps to stabilize the lysosomal membranes by endorsing the acid sphingomyelinase activity.[60] This procedure also helps to protect the tumor cells against cytosolic leakage of lysosomal proteases. Any depletion of Hsp70 leads to a lysosome-facilitated cell death program.[61] In addition, the cancer cell viability is enhanced by cathepsin inhibitors, with augmented lysosomal activity.[62] These findings show that the drug nanoformulations which can target the P13K and the lysosomal Hsp70 accumulation or the cathepsin activity, may work as novel therapeutic agents to make the cancer cells susceptible to lysosome mediated cell death.[63]

Lysosome Targeting in Cancer Cells

For the purpose of proliferation and adaptation to a new environment, cancer cells adapt with increased lysosomal functions. Different factors like p53, Bcl-2 family members, sphingosine, and oxidative stress are altered during the cancerous state and these alterations can lead to increased LMP.[64] Lysosomal disruptions in turn enhance the oxidative stress further, which promotes the lipid peroxidation, mitochondrial dysfunctions, and autophagy. All these alterations lead to cathepsins release, which promotes the degradation of different macromolecules. In addition, these changes can trigger cancer cell death through autosis, apoptosis, or ferroptosis.

Lysosomes perform their role both during catabolic (macropinocytosis and autophagy) and anabolic pathways, as driven forward by mTORC1. All these pathways are potential targets in cancer therapy. Autophagy leads to the delivery of cellular materials to the lysosomes for degradation and it performs multiple functions in cancer progression.[65] As autophagy can have both antitumor and protumor effects, the recent efforts of targeting the cancer cell autophagy as a treatment strategy is given a high priority. Autophagy has the ability to promote the tumor growth and facilitates the chemoresistance during cancer therapy.[66] Macropinocytosis leads to the delivery of extracellular proteins to lysosomes, so can promote the cancer growth, especially in RAS-driven cancers.[67] The inhibition of mTOR functions is widely recognized as anticancer therapy in preclinical trial patients.[68]

Some antimalarial drugs like quinacrine, chloroquine, and hydroxychloroquine have been found to inhibit the lysosomal functions by inhibiting the autophagy cascade.[66] These drugs are used as anticancer agents but have no effect on mTORC1 regulation.[69] Chloroquine possess the DNA binding capacity and its dimerization increases its potency as an autophagy inhibitor. Similarly the dimerization of other antimalarial drugs like quinacrine has been found to surpass the tumor growth.

A simple model of drug action by dimeric quinacrine 661 (DQ 661) in cancer cells is represented in Figure 3 as an example to understand the basic mechanism of its action as anticancer activity. DQ661 has been used as a photo-labeling probe to recognize its molecular target as palmitoyl-protein thioesterase 1 (PPT1). This enzyme is used for depalmitoylation of proteins and DQ661 targets the tumor cells by binding and inhibiting PPT1. In addition, this drug possesses multitarget binding activity as it blocks lysosomal activity and some major catabolic functions of macropinocytosis and autophagy and also inhibits mTORC1. So this drug can block the degradative as well as signaling functions of lysosomes. DQ661 inhibits mTOR by disrupting the complex at lysosomal membrane and prevents the amino acid-dependent regulation of mTORC1 kinase activity (Figure 3).

All these findings show that DQ661 possess in vivo anticancer activity against different tumor models in both immunodeficient and immunocompetent models. These findings show that lysosome inhibition by these drugs is

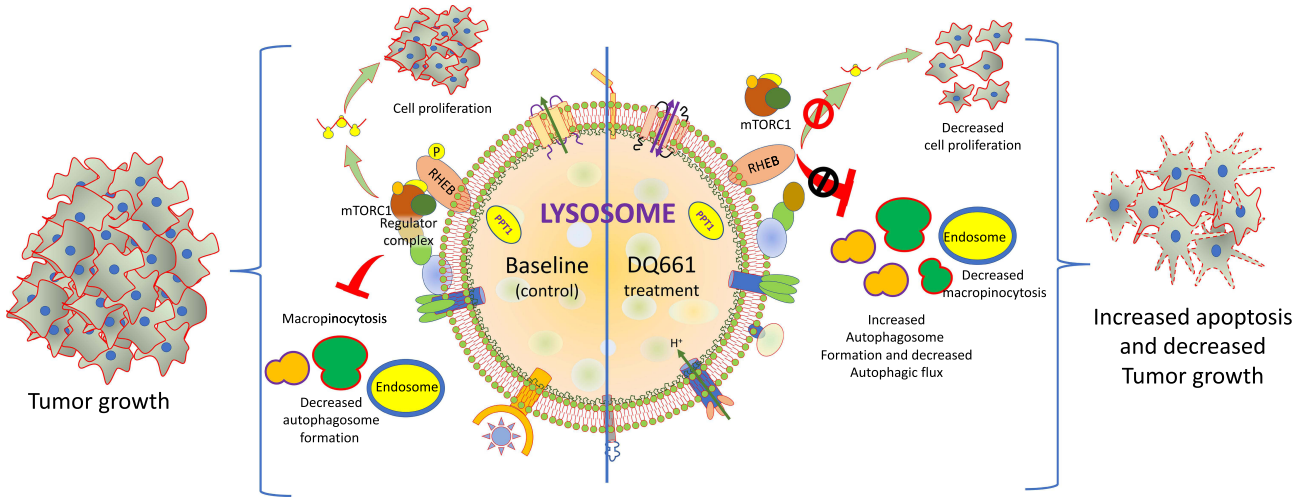

Figure 3 The inhibition of PPT1 by DQ661 and the regulation of multiple lysosome-facilitated signaling processes. The left half of the lysosome median line indicates the basal (control) conditions in absence of DQ661. The right side of the lysosome median line shows the effect of DQ661 binding to PPT1 directly within the lysosomal lumen, resulting in decreased macropinocytosis, autophagic flux, proliferation, and decreased tumor growth and enhanced apoptosis.

critical for the anticancer effect. Furthermore, the inhibition of PPT1 also affects the proper localization of lysosomal v-ATPase activity, which is responsible for maintaining lysosome as acidic by the pH gradient. DQ661 will be useful to illustrate exactly the regulation of mTORC1 complex at the lysosomal membrane. Conclusively, the PPT1 identification as a novel therapeutic cancer target suggests that protein palmitoylation needs deeper investigations.[70]

The cancer cell lysosomes can be targeted at different stages, as discussed here.

Lysosome Membrane Permeabilization

Lysosome membrane permeabilization (LMP) targeting has been fully supported to be a novel therapeutic strategy in different cancers.[71] LMP can either be slight or complete and leads to lipid peroxidation and a partial or complete discharge of lysosomal contents. Some of the contents include cathepsins, which cleave and degrade different proteins.[37] Cancer cells experience altered metabolism with increased ROS that destabilizes the lysosomes and pushes them for LMP.[37] The increased ROS production and the release of lysosomal cathepsins can initiate cell death through mitochondrial dysregulation and ultimately cell membrane permeabilization.[72]

Cancer cells show an altered sphingolipid metabolism that results in an enhanced sphingosine amount which amplifies the LMP. These findings have also been supported when sphingosine is added to some cell lines, which induces their LMP. Some other agents which induce the LMP are tumor necrosis factor-α (TNF-α), DNA damaging drug mediated p53 phosphorylation, which gets translocated to the lysosomes and induces LMP.[22] Cancer cells can smartly regulate this permeabilization through some cellular components like cholesterol, Hsp70, α-tocopherol which minimize the lysosome permeabilization.[73]

Some cell lines when transformed with oncogenes like *Src* and *Ras* have been found to exhibit distorted lysosomal localization and decreased LAMP (-1, -2) expression that primes the cells for LMP.[16] Some cancer cells enhance their lysosomal size, biogenesis, and change in Hsp70 expression, thus creating destabilized lysosomes.[61] These findings show that cancer cells may be sensitive, if approached for lysosomal cell death. Targeting of LMP is a novel strategy to kill different cancer cell types like breast cancer,[35] skin cancer,[74] bone cancer, cervical, ovarian, prostate cancer,[48] colon cancer,[16] lung cancer,[75] and acute myeloid leukemia (AML).[76]

Some of the examples of LMP inducers and their mechanism of action are listed in Table 1.

Lysosomes and Apoptosis

Apoptosis is programmed cell death involving both lysosomal and mitochondrial cooperation and the activation of caspases. A novel strategy of treating all types of cancers non-surgically is by targeting apoptosis. Several anticancer drugs target different stages of both intrinsic and extrinsic pathways.[92] The two common strategies include inhibition of anti-apoptotic molecules and the stimulation of proapoptotic molecules.[93] Some important targets include inhibitors of Bcl-2[94] ligands for death-receptors,[95] X-linked inhibitor of apoptosis protein (XIAP) inhibition,[95] and use of alykylphopholipid (APL) analogs which perform like apoptotic signals.[96] Some important plant derived compounds which possess apoptosis induction capabilities include aloe-emodin, black cohosh curcumin, epigallocatechin-3-gallate (EGCG), genistein, graviola, juglone, and quercetin.[97]

The cancer cells meticulously modulate the central control points of apoptotic pathways, including inhibitor of apoptosis (IAP) and FLICE-inhibitory protein (c-FLIP). The cancer cells precisely suppress apoptosis and develop resistance to apoptotic agents by the expression of anti-apoptotic proteins like Bcl-2 or by downregulating proapoptotic proteins like BAX.

