REVIEW ARTICLE

Nanotherapeutics in autophagy: a paradigm shift in cancer treatment

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Abstract

Autophagy is a catabolic process in which an organism responds to its nutrient or metabolic emergencies. It involves the degradation of cytoplasmic proteins and organelles by forming double-membrane vesicles called "autophagosomes." They sequester cargoes, leading them to degradation in the lysosomes. Although autophagy acts as a protective mechanism for maintaining homeostasis through cellular recycling, it is ostensibly a cause of certain cancers, but a cure for others. In other words, insufficient autophagy, due to genetic or cellular dysfunctions, can lead to tumorigenesis. However, many autophagy modulators are developed for cancer therapy. Diverse nanoparticles have been documented to induce autophagy. Also, the highly stable nanoparticles show blockage to autophagic flux. In this review, we revealed a general mechanism by which autophagy can be induced or blocked via nanoparticles as well as several studies recently performed to prove the stated fact. In addition, we have also elucidated the paradoxical roles of autophagy in cancer and how their differential role at different stages of various cancers can affect its treatment outcomes. And finally, we summarize the breakthroughs in cancer disease treatments by using metallic, polymeric, and liposomal nanoparticles as potent autophagy modulators.

Keywords Autophagy · Autophagosomes · Apoptosis · Cancer · Chemotherapeutic agents · Nanoparticles

Introduction

Autophagy is a catabolic process in which an organism responds to its nutrient or metabolic emergencies. It involves the degradation of cytoplasmic proteins and organelles by forming double-membrane vesicles, termed "autophagosomes," which sequester cargoes, leading them to degradation in the lysosomes. The formation of autophagosomes is controlled by a specific set of autophagy genes called *ATG* genes (Fig. 1). An aberrant behavior in these one or more autophagy genes often leads to various pathological conditions, including tumorigenesis, inflammatory conditions, and neurodegeneration.

Autophagy and cancer association: genetic evidence

Cancer is not a single disease. It is an amalgamation of more than 100 distinct and different diseases. Although there are various types of cancer, all cancers initiate from the abnormal growth of cells [1–5].

Cancer is caused by the malignant transformation of a normal cell into a cancerous cell. It occurs as a result of a successive accumulation of genetic mutations and epigenetic changes that overpower infallible cellular mechanisms such as apoptosis (programmed cell death type I) or oncogene-induced senescence [6, 7].

Some first shreds of genetic evidence point out the relationship between autophagy and cancer. First, the study in 1999 [8] indicated that human breast carcinoma cell lines frequently contain allelic deletions of Beclin1, which was monoallelically deleted in human breast, ovarian, and

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Fig. 1 Mechanism of autophagy: Activation of growth receptor, stress, and nutrient deprivation induces AMPK signaling and inhibits mTOR signaling, which activates the ULK complex (FIP 200, ATG 13, ULK 1/2) and induces the nucleation process. ULK complex further activates PI3K class III complex 1 (Beclin 1, Vps 15, Vps 34, and ATG 14L) and initiates the development of the phagophore. The maturation, as well as the elongation of the developed phagophore, is mediated via the interaction of multiple ATGs like ATG5-ATG12, ATG16, etc. The interaction generates two "ubiquitin-like conjugation systems" which catalyze phosphatidylethanolamine (PE)-conjugated LC3 (LC3II) formation. LC 3II then binds on the surface of autophagosomes. The binding of LC3II on the surface of autophagosomes helps in the internalization of the cellular substrates within the autophagosomes. Further, the presence of exosomal sorting complex (CHMP2A, and VPS4) promotes the closure of the membrane of autophagosomes, which marked the complete maturation of the autophagosomes. The matured autophagosomes amalgamate with the lysosomes in the presence of SNARE proteins and form autolysosomes. The acidic hydrolases existing within the lysosomes provide degradation of nutrients as well as metabolites within the autophagic cargo. These degrading products further get recycled back to the cytoplasm
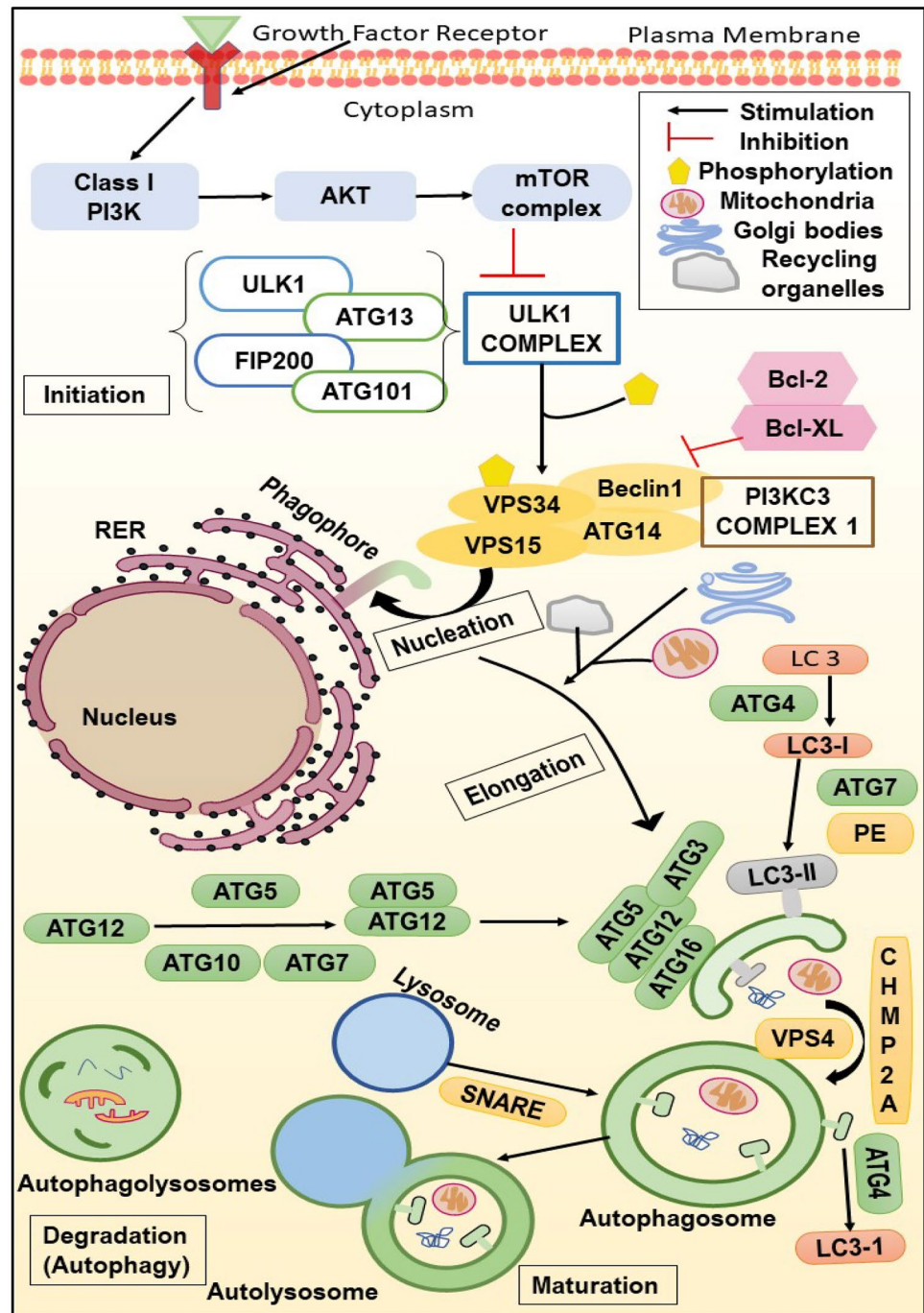
prostate cancers and was expressed at reduced levels in those tumors. The tumor suppressor Beclin1 is part of the phosphatidylinositol 3-kinase class III (PI(3)KCIII) lipid–kinase complex that induces autophagy [9].

Later in 2006, the novel ultraviolet irradiation resistance-associated gene (UVRAG), a tumor suppressor gene, was identified, which is a positive regulator of the Beclin1–PI (3) KCIII complex and hence autophagy. UVRAG was monoallelically mutated at high frequency in human colon cancers [10, 11].

In ensuing studies, targeted mutant mouse models were used, showing that Beclin 1-/- mutant mice die early in embryogenesis (due to disruption in autophagy and not due to apoptosis). In contrast, Beclin 1 +/- mutant mice (hemizygous to Beclin1) are viable but suffer from a high incidence of spontaneous tumors [12, 13]. In 2007, the findings suggested that the complete loss of the gene encoding the UVRAG-binding protein BIF-1 resulted in tumor susceptibility in mice [14]. All these studies, taken together, strongly suggested that autophagy plays a vital

role in human tumor suppression. What is important to note here is that in all the mouse models described above, there was a deletion of the autophagy-related gene in the target cells since their embryonic stage. Hence, all the tumors observed resulted from all-time autophagy impaired cells. Therefore, these experiments gave no clarity about the role of autophagy loss in an established tumor, which is a more common and likely scenario to occur. To find answers to this, robust research was initiated in this field.

Cancer autophagy-related dichotomies

The cytoprotective trait of autophagy is to lessen or moderate the influence of metabolic stress, which occurs due to nutrient and oxygen unavailability. During the growth of tumors, the vascularisation system is hampered, limiting the nutrient supply in the hypoxic regions within tumors [15]. Since autophagy is known to be activated in these regions and could be utilized to keep these tumor cells alive, it seems paradoxical how the inactivation of a process that can keep tumor cells alive benefits tumor development. The quick fix for this contradicting viewpoint is that autophagy evades stress to maintain cellular homeostasis. However, insufficient autophagy can no longer provide nutrients, prevent the accumulation of defective proteins and organelles, manage oxidative stress, and limit inflammation. These factors, along with genomic instability, lead to tumorigenesis.

Autophagy was not only found to promote cell survival in solid tumors [16] but was also found to contribute to cell death [17]. Hence, there were dichotomies and paradoxes in understanding the role of autophagy in both genesis and suppression of cancer. To elude these incongruities coupled with cancer and autophagy, the answer lies in the fact that autophagy's exact role is dependent on the context. This includes the type of tumor in consideration, the stage of tumor/neoplasia, the cellular and metabolic context in which the tumor cell lines, etc. In the following section, we discuss these disjunctions in more detail.

Autophagy in oncogenesis

The role of autophagy in oncogenesis is contradictory. Chronic suppression of autophagy during the initial tumor stage may stimulate oncogenesis. However, once a tumor is established or is in the advanced stage of progression, autophagy might go against the body and promote tumor survival (Fig. 2). This role of autophagy depends on many factors, and its exact mechanism of switching its role is elusive.

Tumor suppressive or pro-death mechanism?

Autophagy is a principal mechanism in maintaining cellular homeostasis and chromosomal stability. Inhibition or lack of autophagy results in increased levels of reactive oxygen species, which leads to accumulation of DNA damage, manifesting itself as gene amplification, increased double-strand breaks, and polyploid nuclei [18, 19]. Under stress conditions, deregulation or loss of autophagy leads to increased DNA damage, which makes the cell more prone to de novo tumor formation and development. How this is achieved and the appropriate mechanism behind it is equivocal. Nonetheless, the cumulation of many corroborations outlines that autophagy acts as a tumor-suppressive or pro-death mechanism under several defined conditions.