The release of cathepsins from lysosomes targets BH3 interacting-domain death agonist (BID) which allow its translocation to the mitochondria where it interacts with Bax and Bak.[98] The antiapoptotic Bcl-2 family members possess the capacity to prevent LMP in addition to control the mitochondrial regulation.[71] It is strongly recommended that there is a link between mitochondrial dysfunctions and the lysosomal disruption. The loss of membrane potential favors the enhanced ROS production which destabilizes the lysosomal membrane through lipid peroxidation and promotes its rupture, which is associated with the activation of caspase 8 and 9.[99] Overall, these findings show that lysosomes possess a remarkable role in either initiating or executing the apoptotic pathways.

A novel synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN/CD437) has been confirmed to induce powerful apoptosis in different cancer cell types. The treatment of human leukemia HL-60 cells with CD437 result in rapid

Table 1 Different Types of LMP Inducers and Their Mechanisms of Action

LMP Inducer	Mechanism of Action and Examples	Reference
ROS	The chemical modification of lipids in lysosomal membrane. The different examples include H_2O_2, redox cycling quinones, napthazarine, fenretinide, etc.	[77]
Lysosomotropic agents	Detergent-like effects lead to LMP. The examples include hydroxychloroquine, sphingosine, LCL204, MDL-72, N-dodecyl-imidazole, BPC, 3-aminopropanol, etc.	[78]
	The proper mechanism is poorly understood and the examples include some detergents like siramesine and MSDH	[73]
	The mechanism is not fully understood and the examples include antibiotics like ciprofloxacin and norfloxacin	[79]
Lipids	The mechanism is poorly understood and the different examples include cholesterol oxidation products, bile salts, fatty acids, palmitate, etc.	[80]
Bcl-2 family proteins	This leads to the formation of lysosomal membrane proteaceous pores and the example includes Bax	[81]
Caspases	Indirect effects and direct lysosome protein digestion and the examples include Caspase-8 and Caspase-9	[82]
Cathepsins	Lysosome protein digestion and the examples include cathepsin B	[83]
Microtubule toxins	The mechanism of action is not known yet and the examples include epothilone B, vinorelbine, vincristine, paclitaxel, vinblastine, etc.	[84]
Photodamage	It damages lysosomal membrane and the examples include ATXs10 and NPe6	[85]
Polyphenols	The mechanism of action is not known yet and the examples are resveratrol	[86]
Receptors	The mechanism of action not yet known and the examples include TNF-α, TRAIL, CD3, and PHA	[87]
Lysosomal proteins	It leads to the formation of pores and the examples include LAPF	[88]
DNA damage	The mechanism of action not yet discovered and the examples include camptothecin, p53, and etoposide	[89]
Silica	The mechanism of action is unknown and the examples are ROS	[90]
Toxins	The toxins directly affect lysosomal membranes and the examples are crotoxin, yesotoxin, and the cobra venom	[91]

apoptosis induction through the caspase activation, mitochondrial functions, and morphological alterations. Treatment with some antioxidants like α-tocopherol acetate effectively inhibits the CD437-mediated apoptosis. In addition, the pretreatment of cells with pepstatin A (cathepsin D inhibitor) blocks the CD437-mediated free radical formation and apoptosis. These observations suggest the role of cathepsin D in initiation of apoptotic cell death. These findings have been confirmed by measuring intracellular distribution of cathepsin D through immunofluorescence, which indicates the release of this enzyme from lysosomes to the cytoplasm. The lysosome labeling with some lysosomotropic agents has confirmed that CD437 induces the lysosomal leakage and apoptosis induction.[100]

Lysosomes and Autophagy

The fusion of lysosomes with autophagosomes results in the formation of autolysosomes, in which the degradation of intracellular and extracellular materials take place. Autophagy performs a significant role in the adaptation of cancer cells to stress, as it protects these cells from death or any induction of its progress.[101] During the normal conditions, the cellular homeostasis is maintained through lysosomes by its biogenesis which occurs through biosynthesis and endocytic pathways. But, during the stressful conditions, the lysosome number gets decreased, as they play a vital role in macromolecular degradation for recycling or removing the damaging organelles. The restoration of lysosomes takes place through autophagic lysosomal reformation (ALR).[102]

The autophagy also regulates the lysosome cycle by permitting to engulf the damaged lysosomes with autophagosomes which later bind with active lysosomes to remove them from the cells.[103] This process leads to the recycling of amino acids and other nutrients to the cell.[104] However, if this process is not properly regulated, the destruction of intracellular structures can lead to cellular collapse and autosis, which is totally dependent on lysosomes.[105]

The alteration of autophagy in cancer cells can prove to be a promising strategy of tumor management. Some drugs are known to target different types of autophagic processes, from its initiation to the degradation step.[106] The suppression of autophagy promotes the therapeutic effects of anticancer agents and leads to apoptosis.[107] Chloroquine has been used as an autophagy inhibitor which enhances apoptosis and the therapeutic effects of photososan-II-mediated photodynamic therapy (PS-PDT) in colorectal cancer cells.[108]

Some common autophagy regulators, such as rapamycin and its derivatives, like temsirolimus, everolimus, chloroquine, and hydroxychloroquine, are regularly used in cancer therapy. Temsirolimus and everolimus induce autophagy by the inhibition of mTORC1, and have been approved by the Food and Drug Administration (FDA) for cancer therapy. Chloroquine and hydroxychloroquine directly inhibit autophagy by the alteration of lysosomal pH, inhibition of autophagic degradation, and accumulation of autophagosomes.[109]

Table 2 lists some common anticancer natural compounds or synthetic drugs which can modulate the autophagy by different mechanisms.

Lysosomes and Ferroptosis

There is an increased metabolic rate and higher turnover of iron-containing proteins in cancer cells, that lead to the accumulation of iron in their lysosomes and the sensitization to ROS-induced LMP.[161] These cells have a higher ROS production rate with more cathepsin release from their lysosomes which can induce their cell death.[162] This lysosome-facilitated cell death offers a novel option to treat the cancer cells which are resistant to general apoptotic cell death. However, cancer cells meticulously evade the lysosome-sponsored cell death by reorganizing their lysosome metabolism.

Cancer cells exhibit an additional iron demand as compared to the normal non-cancerous cells. So this extra requirement for iron can make such cells susceptible to iron-catalyzed necrosis, known as ferroptosis. Ferroptotic cell death is a distinct type which results from the accumulation of iron-dependent ROS.[163] The ferroptosis is regulated by the proteins like ferritin, transferrin, and cysteine antiporter receptors which are responsible for the regulation of iron level.[164] Lysosomes are one of the major storage locations of iron and, in the presence of H_2O_2, the free Fe undergoes Fenton reaction resulting in reactive iron and thus increasing the ROS. A lysosome disrupting compound called siramesine increases the lysosomal pH that results in its leakage which is mediated in part by sphingomyelinase inhibition.[48] All this leads to increased reactive iron and ROS, finally mediating cell death.[73] The actual role of lysosomes in regulating ferroptosis through increased active iron and ROS is still not fully understood and needs further investigations. The ferroptosis-inducer drugs as approved by the FDA bear high expectations for the potential of tumor management as a new promising way to kill cancer cells. Table 3 lists some important drugs that can either induce or inhibit the iron metabolism facilitated ferroptosis with different types of mechanisms involved.

Lysosomotropic Agents

Lysosomotropic agents consist of weak-base cationic or lipophilic amphiphilic drugs which gets accumulated inside the lysosomes. The lysosomal membrane allows the diffusion of these compounds across and get trapped due to their protonation inside the lysosomes.[71] Their excessive accumulation initiates the lysosomal membrane damage and finally causes LMP. Lysosomotropic agents include kinase inhibitors such as ML-9,[179] metal nanoparticles (NPs),[180] and some pharmaceutically important drugs, which include nortriptyline, siramesine, desipramine, clomipramine, imipramine, etc.[48] The chemical structure of some of these compounds is listed in Figure 4.

These compounds have been used in the fight against colon cancer, breast cancer, and CLL cells. In addition, antimalarial drugs like chloroquine and mefloquine have been found to be effective in lymphoma, leukemia, and breast cancer.[181] Further, antiallergic drugs like loratadine and terfenadine have been reported to induce breast and lung cancer cell death.[48] In addition, the treatment with stilbenoid antioxidant, pterostilbene and antipsychotics, thioridazine, chlorpromazine, and aripiprazole have been found to possess good efficacy in leukemia and breast cancers. Except chloroquine, most of these drugs are FDA-

Table 2 Examples of Different Compounds Which Act as Modulators of Autophagy Used as Cancer Prevention and Therapy and the Examples of Different Cancer Where Used

Compound	Mechanism of Action and Cancer Type	Reference
Artemisinin	Leads to the promotion of ROS dependent apoptosis as studied in Lung carcinoma	[110]
Water-soluble artemisinin SM1044	Modulates CaMKK2-AMPK-ULK1 axis as studied in diffuse large B-cell lymphomas	[111]
EF25-$(GSH)_2$	Induces the autophagy facilitated apoptosis as studied in hepatocellular carcinomas	[112]
γ-Tocotrienol	Promotes the accumulation of LC3-II proteins and induces the apoptosis as studied in breast carcinoma	[113]
Bis-dehydroxycurcumin	Promotes the activation of ER stress as studied in Colon carcinoma	[114]
Hydrazinobenzoyl-curcumin	The compound increases in autophagic vacuoles as studied in non-small lung epithelial carcinoma	[112]
Dihydroartemisinin	Promotes the activation of JNKs as studied in pancreatic carcinoma	[115]
	Leads to the inhibition of mTOR kinase as studied in cisplatin-resistant ovarian carcinoma	[116]
	Induces autophagy facilitated apoptosis as studied in esophageal carcinoma	[117]
	Increases the autophagic vacuoles as studied in glioma	[118]
	Enhances ROS production, LC3-II protein expression and caspase 3 activation as studied in myeloid leukemia	[119]
	Increases γH2AX foci and the inhibition of phospho-STAT3 as studied in human tongue squamous cell carcinoma	[120]
Monocarbonyl curcumin, B19	Activation of ER stress as studied in ovarian carcinoma	[121]
Artesunate	The accumulation of LC3-II proteins as studied in breast carcinoma	[122]
	The induction of autophagy facilitate by apoptosis as studied in glioblastoma multiforme	[123]
	The increase in caspase-3, LC3-I/II, and Beclin-1 protein expression as studied in Burkitt lymphoma and colon carcinoma	[124]
Celastrol	Promotes proteotoxic stress as studied in glioblastoma	[125]
	Leads to the induction of autophagy mediated apoptosis as studied in pancreatic and gastric carcinoma	[126]
	Promotes the induction of autophagosomes and the accumulation of LC3B-II proteins as studied in osteosarcoma	[127]
	Induction of microRNA miR-101 as studied in prostate carcinoma	[128]
Paclitaxel	Induces the autophagy facilitated apoptosis as studied in breast carcinoma	[129]
	Leads to the formation of acidic vesicular organelles as studied in lung carcinoma	[130]
	It promotes the accumulation of LC3-II proteins as studied in cervical carcinoma	[131]

(*Continued*)

Table 2 (Continued).

Compound	Mechanism of Action and Cancer Type	Reference
Resveratrol	The induction of autophagy facilitates apoptosis as studied in myeloma ovarian carcinoma, oral carcinoma, hepatocellular carcinoma, and glioblastoma multiforme	[132]
	Suppresses the Wnt/β-catenin pathway as studied in stem cells of breast cancer	[133]
	Leads to the inhibition of NF-κB pathway as studied in cervical carcinoma	[134]
	Results in the modulation of LKB1-AMPK-mTOR pathway as studied in promyelocytic leukemia	[135]
	Leads to the Inhibition of AKT/mTOR pathway as studied in breast and prostate carcinoma	[136]
	Results in the JNK-dependent accumulation of p62 proteins as studied in chronic myelogenous leukemia	[137]
	Modulates Rictor in skin squamous carcinoma	[138]
	Induces p53/AMP-activated protein kinase/mTOR pathway as studied in renal carcinoma	[139]
Synthetic ursolic acid	Results in the increased levels of LC3A/B-II and Beclin-1 as studied in lung carcinoma	[140]
Ursolic acid	The studies on colon carcinoma showed it modulates the JNK pathway	[141]
	It induces autophagosomes and LC3-II protein accumulation, as studied in Cervical carcinoma	[142]
	A study on breast carcinoma showed that it induces ER stress; glycolytic pathway and PI3K/AKT-regulated GSK autophagy pathway, as studied in	[143]
	Leads to the activation of ROS-dependent ER stress, as studied in glioblastoma	[144]
	Modulates Akt/mTOR pathways and Beclin-1, as studied in prostate carcinoma	[145]
	The studies on osteosarcoma have shown its role in autophagy mediated apoptosis	[146]
	Induction of apoptosis mediated by autophagy, as studied in Pheochromocytoma	[147]
Chloroquine and hydroxychloroquine	Induction of autophagy mediated apoptosis studied in bladder carcinoma	[148]
	Pancreatic carcinoma and melanoma has shown the accumulation of LC3-II proteins and induction of apoptosis	[149]
Quinacrine	Colon carcinoma showed the modulation of p53-dependent and p21-dependent mechanisms	[150]
Palm-mixed tocotrienol complex	Breast carcinoma showed the induction of autophagy mediated apoptosis	[151]
	Leads to increased dihydroceramide and dihydrosphingosine intracellularly, as studied in Prostate carcinoma	[152]
Thymoquinone	The head and neck squamous cell carcinoma have revealed the induction of autophagosomes and accumulation of LC3-II proteins	[153]
	A study on glioblastoma showed accumulation of LC3-II and p62 proteins	[154]
	Studies on colon carcinoma showed the induction of autophagy mediated apoptosis	[155]
Curcumin	Inhibits the AKT/mTOR/p70S6 kinase pathway, as studied in malignant glioma	[156]
	The uterine leiomyosarcoma showed the induction of autophagy mediated apoptosis	[157]
	Mesothelioma and chronic myelogenous leukemia has revealed the modulation of PI3K/AKT/mTOR and NF-κB signaling pathways	[158]
	The colon carcinoma has shown the activation of transcription factor EB-lysosome pathway	[159]
	Hepatocellular carcinoma has shown the accumulation of LC3-II protein	[160]

Table 3 Examples of Different Drugs and Other Compounds Which Modulate the Iron Metabolism Mediated Ferroptosis with Different Types of Mechanisms

Drugs	Induction/Inhibition of Ferroptosis and the Mechanism of Action on Target in Different Cancer Types	References
Artesunate	Induction of iron metabolism on ferritinophagy as studied in cervical adenocarcinoma; and hepatocellular carcinoma	[165]
	Induction of iron metabolism on ferritinophagy, as studied in pancreatic cancer	[166]
	Inhibition of lipid peroxidation on NRF2, as studied in Head and neck cancer	[167]
Cisplatin	Induction of iron metabolism on ferritinophagy, as studied in lung cancer	[132]
	Induction of lipid peroxidation and Iron metabolism on GSH-GPXs and IREB2, as studied in colorectal cancer; NSCLC	[128]
Dihydroartemisinin	Induction of iron metabolism on ferritinophagy, as studied in lung cancer and colorectal cancer	[168]
	Induction of iron metabolism on ferritinophagy, as studied in head and neck cancer	[169]
	Induction of iron metabolism on ferritinophagy, as studied in acute myeloid leukemia	[170]
Gemcitabine	Inhibition of lipid peroxidation on GPX4, as studied in pancreatic cancer	[171]
Paclitaxel	Unknown effect of lipid peroxidation on P53:SLC7A11, as studied in colorectal carcinoma	[172]
	Unknown effect of lipid peroxidation on P53, as studied in lung cancer	[173]
Sulfasalazine	Induction of lipid peroxidation on SLC7A11, as studied in sarcoma and colorectal cancer	[174]
	Induction of lipid peroxidation on SLC7A11, as studied in glioma	[175]
Sorafenib	Induction of lipid peroxidation and Iron metabolism on NRF2, SLC7A11, and FTH, as studied in hepatocellular carcinoma	[176]
	Induction of lipid peroxidation on system Xc, as studied in sarcoma	[177]
	Induction of lipid peroxidation on SLC7A11, as studied in sarcoma and colorectal cancer	[174]
	Induction of lipid peroxidation on SLC7A11, as studied in glioma	[175]
Temozolomide	Inhibit of lipid peroxidation on SLC7A11 and transsulfuration pathway, as studied in glioblastoma multiforme	[178]

approved and have been properly investigated in clinical trials.[181] In future, these lysosomotropic agents will provide a significant foundation to be clinically investigated for their therapeutic role in different types of cancers.

Methods, Strategies, and Characterization Used for the Preparation of NPs

The current advances towards the biomedical application of NPs have established different methods to synthesize these entities from diverse materials like metals, metal oxides, semiconductors, ceramics, polymers, etc. So, on the basis of their origin, NPs possess unique structural, physiochemical, and morphological characteristics, which are important for their wide range of applications like center-point drug targeting, bioimaging, molecular tagging, etc. The structural analogs of NPs include liposomes, dendrimers, quantum dots, polymeric micelles, and each structural analoghas specific applications.[182] The synthesis methods of NPs are mainly divided into three categories: physical methods, chemical methods, and bio-assisted methods.

Furthermore, two basic approaches employed for the preparation of NPs include a top-down approach and a bottom-up approach. In the top-down approach, the synthesis of NPs is initialized with a bulk material that leaches out systematically bit-after-bit, resulting in the generation of required NPs. Some commonly used top-down methods include electron beam lithography,

photolithography, milling technique, anodization, ion and plasma etching, etc. The bottom-up approach for NP synthesis involves assembling or coalescence of atoms and molecules generating distinct NPs. Some commonly used bottom-up approaches include sol-gel processing, chemical vapor deposition, laser pyrolysis, plasma or flame spraying, bio-assisted synthesis, and chemical or electrochemical nanostructural precipitation.[183]

The characterization of NPs by different methods is equally important to control their desired in vivo and in vitro behavior. These entities are characterized by their morphology, size, and surface charge by utilizing highly advanced microscopic techniques such as transmission electron microscopy, scanning electron microscopy, and atomic force microscopy. The colloidal stability of NPs is determined through zeta potential, which is an indirect measure of surface charge. The NPs and drug interaction are characterized by using differential scanning calorimetry. In addition, the binding and internalization of targeted nanoformulations against specific cells is determined by cell uptake assays. The biodistribution, intracellular uptake, and subcellular localization of these NPs could be confirmed by confocal microscopy.[184]