BECN-1

The first link between autophagy and tumor suppression came from the discovery that BECN (the gene that encodes for Beclin1) is a haploinsufficient tumor suppressor gene and was found to be monoallelically deleted in approximately 50% of human breast, ovarian and prostate cancers and expressed only at low levels in brain tumors [9, 20].

ATG5 and ATG7

ATG5 and ATG7 are two other critical autophagy regulators whose deletion and subsequent aftereffects have been analyzed in experimental animal models. In contrast to the embryonic death in the case of BECN1, it was found that mice with systemic mosaic deletion of Atg5 and liver-specific Atg7−/− mice develop benign liver adenomas [21, 22]. As Atg7 and Atg5 are essential for amino acid supply in neonates and starvation-induced bulk degradation in mice, the depletion of these genes leads to the death of both mice within 24 h of their birth. This occurs as a result of autophagy inhibition, due to which transplacental nutrient supply is suddenly interrupted, and neonates face severe starvation and ultimately death [22, 23].

ATG4C

The most widely expressed mammalian orthologue of yeast Atg4 is ATG4C (autophagin-3). Mutant mice deficient in ATG4C were generated and found fertile and viable without any abnormalities in the early neonatal period. However, tissue-specific autophagy alterations were leading to decreased autophagy in the diaphragm. To boot, animals deficient in Atg4C show an increased susceptibility to develop fibrosarcomas induced by chemical carcinogens [24]. Giving
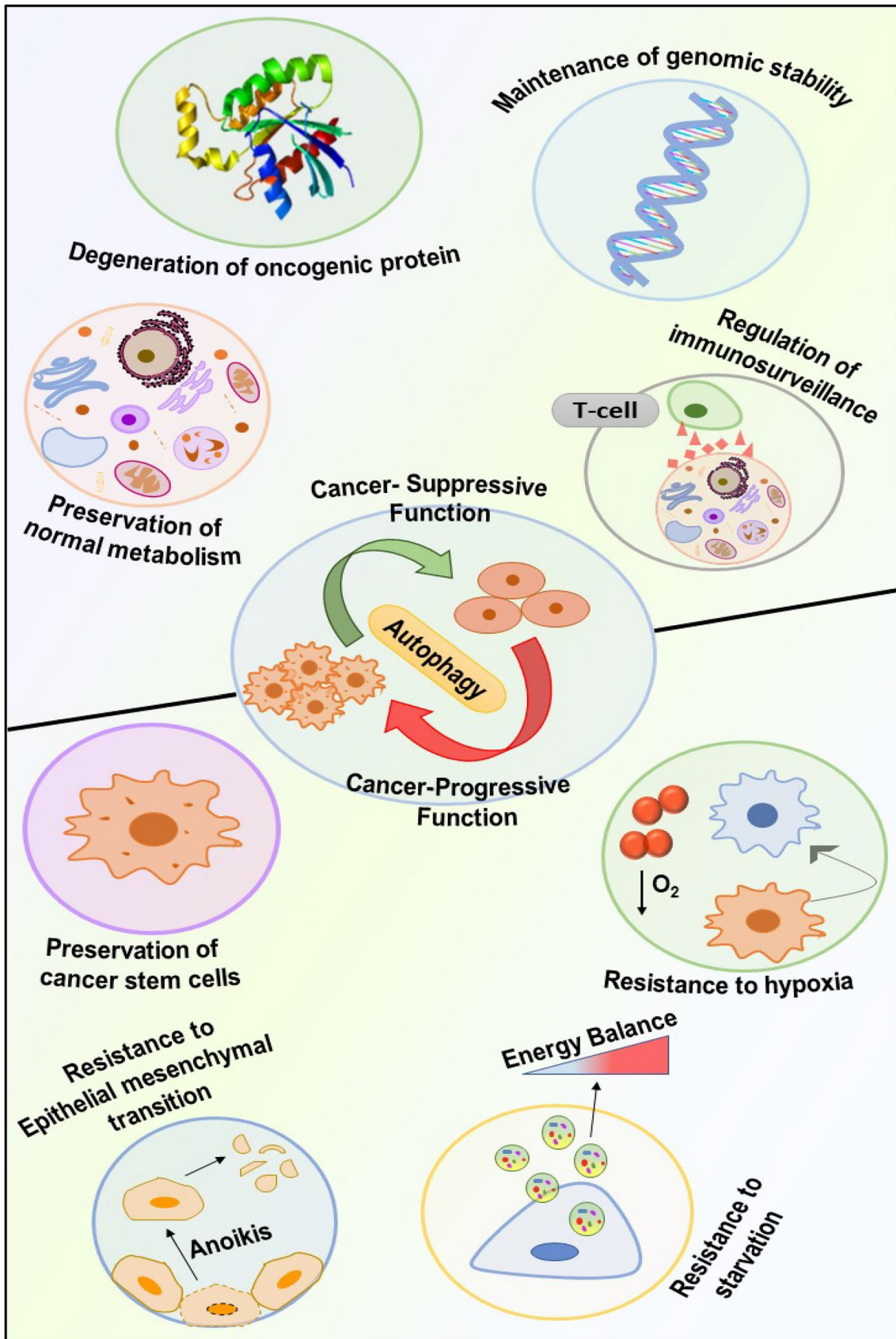
◄**Fig. 2** Conflicting role of autophagy during oncogenesis: The onco-suppressive properties of autophagy include preservation of normal metabolism, degradation of oncogenic proteins, maintenance of genomic proteins, and regulation of immunosurveillance. On the other hand, autophagy speeds up the cancer progression and undergoes resistance to chemotherapy, once the cancerous transformation has occurred. The cancer-supporting properties depict the capacity of autophagy in preserving the cancer stem cells, promoting resistance in cancerous cells toward various endogenous conditions like anoikis, hypoxia, nutrient deprivation, and epithelial-to-mesenchymal transition (EMT) that could otherwise lead to cell death

substance to this is the study [25], which showed that the expression level of ATG4C is deeply associated with the prognosis of glioma patients.