Lysosomal Targeting with Drug Nanoformulations

The practice of using a single unit drug nanoformulation as a therapeutics and diagnostics (theranostics) composite is now well-known as a novel approach of the drug delivery system.[185] There are several advantages of using theranostic nanoformulations as compared to the conventional systemic administration of native drugs. Native drugs have the problems of limited solubility, easy inactivation, and fast biodegradation. Some advantages of using drug nanoformulations include extended circulation time, higher concentration at tumor site, multiple synergistic drugs, and diagnostic system delivery.[186] Some more advantages include controlled drug release at the tumor site through stimulus-sensitive delivery systems (eg, temperature, pH, enzyme-sensitive nanoformulation, overcoming multidrug resistance and enhanced therapeutic efficacy. The approach of a drug delivery system even up to the organelle level (third level drug targeting) with the aid of different nanoformulations has revolutionized the therapeutic approach for different diseases, including cancer.[187]

Lysosomes are considered as novel targets for anticancer therapeutics as cancer cells can bypass cell death through the classical caspase-dependent apoptosis pathway. This enables us to focus on targeting apoptosis and drug-resistant cancer cells as a novel therapeutic strategy.[188] Cancer cell specific and particularly organelle-directed drug targeting is one of the major challenges in pharmaceutical research. This area requires a multidisciplinary approach for center-point delivery of novel therapeutics without affecting nearby healthy tissues. These drug delivery system nanoformulations are constructed by keeping certain criteria in observations. Several organelle-specific small molecules like heterocyclics, peptide substrates, and oligonucleotides have been widely used.[189] Recently, different polymeric carriers like N-(2-hydroxypropyl) methacrylamide (HMPA) and mesoporous silica nanoparticles (NPs) (MSNPs) have been used to target several cancerous tissues.[190] These nanoformulations act by passive targeting by taking the benefit of enhanced permeation and retention effect (EPR) of the cancerous tissues. These nanoformulations are hardly selective but get simply distributed by blood circulation. So most of these administered nanoformulations get accumulated within the lungs, spleen, and liver. The researchers have constructed novel drug delivery systems by focusing on different cellular proteases as target sites.[191]

Among different organelles, lysosome targeting in tumor cells is reported to be one of the potential ways of cancer treatment.[192] As lysosomes are rich in proteases, the cathepsin family of enzymes are considered as potential targets of therapeutic strategy in cancer management. In cancer cells, the lysosomal cysteine proteases, including cathepsin B (Cat B), are highly upregulated at mRNA and protein levels.[193] The overexpression of Cat B is reported in breast cancer, oesophageal cancer, and other tumors.[194] So targeting the Cat B appears to be a promising strategy for novel drug delivery against different types of cancer cells. A tetrapeptide, Gly-Leu-Phe-Gly (GLPG) possesses higher plasma stability and the least hydrolysis by Cat B.[195] So the novel targeting of Cat B-enriched cancer cells enhances the efficacy of anticancer drugs, and minimal toxicity to the normal cells.

A synthetic Cat B peptide sequence, GLPG has been engineered by conjugation with a sorbitol core attached with multiple guanidine residues, which targets the cancer cell lysosomes. This strategy mimics the Arg-8-mer or Tat, which shows potential translocation across the blood–brain barrier, cell membrane, and mitochondria.[196] There are some advantages of using sorbitol as a delivery carrier, as it possesses the highest density of functionality among

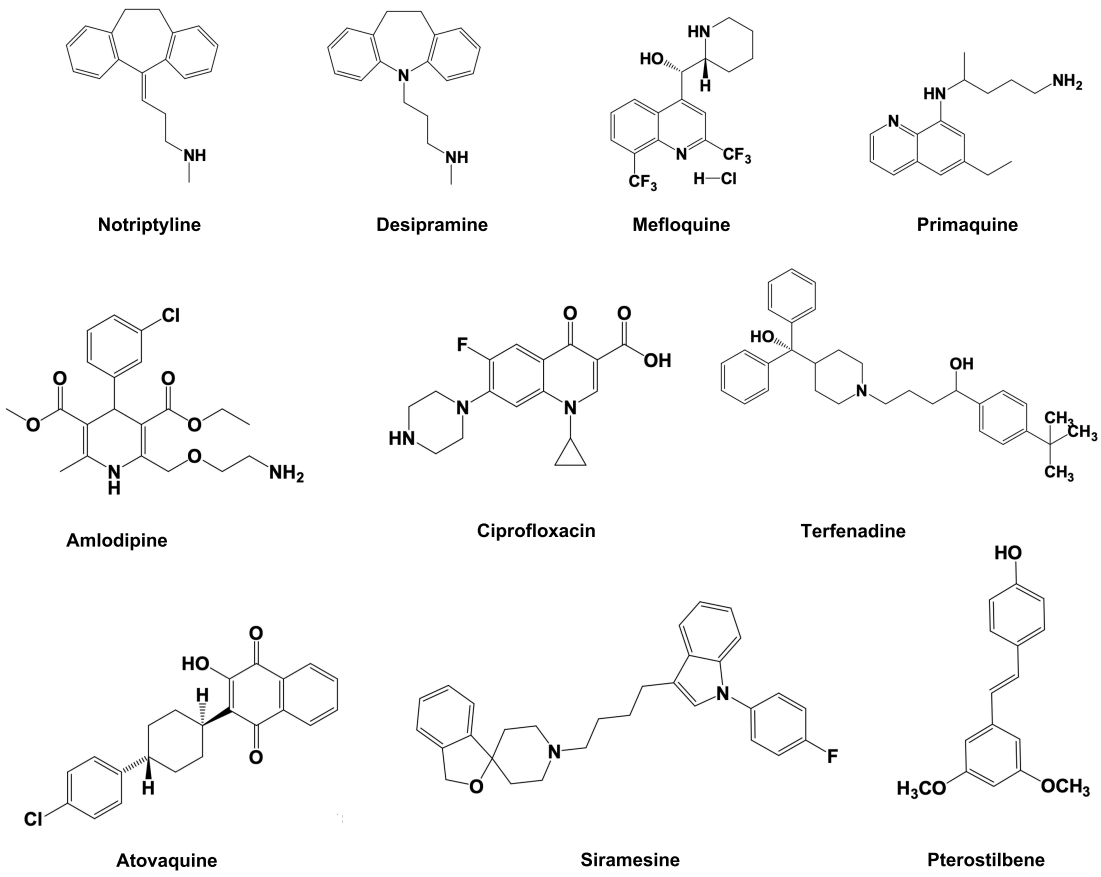

Figure 4 Chemical structure of some important lysosomotropic compounds used to induce LMP.

other organic compounds having multiple hydroxyl groups. Sorbitol is naturally occurring and devoid of toxicity with the positively charged guanidine groups displaying an association with negatively-charged phospholipids present on cell membrane and other organellar membranes, thus facilitating its entry through the lipid bilayer.[197] Doxorubicin, a potential antitumor drug, has been delivered in different cancer cells by using sorbitol as a carrier system conjugated with Cat B cleavable peptide sequence. However, only a few nanoformulations have been tested so far, and these nanoformulations also exhibit little weaker cancer selectivity, but have been found to be effective during combination therapies.[188]

Lysosomal Targeting with Free or Peptide-Facilitated Gold-Nanoparticles

Recently, different types of NPs, including gold NPs (AuNPs), dendrimers, fullerenes, neodymium oxide, and quantum dots have been shown to be autophagy inducers.[198] The AuNPs are one of the most commonly used nanoformulations which initiates the induction and accumulation of autophagosomes. These particles are engulfed by the cancer cells via endocytosis in a size-dependent manner. These NPs ultimately show lysosomal accumulation, which leads to their degradation through the alkalinization of lysosomal pH. Further, it has been observed that AuNPs induce the accumulation of autophagosome and the processing of autophagosome marker protein, microtubule-associated light chain 3 (LC3). However, the degradation of p62, an autophagy substrate, is blocked in AuNP-exposed cells, indicating that the accumulation of autophagosome is the consequence of autophagy flux blockade, rather than autophagy induction. So the AuNPs accumulation of autophagosome clarifies its role on lysosomes. The use of AuNPs and their lysosomotropic-agents tagging can be a novel strategy of targeting specific tumor cells as a cancer therapeutics strategy.[199]

Gold nanoparticles (AuNPs) with a diameter of about 13 nm tagged with cell membrane penetrating peptide (CPP) and lysosomal sorting peptides (LSPs) have been

targeted to lysosomes. The results of this study have confirmed that LSP is quite efficient in transporting AuNPs tagged with CPP up to lysosomes and other lysosome like structures. There are some novel advantages of using LSPs tagged with different types of metal and non-metal based NPs to treat different types of enzyme replacement therapies (ERT) or targeted drug delivery within the lysosomes.[200]

Co-Targeting of GRP78 and Lysosomes with TPP-PEG-Biotin Self-Assembled NPs

Glucose-regulated protein 78 (GRP78) is a type of Hsp70, a marker protein overexpressed in cancer cells.[201] In tumor cells, GRP78 is used to combat the stressful environment and promotes the proliferation, metastasis, survival, and resistance to different anticancer drugs.[202] In addition, the lysosomes in cancer cells help in recycling of dysfunctional organelles and other contents by autophagy to reuse the basic components.[203] These cells require a continuous supply of functional proteins for their faster cell division, as compared to the normal cells. So, these cells overexpress the molecular chaperones like GRP78 to regulate the overloading of proteins within ER.