UVRAG

As mentioned earlier, in "Autophagy and cancer association: genetic evidence" section, UVRAG (a tumor suppressor gene) is found to be monoallelically mutated at high frequency in human colon cancers. In contrast, in the xenograft studies, UVRAG's expression in human HCT116 colon carcinoma cells makes them less susceptible to tumorigenicity [10, 11]. Frameshift mutations of UVRAG are also present in gastric carcinomas in addition to colorectal carcinomas with microsatellite instability and promote tumorigenesis [26, 27]. Furthermore, disruption of the association of UVRAG with centrosomes causes centrosome instability and aneuploidy, which is a hallmark of cancer [28].

BIF1

Bif-1 (also known as endophilin B1 or SH3GLB1) is another protein that joins the UVRAG–Beclin 1 complex as a potential activator of autophagy and tumor suppressor. Unlike Beclin-1-deficient mice, which were embryonically lethal, Bif-1-/- developed normally and were indistinguishable from their wild-type littermates, except for an enlarged spleen. However, these mice had an 89.7% chance of developing spontaneous tumors at an average age of 12 months. Hence, this showed that the knockout of Bif-1 significantly enhances the development of spontaneous tumors in mice [14].

Accumulation of p62

p62 (also known as sequestosome-1 or SQSTM 1) is a multi-domain protein that interacts with the autophagy machinery as a key adaptor of target cargo by linking LC3 with ubiquitin moieties on misfolded proteins. Autophagy, therefore, mediates the clearance of p62 together with ubiquitylated proteins. Hence, suppression of autophagy results in p62 accumulation and contributes to tumorigenesis [29]. Increased evidence of p62 upregulation and/or reduced

degradation has been implicated in tumor formation, cancer promotion as well as in resistance to therapy. The growth of liver tumors caused by inhibition of autophagy was greatly reduced by p62 deletion [21]. Chronic p62 elevation contributes to hepatocellular carcinoma by preventing oncogene-induced senescence and death of cancer-initiating cells and enhancing their proliferation [30]. Tumor promotion activity of p62 was observed in hepatitis B virus (HBV)-associated hepatocarcinoma [31], breast [32], gastric [33], and prostate cancers [34].

Oncogene-induced senescence

Autophagy is regarded as an effector mechanism of senescence which is essential for the rapid protein remodeling required to make the efficient transition of a tumor from a proliferative to a senescent state. Furthermore, inhibition of autophagy delays the senescence phenotype. Hence, autophagy might be required in this process as a tumor suppressor [35].

Tumor growth or pro-survival mechanism?

As seen in the previous section, insufficient autophagy can promote a normal cell to a state of a malignant tumor. However, increasing evidence also suggests that the tumor in the advanced stages of tumorigenesis is strongly dependent on autophagy for survival and proliferation. Poor sensitivity of anticancer therapies showed that autophagy, rather than inducing autophagic cell death (as presumed earlier), is helping cancer cells cope with the metabolic stress and hypoxia progressing to tumor growth. Many observations suggest the need for autophagy in cancer development.

BECN-1

In contrast to the previous study, which depicted decreased beclin-1 expression in breast cancer cells compared to the normal breast cells [8], another study indicated that expression of beclin-1 was detected in 95% of the colorectal carcinomas and 83% of the gastric carcinomas. In contrast, normal mucosal cells of both stomach and colon showed no or very weak expression of beclin-1. This identification suggests the possibility that neo-expression of beclin-1 may play a role in both colorectal and gastric tumorigenesis [36].

Another study showed that monoallelic deletion of beclin-1 blocks or delays tumor formation in TSC2 + / − and ATM − / − mice, respectively [37, 38].

FIP200

Again, in contrast to monoallelic deletion of beclin-1 leading to tumor progression, the knockout of essential autophagy

proteins including FIP200 (FAK family-interacting protein of 200 kDa) in various tissues did not lead to malignant tumor development in vivo. Furthermore, inhibition of autophagy by FIP200 ablation suppresses mammary tumor initiation and progression in a mouse model of breast cancer. This provided strong evidence for a pro-tumorigenesis role of autophagy in oncogene-induced tumors in vivo [39].

ATG5 and ATG7

It was observed that both the mRNA and protein levels of ATG5 and ATG7 were increased in cells overexpressing K-RasV12. K-RasV12 overexpression leads to malignant transformation in human breast epithelial cells. Hence, this provided the first evidence that autophagy is essential for oncogenic K-Ras (K-RasV12)-induced malignant cell transformation [40].

Cytoprotective traits

The cardinal role of autophagy in established tumors is to fulfill their nutrient/oxygen requirements and allow them to survive in conditions of metabolic and cellular stresses.

It was seen that normal cells die within 24 h when subjected to extreme nutrient starvation. On the other hand, liver cancer cells died within 36 h, > 50% of pancreatic cancer cells survived, even after starvation for 48 h, 1 of 3 gastric cancer cells survived > 36 h, and > 50% of the colon cancer cells survived > 36 h [41]. In solid tumors, enhanced autophagy is frequently found in the hypoxic regions contributing to cell survival [42]. Due to increased cell proliferation, cancer cells have a high demand for nutrients and oxygen. When the blood supply is insufficient, particularly during the initial steps of tumor formation or in the poorly vascularized regions of solid tumors, cancer cells encounter metabolic stress and induce autophagy to allow them to survive [43, 44].

Induction of dormancy

Dormancy is a stage in cancer progression where the cells cease dividing but survive in a quiescent state while waiting for appropriate environmental conditions to begin proliferation again [45]. It is seen that autophagy has a crucial role in inducing tumor cell dormancy under stressful conditions.

Pharmacologic or genetic inhibition of autophagy in dormant breast cancer cells results in significantly decreased cell survival and metastatic burden in mouse and human 3D in vitro and in vivo preclinical models of dormancy [46]. Another study shows that the tumor suppressor gene aplasia Ras homolog member I (ARHI; also known as DIRAS3), in an ovarian cancer xenograft mouse model, leads to acute cell death and tumor regression initially. However, subsequently, the tumoral resurgence is furnished when ARHI expression and autophagy are terminated. This showed that autophagy helps the cells to remain dormant and still survive [47, 48].

Inhibition of anoikis

Anoikis is a programmed cell death induced upon cell detachment from the extracellular matrix, thus avoiding colonizing of distant organs [49]. Inhibition of cell death by anoikis due to autophagy induction during the later stages of tumor progression, such as tumor cell dissemination and metastasis, can lead to tumor cell survival [50]. Due to anoikis inhibition, the cancer cells undergo malignant transformation and can be transferred through blood or lymphatic circulation to a distant location, undergoing metastasis [51]. It was shown that the knockout of Atg5 and Atg7 genes in the 3D culture model of MCF-10A induced pro-survival autophagy in the epithelial cells promoting cell survival during anoikis [50, 52]. Furthermore, clinical studies deduced that the under-expression and overexpression of autophagy in melanomas were associated with poor prognosis. However, the latter manifested metastases much earlier than the former [53]. Similarly, hyperactivation of autophagy in HCC (hepatocellular carcinoma) facilitates malignant tumor differentiation, which results in a more aggressive cancer cell phenotype and poor prognosis of HCC [54].

Adaptation to reprogrammed metabolism

Cancer cells are observed to rewire their metabolism and energy production networks to support their increased biosynthetic needs [55]. In 2011, Drs. Hanahan and Weinberg have recognized cancer metabolic reprogramming as one of the ten cancer hallmarks and published it in their seminal review paper [6]. In the 1920s, the legendary German biochemist Otto Warburg established the first link between tumorigenesis and metabolic reprogramming and is also known as the "Warburg effect." Normally differentiating cells rely primarily on mitochondrial oxidative phosphorylation for fulfilling their metabolic requirements and energy generation. In contrast, high levels of aerobic glycolysis activity were observed in cancer cells [56]. The hypothesis is that mitochondrial dysfunction leads to its repurposing, facilitating the uptake and incorporation of nutrients into the biomass (e.g., nucleotides, amino acids, and lipids) needed to produce a new cell [56, 57].

Shifting of the metabolic pathway from oxidative phosphorylation to aerobic glycolysis is induced and regulated by several oncogenes. In cells transfected with activated RAS and SRC oncogenes, the rate of glucose uptake was markedly increased, which is a sign of their metabolic reprogramming [58, 59]. Activation of RAS can also induce autophagy in tumor cells [60–62]. There is an accumulation

of abnormal mitochondria and reduced oxygen consumption in Ras expressing cells. As mitochondria sustain the viability of Ras expressing cells in starvation, autophagy is required to maintain a pool of functional mitochondria necessary to support Ras-driven tumors' growth [60, 62]. Hence, inhibiting autophagy virtually abolishes the tumorigenicity of RAS and reduces glucose metabolism.

The MYC oncogene, which contributes to the genesis of many human cancers, encodes a transcription factor c-Myc, which links altered cellular metabolism to tumorigenesis [63]. Similarly, the constitutive activity of the serine/threonine kinase AKT (also known as protein kinase B (PKB)) is a common perturbation observed in malignant cells. It was observed that Akt activation did not lead to the increased proliferation of malignant cells but stimulated glucose consumption in transformed cells without affecting the rate of oxidative phosphorylation [64, 65]. All these studies suggested that activation of oncogenes, such as RAS, SRC, MYC, and AKT, seems to orchestrate the metabolic changes associated with cell transformation [66].

Survival and self-renewal of cancer cells

Since the establishment of the cancer stem cell (CSC) theory and the discovery of CSCs in individual cancer types, autophagy has been proposed to be a key mechanism in their homeostasis, dismissal, or spread [67]. Cancer stem cells (CSCs) are a small subpopulation of cells within tumors with capabilities of self-renewal, differentiation, and tumorigenicity when transplanted into an animal host [68]. Pluripotency is the key feature of CSC, which allows them to divide indefinitely and maintain the undifferentiated state [69].

From the study of breast cancer CSCs, it was found that autophagy regulates breast CSC maintenance in autophagy-dependent breast cancer cells by modulating IL6 secretion, hence maintaining the pluripotency of the CSCs [70, 71]. Furthermore, autophagy is found to be upregulated in the mammospheres [72, 73], compared to adherent cells, and both Beclin 1 and ATG4, two essential autophagy proteins, are needed for their maintenance and expansion [74].