A novel strategy of co-targeting GRP78 and lysosomes and destroying them can lead to the accumulation of by-products including unfolded proteins. This significant co-targeting strategy is desperately needed as an anti-cancer therapeutic approach. In this regard, Ruthenium (II, III) complexes have been found to possess strong affinities for thiol containing proteins like glutathione (GSH), transferrin and bovine serum albumin (BSA), etc.[204] The strong affinity of ruthenium for transferrin is novel, as it has more specificity for cancer cells as compared to the normal cells due to the highly cancer active metabolism requiring Fe^{2+} ions.[205]

A photo-dynamic therapy substance made from tetraphenylporphyrin (TPP)-polyethylene glycol (PEG)-biotin produces ROS when irradiated at 660 nm.[206] After the covalent bonding between TPP and PEG, this material undergoes self-assembly making novel photosensitizer-NPs. The TPP-PEG-biotin can be efficiently delivered to different cancer cells, like HepG2 and MCF-7, with over-expressed biotin receptors. The TPP-PEG-biotin formulation has been localized within lysosomes of MCF-7 cells.[206] These self-assembly NPs of TPP-PEG-biotin can also encapsulate chemotherapeutic drugs targeting multiple organelles.

The nanoformulation made from TPP-PEG-biotin loaded with Ru-1 has been used to co-target GRP78 and lysosomes as a recent novel anti-tumor therapeutic strategy. In comparison to the previous approaches, based on doxorubicin encapsulation using TPP-PEG-biotin,[206] this approach is based on co-targeting strategy. The co-targeting of different locations within the cancer cells adds the damaging sites and proves to be more lethal to them. The co-targeting of lysosomes and GRP78 has been proven to be a very efficient anti-cancer therapeutic strategy.

Lysosome Targeting with Octadecyl-Rhodamine-B Liposomes

The Gaucher disease type 1 (GD1) is recognized by increased incidence of gammopathy and risk of developing multiple myeloma and possibly other hematological malignancies.[207] This disease is caused by a deficiency of the lysosomal hydrolase, acid β-glucosidase and results in accumulation of its primary substrate, glucosylceramide (GC), which in the systemic circulation is derived primarily from the turnover of senescent blood cell membranes.[208] The liposomes loaded with β-glucosidase from human origin have been found to degrade GM1-ganglioside within feline fibroblast lysosomes and drastically reduced the 70–80% accumulation of its substrate, galactocerebroside.[209] However, these liposome-based formulations are not used clinically for ERT, so there is a drastic need to use such nanoformulations, having enhanced enzyme loading efficiency and proper targeting within the lysosomes.

The surface modification of liposomes with novel lysosomotropic octadecyl-rhodamine B has significantly increased the proper delivery of such nanoformulations within the lysosomes of HeLa cells.[210] Octadecyl rhodamine B (Rh) has significantly improved the delivery of a model marker fluorescein isothiocyanate (FITC)-dextran within HeLa cell lysosomes.[211] These lysosomotropic agent modified liposomes can prove to be a novel therapeutic strategy for the treatment of Gaucher's fibroblasts and model diseased cells. Keeping this in mind, lysosomotropic Rh tagged liposomes were loaded with velaglucera alpha (VPRIV) and their intracellular and lysosomal delivery has been investigated.

In parallel, some novel liposomal nanoformulations loaded with varied therapeutic enzymes have proved to be a promising strategy of ERT.[212] The biodistribution of

β-fructofuranosidase containing liposomes has demonstrated up to 50% enzyme activity build-up in liver cell lysosomal fractions.[213] In parallel, liposome encapsulated neuraminidase, α-mannosidase, and β-glucosidase have been intravenously administered for their therapeutic purposes.[214]

Induction of Lysosome-Mediated Cell Necrosis by Cationic Liposomes

Cationic liposomes (CLs) are commonly used as gene delivery vectors, having excellent biocompatibility and biodegradability. The CLs induce enhanced autophagy and promote cell death by significant accumulation of autophagosomes.[215] It has been hypothesized that CLs induce LMP through mTOR-independent autophagic flux.[216] In addition, the autophagic flux is altered by CLs at early stage and inhibiting at the later stages. This leads to the induction of cellular toxicity by the induction of LMP and the inhibition of autophagic flux. These hypotheses have been supported by the study of CLs on autophagic flux and dysfunction of lysosomes in human liver epithelial cell lines. The results have shown cellular toxicity by the induction of LMP and the inhibition of autophagic flux, with the release of cytoplasmic cathepsin B, enhanced ROS production, and mitochondrial dysfunctions, the key mediators of cellular toxicity.[217]

Besides this, the cationic liposomes (CLs) have also been used to impair Na^+/K^+-ATPase in lung cells to induce cell necrosis.[218] Furthermore, the CLs can destabilize the plasma membranes by inducing the formation of nonbilayer lipid structures and promotes pronounced cell membrane disruption.[219]

Carbon Nanotubes-Mediated Autophagy Blockade

Multiwalled carbon nanotubes (MWCNTs) induce an abnormal accumulation of autophagosomes, possibly because of autophagy blockade.[220] The autophagy blockage occurs through the modulation of synaptosomal-associated protein (SNAPIN) expression. The chemical nature of NPs and its shape, length, size, crystal phase, and surface properties impact differently on autophagosome accumulation. In an interesting study by Cohignac et al,[268] the shape of NPs (CNTs versus spherical carbon or titanium NPs) impacts the induction or blockade of autophagy. Indeed, MWCNTs promote the blockade of autophagic flux, whereas spherical NPs (TiO_2 NPs) lead to the activation of functional autophagy.

Furthermore, CNTs have been found to exert cellular toxicity in many cell types through LMP. The LMP leads to enhanced oxidative stress, mitochondrial dysfunctions, and cathepsins release.[221] In addition, LMP is a potential mechanism of autophagic flux inhibition, through the blockade of lysosome-autophagosome fusion. This leads to the accumulation of autophagosomes and their substrates like ubiquitinated protein aggregates.[215]

Lysosomal Targeting by Saposin C Protein Nanovesicles

Saposins or sphingolipid activator proteins (SAPs) are nonenzymatic glycoproteins present in lysosomes. These glycoproteins are usually smaller in size and are essential for the degradation of sphingolipids and membrane digestion. These glycoproteins are comprised of five types as saposin A-D and the GM2 activator protein.[222] Saposins play an important role in lipid transport, lipid microdomain assembly, lipid membrane binding capability and reorganization of the biological membranes.[223]

It has been found that phosphatidylserine is abundantly found in cancer cells,[224] so it could be a novel target for saposin C. Several researchers support the idea of a linkage between cellular membrane aberrations and ceramide-facilitated initiation of apoptosis in cancer cells.[225] Therefore, some novel agents have been identified which interfere with cancer cell membranes including lysosome membranes and modulate their organization, signal transduction, fluidity, and metabolic activities.[226]

Saposin C-dioleoyl phosphatidylserine (Sap-C-DPS) nanovesicles with a diameter of almost 190 nm were prepared and these entities presented specific cancer cell targeting. Following the administration of Sap-C-DPS nanoformulation, it got preferentially accumulated in cancer cells in tumor-induced mice. Sap-C-DPS led to the induction of apoptosis preferentially in different cancer cell types and spared the normal cells and tissues. The mechanism of Sap-C-DPS-mediated apoptosis has been found to be through the elevation of intracellular ceramides followed by the activation of caspases. The Sap-C-DPS nanoformulation has been found to significantly inhibit the growth of malignant peripheral nerve sheath tumor and preclinical xenograft. These nanoformulations can prove to be novel cancer-specific agents for the treatment of a broad range of tumors.[227]

Lysosome Targeting with Mixed-Charged NPs

There are some limitations with unicharged (cationic or anionic) NPs as pure anionic NPs are slowly internalized by target cells while cationic NPs, due to their strong electrostatic attractions with membranes, depolarize the membranes and generate hydrophilic membrane pores promoting their membrane permeabilization.[228] Unfortunately, these NPs are non-selectively cytotoxic.[229] These issues have been resolved by using mixed charged NPs in different proportions.

The AuNPs functionalized with positively charged N, N,N-trimethyl(11-mercaptoundecyl) ammonium chloride (TMA) and negatively charged 11-mercaptoundecanoic acid (MUA) ligands were prepared to form mixed charged NPs.[188] Surprisingly, these mixed charged NPs have been found to possess several intriguing properties, as these are much stable, and can be precipitated or crystallized out at different pH values.[230] It is assumed that these pH-dependent mixed charged NPs could prove to be novel for selective targeting of cancerous cells and their lysosomes.