In recent studies, autophagy's relationship with various CSCs has been identified and documented. These include breast [75, 76], pancreatic, liver [77], osteosarcoma [78], ovarian [79], and glioblastoma [80] CSCs, in which autophagy impairment negatively affects the expression of staminal markers and consequently the cell self-renewal capacity [74].

Autophagy in different stages of oncogenesis

From various studies, it has been observed that the process of autophagy always acts as a means of protector during oncogenesis, even if it serves dual roles of tumor suppressor and promoter in various stages of oncogenesis. Autophagy safeguards healthy cell homeostasis during the early stages of oncogenesis via restricting the genome instability by retarding the stem cells involved damage/repair cycle and prohibiting the formation of inflammation or tumor microenvironment. During the late stage of oncogenesis, autophagy safeguards the survival of tumor cells by providing support for metabolic demand and reducing metabolic damage. Further, autophagy increases the migratory behavior of cancer cells by initiating the anoikis resistance and dormancy [81].

Autophagy modulation: overcoming resistance to cancer therapies

As discussed previously, the bipolar nature of autophagy leads to its completely contrasting traits in various cancers. It can be upregulated or downregulated at different stages and conditions of cancers. Additionally, this behavior impacts the anticancer therapies devised for multiple cancer types. Depending on its subsequent roles, it can have either a beneficial or a detrimental influence on the patients undergoing cancer treatment therapies. Let us see how.

Drugs inhibiting autophagy

As discussed in "Drugs inducing autophagy" section, autophagy acts as a pro-survival mechanism by aiding the hypoxic and nutrient-deficient tumor cells to survive such hostile conditions. In this case, the activation of autophagy leads to tumorigenesis. Similar to this, recent studies have demarcated growing evidence of autophagy's potential for protecting cancer cells against chemotherapy by mediating their acquired resistance phenotype.

Recent studies have provided increasing shreds of evidence that autophagy is seen to upregulate in tumor cell lines on exposure to various anticancer therapies. These can include radiation therapies, chemotherapy, and targeted cancer therapies [82, 83]. Hence, pre-studies demonstrated that introducing drugs that could inhibit autophagy, along with anticancer drugs, resulted in the increased sensitization of tumor cells to anticancer therapeutic agents [84] (Fig. 3).

Many in vitro and in vivo studies have been carried out to validate this hypothesis. In human colon cancer cell lines, autophagy was seen to have a cytoprotective role against 5-fluorouracil (5-FU)-based adjuvant chemotherapy. However, autophagy inhibition by 3-methyladenine (3-MA) or small interference RNA targeting Atg7 (Atg7 siRNA) produced a synergistic effect along with 5-FU by significantly augmenting 5-FU-induced apoptosis [85]. A similar observation was noted when 5-FU was used with another autophagy inhibitor, chloroquine (CQ) [86]. In hepatocellular carcinoma (HCC) tumors, the anticancer effects of
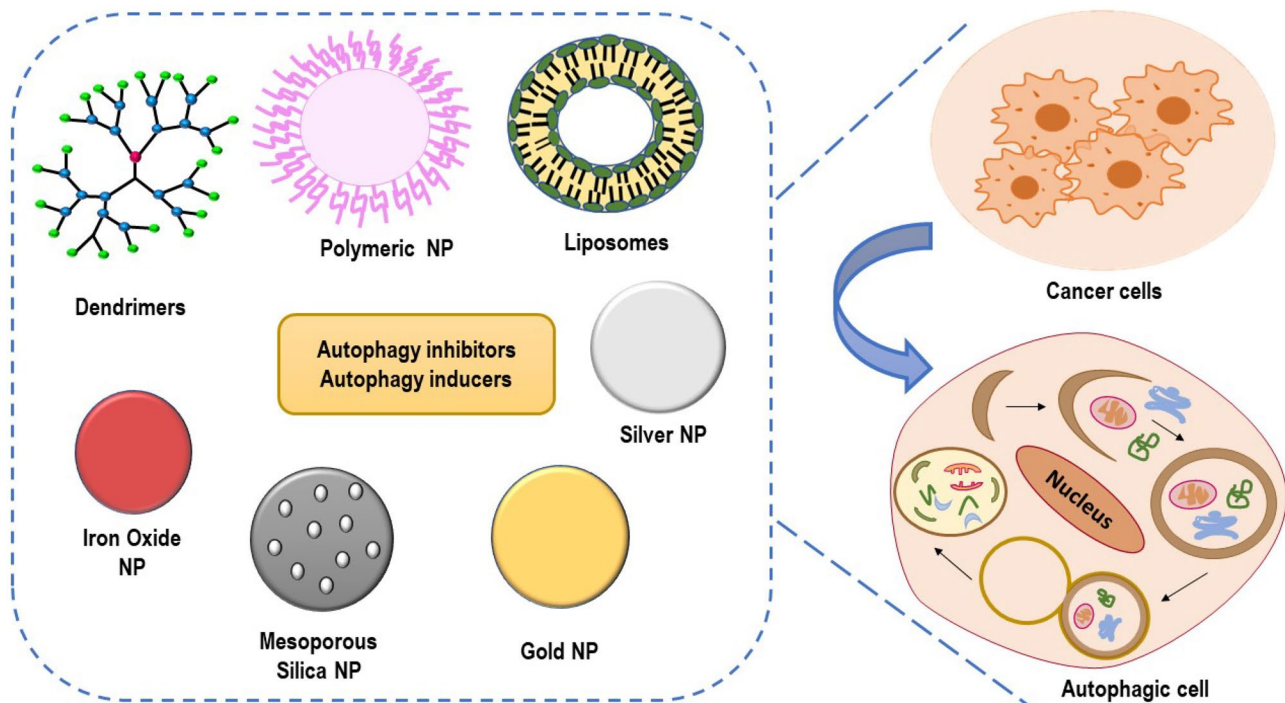
Fig. 3 Induction and inhibition of autophagy cancer therapy: Cancer cells experience either apoptotic or autophagic cell death or both when treated with autophagic inducers in combination with chemotherapeutics entrapped in nanoparticles. Such an approach provides a cytotoxic process. While some cancerous cells activate autophagy as a response to alleviate the stress induced by the chemotherapeutics and provide cytoprotective autophagy which promotes cell survival. In such cases, nanoparticles are administered comprising of a combination of autophagic inhibitors and chemotherapeutics. Autophagic inhibitors sensitize the cancerous cells toward the chemotherapeutics, as a result, increase the cytotoxic effects induced by the chemotherapeutics leading to apoptosis