Based on this innovative strategy, lysosomes have been targeted with mixed charged NPs, which gradually disrupt the lysosomal membrane integrity, finally initiating lysosome-facilitated cell death, quite selectively in cancer cells (Figure 5). These NPs cluster at cell surface, followed by the internalization of about 50–100 nm NPs through endocytosis and their gradual accumulation within multivesicular endosomes, which finally leads to shipment to the lysosomes. These mixed charged NPs form superacrystals within the lysosomes. This promotes the lysosomal swelling with a gradual weakening of lysosomal membrane integrity and finally promotes the cell death. In contrast to cancerous cells, in normal cells these mixed charged NPs show limited aggregation, and are excluded through exocytosis, so these cells get least harm. So the use of these mixed charged NPs against different cancers is presumed to be a novel strategy in fighting against cancer (Figure 5).[188]

Lysosome Targeting with Au-ZnO Hybrid NPs

Zinc oxide NPs (ZnONPs) have been conjugated with lysosome targeting peptide, to selectively enter the cancer cells through the endocytic pathway. These particles get rapidly accumulated within the lysosomes and initiate ROS generation, thus might propose a new strategy of LMP-dependent apoptotic cell death progression.[231] ZnONPs were combined with AuNPs to check the progression of LMP in real-time by fluorescence quenching to understand ROS mediated lysosomal death pathways. These hybrid NPs combine the merits of both ZnO and AuNPs, achieving excellent catalytic activity and fluorescence quenching. Furthermore, these hybrid NPs have been conjugated with FITC-labeled cathepsin B substrate sequence (Arg-Arg, RR)[71] and also the $\alpha_v\beta_3$ integrin-targeting peptide (RGD).[232] The resulting FITC-RR-ZnO-

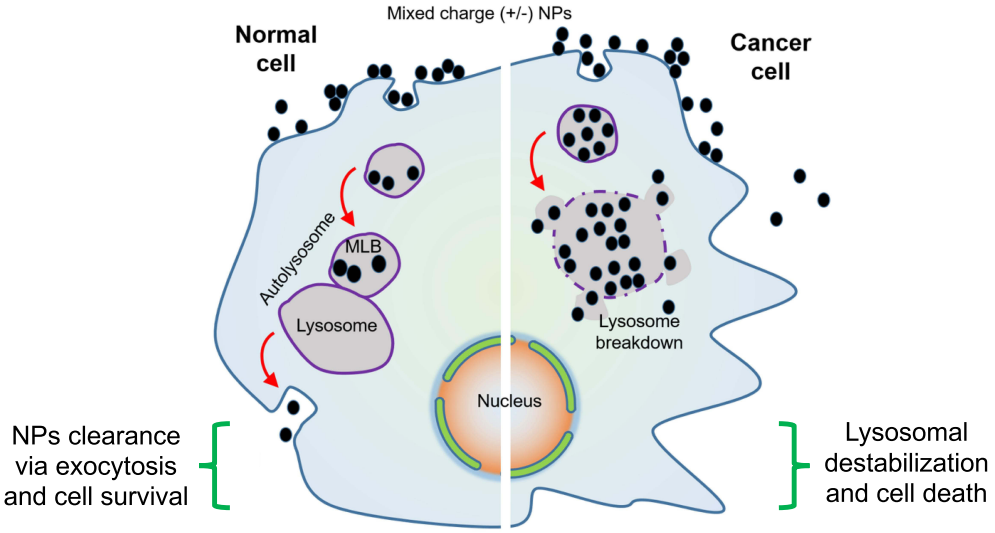

Figure 5 The role of mixed charged NPs within normal and cancer cells. Normal cells clear these NPs through proper exocytosis and are least damaged. In cancer cells these NPs form superacrystals and lead to lysosome breakdown resulting in cell death.

Au-RGD NPs bind specifically to integrin $\alpha_v\beta_3$-rich HepG2 cells, accumulate within their lysosomes, and mediate ROS production and also enable real-time monitoring of LMP-dependent apoptosis in these tumor cells.

Lysosomal Membrane Permeabilization by Targeted Magnetic NPs

Targeted lysosomal dysregulation by magnetic NPs is considered as a novel alternative to overcome cancer resistance. These NPs selectively target cancer cells and enhance its LMP, so these NPs demonstrate as powerful tools in tumor therapeutics. In a novel study, iron oxide magnetic NPs (FeONPs) have been engineered to selectively target epidermal growth factor receptor (EGFR) overexpressed on cancerous cells. These NPs induce LMP under the action of an alternating magnetic field. The enhanced LMP led to the production of excessive ROS that resulted in decreased tumor viability. In these cells, the cytosolic activity of lysosomal protease cathepsin B was confirmed by confocal microscopy. These innovative findings suggest that the lysosomal death pathway can be remotely controlled in cancer cells by triggering their membrane permeabilization through the administration of magnetic FeONPs.[233]

Mesoporous Silica NPs Can Alter the Lysosomal Exocytosis Rate

Mesoporous Silica NPs (MSNPs) have gained much attention due to some specific properties like large internal pore volume, large surface area, good chemical and thermal stability, and tunable pore size.[234] Cancer cells engulf these NPs by energy dependent endocytosis and the majority of these NPs are colocalized within endo/lysosomal compartments.[235] It has been reported that MSNPs can be used as efficient imaging vectors and drug delivery vehicles in different types of cancers.[236] Different animal studies have demonstrated the role of MSNPs in cancer growth inhibition by targeting lysosomes.[237] The ultimate fate of MSNPs after the engulfment by cancer cells has not been fully understood. The cellular mechanism involving the exocytosis of these NPs from the cells also needs to be investigated in detail. The possible mechanisms include co-localization of these NPs with the lysosomes and may enter the Golgi apparatus for excretion or undergo lysosomal exocytosis.

In a previous study, the exocytosis of MSNPs was observed when these entities were not surface modified. But coating the surface of these NPs with 3-(trihydroxysilyl) propyl methylphosphonate improves the dispersibility of these NPs, so can be used to carry targeting moieties, thus improving its cancer therapy.[238] In one study, phosphonate-modified MSNPs (P-MSNPs) have been examined to study lysosomal exocytosis. It has been observed that these NPs recover intact after their cellular excretion and this is mediated by the fusion of lysosomes with the plasma membrane. Further, it has been demonstrated that the exocytosis of these P-MSNPs can be regulated by controlling the lysosomal exocytosis. The anticancer drug is released from these NPs via diffusion, so the exposure time of these NPs within the cells could influence the amount of drug release. By decreasing the rate of exocytosis of these NPs, the camptothecin loaded P-MSNPs have been found to improve the cellular effects of drug delivery.

T-Cell Lysosome Targeting for Cancer Immunotherapy

In solid tumors, T-cell immunotherapy faces a great challenge, due to minimal activation, synthesis, and release of lysosome specific therapeutic proteins like granzyme B and perforins. In a novel study, a special type of NPs [mineralized metal-organic framework (MOF)] coupled with CD63 (lysosome targeting aptamer) have been engineered. These NPs target the lysosomes of T-cells and enhance their anticancer potential. The MOF is synthesized from dimethylimidazole and Zn^{2+} and Calcium carbonate ($CaCO_3$) is used for the mineralization of these NPs. $CaCO_3$ improves the composite material stability for encapsulating the therapeutic proteins and provides calcium ions with synergistic potential. In addition, these NPs are ideal lysosome delivery vectors and possessan efficient protein encapsulation capacity besides having acid sensitivity. Before mineralization, T-cell required therapeutic proteins (granzyme B and perforins) are preloaded with the MOF. The treatment of a specific cancer also involves the T-cell pretreatment with processed tumor-specific antigens to produce or activate memory before reprograming their lysosomes. By using these novel NPs, a significant control of breast cancer enhancement has been achieved.[239]

Table 4 elaborates some more examples of different types of NPs used to target lysosomes. This table also briefs the NPs size, cellular uptake mechanism, in vitro

and in vivo models used, and the imaging and therapy applications (Table 4).

Nanoparticles and Immune System

The therapeutic applications of NPs are often challenged by its toxicity concerns involving their interaction with various components of the immune system. It is now well known that NPs size, shape, surface charge, steric effects, and hydrophobicity/hydrophilicity can dictate NPs compatibility with the immune system.[277] NPs are constantly engineered to either avoid recognition by the immune system or specifically inhibit or enhance their immune response. NPs can be engineered to modulate the cellular trafficking, thus influencing the immune system. Lysosomes are the principal subcellular catabolic organelles which are meant for degradation and recycling of both intracellular and extracellular materials, which are the final steps in phagocytosis and autophagy. Autophagy and phagocytosis in macrophages play an essential role and serve as a bridge between innate and adaptive immunity. Exposure of macrophage lysosomes with different pathogens leads to distinct alterations in its proteomics, which is closely associated with macrophage immune functions like antigen presentation, toll-like receptor activation, and inflammation.[278] In addition, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells play a vital role in the immune system, as they eliminate both virally infected and tumorigenic cells. Regulated exocytosis of perforins and proapoptotic granzymes from the secretory lysosomes of these cells helps in clearance of target cells.[279]

NPs elicit the immune response by either direct immuno-stimulation or by direct interaction with antigen presenting cells (APC) or by delivery of antigens to specific cellular compartments.[280] Metal drug nanoformulations are well known to possess cytotoxic anticancer potential and can interact with the cancer-immune interface and can reverse immune evasion aspects. Metal drug nanoformulations have the fidelity to induce a long-lasting anticancer immune response.[281] After conventional chemotherapy, the anticancer immune response may contribute to control the cancer. In addition, treatment with radiotherapy and some chemotherapeutic drugs, like anthracyclines, can induce specific immune responses leading to immunogenic cancer cell death. The residual cancer cells are eliminated by this anticancer immune response which can also maintain micrometastases in the dormancy stage.[282]

Within the biological system, different molecules interact with NPs and ultimately lead to the formation of a protein coat around it called the protein corona. This protein corona of NPs plays a significant role in modulating the macrophage behavior.[283] NPs are usually first picked up by macrophages which can lead to immunosuppression or immunostimulation, and can promote autoimmune or inflammatory disorders or increase the host's susceptibility to infection and cancer. The immune cells can inadvertently recognize the NPs as foreign entities which can result in multilevel immune response resulting in toxicity and diminish their therapeutic efficacy. For example, granuloma formation has been reported in the skin and lungs in animals exposed to CNTs.[284]

When NPs are engineered to behave as self without immune recognition by the host, this is considered the first success in the field of bioimaging or drug delivery. NPs are provided a hydrophilic environment by tagging with polyethylene glycol (PEG) or other polymers, to shield from immune recognition.[285] But some data reveals that even after PEGylation some NPs can elicit antibodies. These antibodies promote faster clearance of such NPs from blood and change their pharmacokinetic profile.[286] So, NP-specific antibodies can affect the safety and efficacy of their therapeutic potential.