bevacizumab and sorafenib were augmented by using them in combination with chloroquine or 3-MA and CQ, respectively [86, 87]. Furthermore, in human lung cancer cell lines, EGFR tyrosine kinase inhibitors, gefitinib, and erlotinib, when solely used, were seen to acquire therapy resistance by inducing high levels of autophagy. However, cytotoxicity induced by these drugs was significantly enhanced when used in amalgamation with autophagy inhibitors like CQ and siRNAs targeting ATG5 and ATG7, which are essential autophagic components [88, 89]. Also, in CML, the drug suberoylanilide hydroxamic acid (SAHA), which is a histone deacetylase (HDAC) inhibitor, when used in combination with 3-MA, CQ, or bafilomycin A, helps overcome autophagy-aided drug resistance [90].

Multiple potential anticancer therapies and autophagy inhibitor drug combinations have been identified and documented. It is seen that different autophagy inhibitors work in association with different anticancer therapies by blocking the autophagic process at different stages. Some examples include CQ, HCQ (anti-malarial drugs), and bafilomycin A1 sensitize tumor cells to therapy by inhibiting autophagosome fusion with lysosomes and its degradation. 3-MA, LY294002, and Wortmannin lead to Class III PI3K

inhibition, impeding autophagy's initiation or expansion stage. Furthermore, autophagic inhibition is also achieved by genetic silencing of autophagy regulatory genes [91–94].

Drugs inducing autophagy

As discussed in "Drugs inhibiting autophagy", autophagy is also a self-cannibalistic process despite its clear pro-survival role and cytoprotective traits. This is due to its ability to induce cell death of cancer cells in case of excess or enhanced autophagy. Increasing evidence has been provided stating it exhibits a pro-death mechanism in cases of hampered or defective apoptosis [95–98]. This role of autophagy contributes to the efficacy of anticancer drugs by generating an autophagy-mediated cell death mechanism (Fig. 2). Anti-tumor action of cannabinoids (autophagy inducers) was enhanced in glioma cancer cells by the inhibition of mTORC1 and thereby activation of an ER stress response which promoted autophagy [99]. Similarly, the activity of gemcitabine (used in the treatment of advanced pancreatic adenocarcinoma) was augmented by adding cannabinoids. Their combined effect strongly inhibited the growth of human pancreatic tumor cells xenografted in nude mice without apparent toxic effects [100]. Furthermore, cannabinoids were also found

to generate an anti-tumoral action on hepatocellular carcinoma by activating the AMPK pathway and autophagy [101]. These studies highlighted the possibility of designing new therapeutic strategies that could exploit autophagy's pro-death mechanism. In Atg5 and Atg7 knockout cultured colon cancer cells, the cytotoxicity of FK-16 (derived from anticancer peptide LL-37) was seen to be attenuated, which pointed toward the pro-death nature of FK-16-induced autophagy [102]. The cell death induced by temozolomide in the glioblastoma cell line was potentiated by the mTOR inhibitor, RAD001 [103].

All these results strengthen the connection between autophagy and anticancer sensitivity. Hence, all these results point toward autophagy induction being considered as a new hope for possibilities in targeting and designing treatments for these malignancies [104].

Targeting autophagy in cancer: nanotechnology-based approaches

We have seen how the contrasting roles of autophagy and its modulation in cancer can be exploited to understand the designing of chemotherapies and treatments for cancer patients. On the one hand, in tumors with elevated levels of autophagy, anticancer agents are administered along with autophagy inhibitors. This inhibits tumor cell survival under metabolic and chemotherapy stress. On the other hand, overstimulation of autophagy, especially in apoptosis defective cells, by the use of autophagy inducers along with cytotoxic drugs may lead to autophagy-mediated tumor cell death [104]. Hence, the importance and utility of conventional chemotherapeutic drugs could not be denied; however, they do carry a variety of side effects along with them [105]. Therefore, new and improved treatments involving the role of nanotechnology provide novel approaches to harness more possibilities in cancer treatments [106]. This section covers the various aspects and advances in the use of nanoparticle-based strategies as potent modulators of autophagy and their therapeutic implications in cancer.

Why are nanoparticles (NPs) compelling candidates compared to common autophagy-inducing drugs?

Conventional drugs pose certain obstacles to offering efficient, safe, and innovative agents to patients for fighting cancer. Various side effects are triggered by conventional drugs, which include poor specificity, uneven tissue, and organ distribution, rapid clearance of the drug, and its biodegradation [105]. The use of nanomedicine addresses these issues and provides multiple advantages faced by traditional drugs [107–111].