NPs mediated immunosuppression can be either desirable or inadvertent. On one hand immunosuppression can lower the defense against cancer cells and infections, but on the other hand, it may augment the therapeutic advantages of treatment for autoimmune diseases and allergies and can also prevent the transplanted organ rejection. Most studies focus on inflammatory potential of NPs, while few studies have demonstrated the immunosuppression by the inhalation of CNTs suppressing B-cell functions and the production of TGF-β by alveolar macrophages.[287] NPs can aid to deliver immunosuppressive drugs and prevent immunosuppressive properties of some other drugs.[288] In one study, poly(D,L-lactide-co-glycolide) (PLGA) NPs have been used to deliver glucocorticoids in mouse model inflamed joints as a treatment for arthritis.[289]

Some breast cancer patients treated with Abraxane (paclitaxel bound to human serum albumin) NPs have demonstrated grade 4 neutropenia (decreased number of neutrophils), a form of myelosuppression.[290] Some immunosuppressive agents (eg, corticosteroids, cadmium, tetrachlorodibenzo-p-dioxin) act by impairing the development and functions of T-cells. So the use of quantum dots

Table 4 Different Lysosome-Targeted Nanoformulations Used for Therapy and Imaging Purpose Based on Autophagy and Non-Endocytic Uptake in Different Cancer Cell Types

Nanoparticle Type and Size	Cellular Uptake Mechanism	In vitro/in vivo Models	Imaging/Therapy and Applications	References
Au–ZnO hybrid NPs decorated with cyclic RGD	Integrin receptor-mediated endocytosis	In vitro (HepG2 cells integrin positive) and HL7702 (integrin negative) cells	ZnO mediated ROS generation, LMP-dependent apoptosis	[240]
HApt grafted Au NS (410 ±10 nm)	HER 2 mediated endocytosis	In vitro (SK-BR-3 cells)	HER 2 inhibition, lysosomal degradation cell cycle arrest (Go/G1), apoptosis	[241]
Nucleic acid decorated Au NPs	–	In vitro (HeLa cells)	Acid-sensitive DOX delivery	[242]
Lyso-Ru-NO@FA@C–TiO_2 NPs	Folate receptor mediated endocytosis	In vitro HeLa cells (FA positive) & MCF-7 (FA negative)	NO delivery (Φ_{NO}=0.0174 ±0.002 mol E^{-1}) and PDT ($\Phi_{\Delta=}$ 0.23–0.28) at 808 nm irradiation	[243]
NGO-PEG-BPEI NPs	Energy-dependent endocytic pathway	In vitro (HeLa cells)	PDT (Cholin Ce6)	[244]
Gastrin grafted magnetic NPs (8.7±1.6 nm)	–	In vitro, INRIG9-CCK2R cells	PTT and ROS production (Fenton reaction), caspase 1, and cathepsin B dependent apoptosis	[245]
L-tyrosin and poly(ester-urethane) based NPs (100 ±10 nm)	Energy-dependent endocytosis	In vitro (MCF-7 and HeLa cells)	Thermo and lysosomal esterase responsive DOX and CPT drug delivery	[246]
Ru-CD-RGD NPs (61 nm)	Integrin receptor mediated endocytosis	In vitro (U87MG cells (Integrin positive) MCF-7 cells	ROS production, caspase dependent apoptosis	[247]
Iron oxide-based MG-IONP-DY647 NPs	β-arrestins, clathrin-pits, and dynamin dependent endocytosis	In vitro, HEK293 (CCK2R positive) cells	Lysosomal dependent apoptosis	[248]
FA-conjugated FA-SPIONs (67 nm)	Folate receptor-mediated endocytosis	In vitro and in vivo (MCF-7 cells)	MRI imaging and acid sensitive DOX delivery	[249]
Biotinylated chitosan $CaCO_3$ NPs (200 nm)	Biotin receptor mediated endocytosis	In vitro (MCF-7/ADR DOX resistant and HeLa DOX non-resistant cells	Acidic pH-dependent DOX and TQR (P-gp inhibitor) delivery	[250]
Meso-silica based MSNs-siRNA@DOX-PEG-FA NPs	Folate receptor mediated endocytosis	In vitro (MCF/ADR cells), In vivo (MCF/ADR	Acidic pH-sensitive DOX release, and SiRNA delivery	[251]
Fe_3O_4/CPs based NPs (150 nm)	Endocytosis	In vitro (HepG2 cells), In vivo (H22 tumor xeno-graft, *i.v.* administration)	pH-dependent Zn^{2+} ion release, ROS production and LMP triggered apoptosis	[252]
Bis-styryl BODIPY & DSPE-mPEG5000 NPs	–	In vitro (A549 cells) and in vivo (A549 tumor xenograft)	pH-dependent PDT (730 nm irradiation)	[253]
Fluo-Mor NPs	–	In vitro (HT-20 cells)	pH-dependent PDT (Φ_{Δ}=0.65 at pH 3, 365 nm irradiation)	[254]
Lysosomal escaping and lysosomal toxicity nanoformulations				

(*Continued*)

Table 4 (Continued).

Nanoparticle Type and Size	Cellular Uptake Mechanism	In vitro/in vivo Models	Imaging/Therapy and Applications	References
$NaYF_4:Eu^{3+}$ NPs NY50 (50 nm) NY200 (200 nm)	–	In vitro (BMSC cells)	LMP, lysosomal swelling, cathepsins B and D, ROS generation leads to necrosis	[255]
SiRNA loaded nanogels (siNGs) incubated with CADs	Endocytosis	In vitro (H1299 cells)	siRNA delivery	[256]
S-NP/DNA NPs	Endocytosis	In vitro (HeLa cells)	DNA delivery	[257]
pH-responsive R-P@MSN–DTX NPs	Endocytosis	In vitro (HeLa cells), In vivo (HeLa tumor-bearing mice)	pH Responsive DTX delivery	[258]
INF-7 peptide modified magnetic NPs	–	In vitro (Caco-2Luc cells)	siRNA delivery	[259]
Gd_2O_3@albumin NPs (GA-NP) conjugated with Ce6 PS (10.1 nm)	–	In vitro (4T1 cancer cells), In vivo (4T1 tumor-bearing mice)	MRI-guided PDT and PTT (Φ_Δ =0.1, temperature rise at tumor ~13°C)	[260]
Unimolecular NPs	EGFR-mediated endocytosis	In vitro (MDA-MB-468 cells)	pH/Redox dual sensitive siRNA siRNA delivery	[261]
EGF-HMSNs-5-FU (120 nm)	EGFR mediated endocytosis	In vitro (SW480/ADR cells) MCF-7 cells (integrin negative)	5-FU delivery	[262]
VM-RGD-NPs (2.75 nm) ZnO NPs	Endocytosis	In vitro (BEL-7402/MDR tumor cells), In vivo (BEL-7402/MDR xenograft nude mice) In vitro (SHSY5Y cells)	Verapamil and mitoxantrone delivery ROS generation by zinc ions delivery	[263]
Nanolipoplexes (Nx NPs)	Endocytosis	In vitro (THP-1 macrophages & HIV TZM-bl cells)	siRNA delivery	[264]
Autophagy and non-endocytic uptake nanoformulations				
Multiwall carbon nanotubes (MWCNT)	–	In vitro (RAW264.7 macrophages)	Toxic effects due to increased autophagy, the fusion of lysosomes and autophagosomes	[265]
Arginine functionalized gold NPs as a nanoparticle-stabilized nanocapsule (NPSC)	Non-endocytic uptake pathway	In vitro (HEK293 cells)	Delivery of siRNA, Depleted the PLK1 expression in cancer cells	[266]
Gold nanoparticles (AuNPs) and HIV-1 Tat CPPs.	Direct translocation through the cell membrane (non-endocytic pathway) Non-endocytic pathway	In vitro (human bronchial epithelial cells)	The shape of the cationic object is crucial in the translocation of the cell membrane	[267]
Single-walled carbon nanotubes (SWNT)	Endocytosis	In vitro (CRND8 glial cells)	Reversal of lysosomal proteolysis deficiency and restored the normal mTOR signaling	[268]
Silica NPs (SiNPs)	Endocytosis	In vitro (L-02) and HepG2 cells	Induced autophagy and inhibited the autophagic flux	[269]

(*Continued*)

Table 4 (Continued).

Nanoparticle Type and Size	Cellular Uptake Mechanism	In vitro/in vivo Models	Imaging/Therapy and Applications	References
QD decorated with arginine-based cell-penetrating poly (disulfide) s linkage (CPD-QD) NPs	Direct translocation	In vitro, *Drosophila* S2 cells	Delivery of QDs and delivery of GFP or anti-GFP nanobodies	[270]
Palladium nanoparticles (PdNPs) (20 nm)	Endocytosis	In vitro (HeLa cells)	Autophagic flux blockade and cell death	[271]
Photoactivated nanoparticles (paNps)	–	In vitro, fatty acid–treated INS1 rat- pancreatic beta cells	Reversal of normal lysosomal acidic levels under UV photoactivation	[272]
Graphene oxide NPs and acid-functionalized single-walled carbon nanotubes	–	In vitro (Mouse peritoneal macrophages)	Toxic effects due to the induced autophagy	[273]
Gold nanospheres	Endocytosis	In vitro (HeLa cells)	Trigger more autophagosome accumulation	[274]
Cobalt oxide (Co_3O_4) NPs	Non-endocytic pathway	In vitro (*Xenopus laevis* oocytes)	Calcein-fluorescence quenched after non-transfected (NT) Calcein- injected oocytes exposed to (Co_3O_4) NPs	[275]
Arginine-terminated QD NPs	Direct translocation	In vitro (HeLa and HT22 cells)	Delivery of DQs	[276]

(cadmium containing NPs) on thymus needs adequate studies to understand their therapeutic potential.[291]

NPs are also evaluated for their potential to stimulate adaptive and innate immune responses. The activation of a complement system can be damaging if the NPs inadvertently, or by their design, face the systemic circulation, which can lead to anaphylaxis and other hypersensitivity reactions.[292] The nanoformulation size is considered as a major factor to determine whether it induces type I (interferon-γ) or type II (Interleukin-4) cytokines, thus contributing to different types of immune response.[293]

The basics of NPs mediated immune recognition and examples of the allergic reactions due to its exposure in humans and laboratory test animals have been reported somewhere else.[294] Some allergic reactions are constantly reported during the occupational hazards as exposure to nanoformulations. For example, toxic epidermal necrolysis-like dermatitis has been reported in workers manufacturing dendrimers.[295]

NPs loaded with immunotherapy particles can elicit a strong antitumor response. The immune cells have the capacity to proliferate and propagate the response further by activating complementary immune cells. Immunotherapy-loaded NPs can be directly conjugated to the surface of T-cells. This approach is used to release payloads to augment the functions of either T-cells themselves or to release the payload to modify the tumor microenvironment.[296] Some standard payloads include cytokines like IL-21,[297] and cytotoxic drugs like SN-38. Unlike free NPs, T-cells can concentrate their payloads in tumors by two orders of magnitude. The immunomodulatory NPs can be conjugated to the surface of any leukocyte population, thus facilitating a broad utility for cancer management.

Clinical Trials and Cancer Management

The research over cancer management by lysosome-targeted drugs and its nanoformulation is going on to evaluate the clinical trials and its phases. More than 40 clinical trials have been performed by using hydroxychloroquine (HCQ) on humans and dogs worldwide.[298] Six Phase I/II clinical trials have been accomplished in

patients having melanoma, glioblastoma, refractory myeloma, and other cancers.[299–301] These clinical trials also comprise some combination therapies planned from preclinical investigations.[302–304] These trials have demonstrated that cancer management in humans can be efficiently achieved safely through autophagy inhibition. These trials reported the accumulation of autophagic vesicles in peripheral blood mononuclear and cancer cells. The treatment combinations were tolerated even to higher doses without any metabolic dysfunctions, liver damage, or some neurological impairments.[298] However, some dose limiting toxicities have also been reported by using HCQ–cancer drug combinations. The Phase II clinical trials have also revealed that some more potent drug formulations are required to have a better outcome as higher doses of HCQ alone did not demonstrate better therapeutic efficacy for previously treated metastatic pancreatic cancer.[305] The CQ dimerization led to the formation of Lys05, which is far more potent as a single agent and in combination with B-Raf protooncogene serine/threonine protein kinase (BRAF) inhibitors.[306]

Future Aspects of Targeting Lysosomes and Cancer Management

The recent findings clearly mention that the degradative functions of lysosomes are closely linked to multiple pathways, which control the overall cellular homeostasis. Now past are the days when lysosomes were considered as isolated organelles with limited functions and little contacts with other organelles and processes. Some recent research has elicited the role of lysosomal activity involving cell trafficking, nutrient sensing, kinase signaling and death signaling. The new studies must report the lysosomal functions in the context of the entire cell and organism.

Despite the recently published massive research about lysosomes, its connection with other organelles and cellular processes, it just represents the tip of an iceberg. The number of new queries build up much faster than the previous doubts are cleared. Some of the new queries to be resolved in the future are to understand the role of hundreds of peripheral and integral lysosomal membrane proteins whose functions have not been discovered yet. In addition, how do ion and nutrient transporters respond to cellular metabolism and connect lysosomes with cellular environment, needs to be solved.

Regarding the cancer cells, the knowledge about lysosomes is still in its infancy. The future tasks include to know the role of lysosomes in a varied cellular environment, and to know if these variations are the same in all cancer cells or change from cell-to-cell or vary at different stages of cancer cells. The approach of direct targeting lysosomes with anticancer drug nanoformulations within the tumor cells will dramatically decrease the drug action at non-specific locations, side-effects, and unwanted higher drug load. The specific pin-point drug targeting tactics are the ultimate goal of future therapeutics in cancer management.

Some novel biomarkers availability for the assessment of drug efficacy is one of the major limitations for clinical trials. The current methods for the visualization of autophagic vesicles accumulation in cancer cells include electron microscopy along with Western blotting and immunohistochemistry. Some recent evidence has encouraged that more potent autophagy inhibitors will be available which can be used synergistically with radiotherapy and conventional chemotherapy. Although in recent years, the knowledge about NPs and their interaction with immune system components have improved, but still many questions require a thorough understanding and deeper investigation. Some more mechanistic studies are needed to investigate the NPs immunomodulatory effects to improve the understanding of physicochemical parameters in relation with the immune system.

The organelle-targeting nanoformulations face tremendous challenges because of wide variations in biological systems. Different nanoformulations like micelles, liposomes, dendrimers, CNTs, etc. tagged/loaded with different lysosomotropic agents and anticancer drugs are being worked out in current research. However, it is of utmost importance to have comprehensive research to comprehend appropriately the safety aspects of these nanoformulations when used in human subjects. Furthermore, it is a very challenging task to prepare perfectly targeted oriented lysosomal drug nanoformulations in cancer cells without any toxicity to nearby normal tissues.

To overcome these challenges, it is very important to know the physico-chemical properties of different nanoformulations like size, shape, charge, drug loading, and its release capacity in addition to specific targeting. So the challenges of cancer management by lysosome targeted drug nanoformulations are quite tough, which are expected to be resolved appropriately by undergoing vigorous research in this area.

Conclusion

The recent updates about the structure and function of lysosomes as well as their role in different diseases including cancer is quite fascinating. The lysosomes are now well recognized as the central communication hub of the major metabolic activities within the cell. The knowledge about the changes within lysosomes in cancer advancement and treatment is still quite young, but some current innovative advances in this area promise speedy progress in the near future. The recent updates about the lysosome ultrastructure, role in different diseases, its cross-talk with other organelles, and the use of some drug nanoformulations, which directly or indirectly target this organelle within the tumor cells, is currently being mentioned to boost the therapeutic strategies. In different cancer cells, the lysosomes have been targeted with different lysosomotropic drug nanoformulations to perturb its signal transduction cascades, pH, Ca^{2+} homeostasis, membrane permeabilization, and disruption in autophagy and apoptosis.

One of the distinguished hallmarks of cancer is its ability to escape or dodge the immune response. Some more recent scientific advances elucidate the implementation of innovative approaches for immunotherapies to eradicate or treat diverse cancers. NPs can facilitate the location, pharmacokinetics, and co-delivery of special immunomodulatory drugs eliciting the anticancer responses which cannot be achieved by free drugs. The convergence of biotechnology, nanotechnology, cancer immunotherapy, and drug delivery approaches can now be employed to eradicate the cancer menace in the not-too-distant future. Only a few nanoformulations like peptide facilitated-AuNPs, TPP-PEG-biotin, octadecyl-rhodamine B, cationic liposomes, and mixed charged NPs have been engineered and satisfactorily used to target lysosomes in cancer cells. Despite the initial success of some free drugs or drug-conjugated nanoformulations targeted to lysosomes, systematic pre-clinical and clinical surveys are required for their authentic use in final clinical settings.

Abbreviations

AKT, serine-threonine–specific protein kinase; AMP, adenosine monophosphate; AMPK, 5′-adenosine monophosphate-activated protein kinase; CaMKK2, calcium/calmodulin-dependent protein kinase kinase 2; EB, transcription factor EB; EF25-(GSH)2, 3,5-bis(2-hydroxybenzylidine)tetrahydro-4*H*-pyran-4-1 glutathione conjugate (a water-soluble curcumin analog); ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; FNIP, folliculin interacting protein; γH2AX, γ-H2a histone family member X; GAL-3,-8,-9, galactins; GSK, glycogen synthase kinase; GTPase, guanosine triphosphatase; JNKs, c-Jun NHκ-terminal kinases; LBP8, lipid-binding protein 8; LC3A/B-II, microtubule-associated protein 1A/1B light chain 3B, modified lipid form; LC3-II, light chain 3 phosphatidylethanolamine conjugate; LKB1, liver kinase B1; miR-101, microRNA 101; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; NF-κB, nuclear factor κ light chain-enhancer of activated B cells; OCRL, oculocerebrorenal syndrome protein; OEA, oleoylethanolamide; p21, cyclin-dependent kinase-interacting protein 1; p53, tumor protein p53; p70S6, ribosomal protein S6 kinase; PI3K, phosphoinositide 3-kinase; PIP5K, phosphatidylinositol 4-phosphate 5-kinases; RAG GTPases, Ragulator GTPase; ROS, ROS proto-oncogene, receptor tyrosine kinase; TFEB, transcription factor EB; TLR9, toll-like receptor 9; TRIM16, tripartite motif-containing protein 16; TSC, tuberous sclerosis complex; ULK1, Unc-51-like kinase 1; Wnt, wingless/integrated.

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