NPs have specific properties, making them versatile carriers contributing to a wide range of biomedical and pharmaceutical applications [112–116]. The first property includes NPs platforms which are "scaffolds" for assembling defined multi-functional structures. These provide abilities to generate a wealth of possibilities by simply incorporating slight variations in composition, surface coating, and ligand choice [117–119]. Secondly, they have a high surface-to-volume ratio. This property provides vital importance for optimizing drug payloads and other NP-based interactions [120–122]. Thirdly is its controlled shape and size. The shape of the NPs directly affects their function in vivo [123–126]. Further, the careful size modulation of these structures can provide a perfect transition from effective renal clearance (for smaller NPs) to accumulation in the body (for larger NPs), depending on the therapy requirements [127–129]. Lastly, the optical properties of NPs help in facilitating an image-based drug delivery hence combining diagnosis and treatment [130]. Thus, these four properties broadly answer the reason for an enthusiastic interest in this field, which tends to mark a paradigm shift in cancer treatment [131].

Elaborating on the importance of NPs concerning cancer specifically, it is found that all these properties, though useful, do lead to issues like NP toxicity.

Hence, NPs can enhance the effectiveness of standard chemotherapy treatment by acting as cytotoxics [132, 133]. NPs can be classified into four categories based on their size, shape, morphology, and chemical properties. These categories include metals, liposomes, polymers, and carbon-based NPs.

Modulation of autophagy by metallic nanoparticles

Metallic NPs can selectively overstimulate autophagy. They cause this "selective" overstimulation by creating imbalances in the cellular signaling pathways, which do not affect the non-cancerous cells. This approach provides an exciting area of exploration and carries the potential of designing therapies for various cancers.

Silver-based nanoparticles

When silver nanoparticles (AgNPs) were embedded into specific polysaccharides (EPS), then it was observed that they produced pronounced cytotoxic activity against breast cancer cell lines. This mode of action of AgNPs was elucidated by using SKBR3 breast cancer cells. In particular, AgNPs were found to increase ROS generation, supporting cell death primarily through autophagic cell death mechanism and minorly through apoptotic cell death [134] (Fig. 4). In another study for identifying the underlying molecular mechanism of AgNPs against pancreatic cancer cells, it was found that these
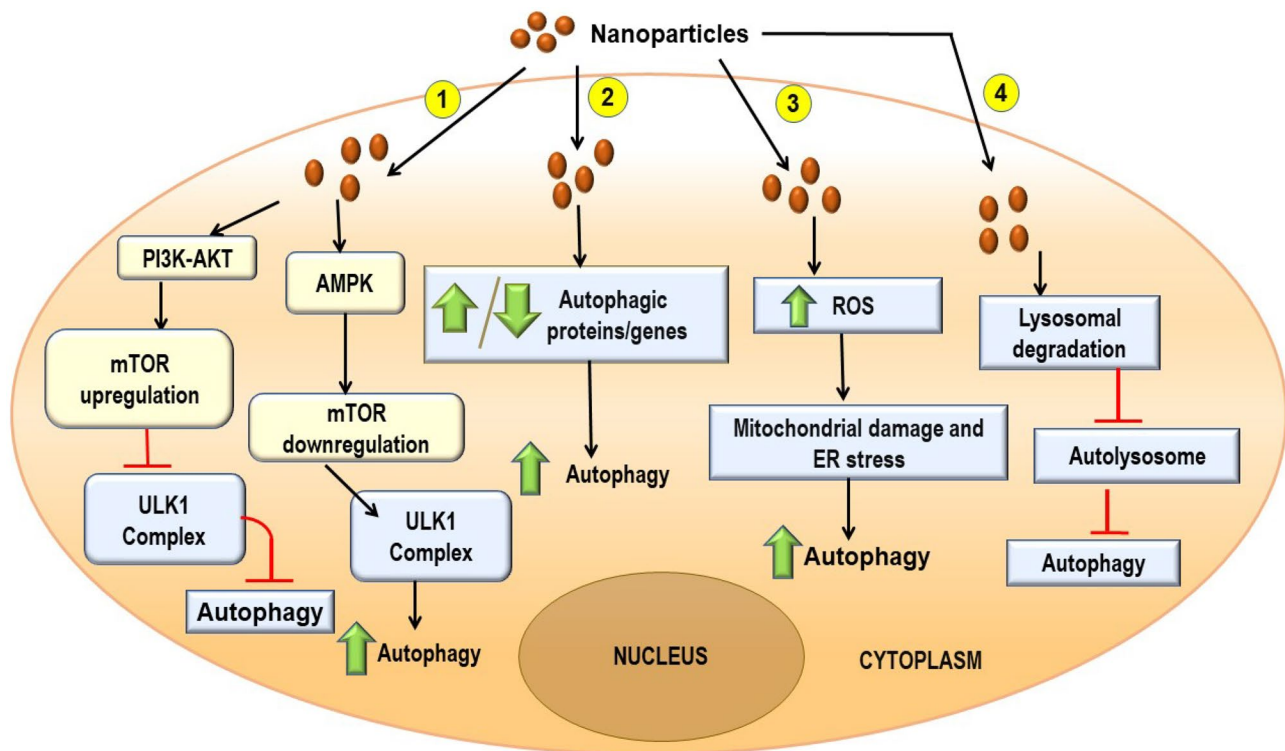
Fig. 4 Modulation mechanism of NP in autophagic-signaling pathways: Current evidence suggests that the nanoparticles (NP) are regarded as an endosomal pathogen to the cells. So, like the cellular substrates, the NP also undergoes ubiquitination which leads to their sequestration via phagophore. NP can induce autophagy by inducing mitochondrial damage and ER stress, by altering autophagic-dependent genes and proteins, and by suppressing and downregulating Akt-mTOR signalings. NP can also inhibit autophagy by inducing lysosomal degradation, and upregulating Akt-mTOR signalings

NPs induced tumor cell death through necrosis and apoptosis, occurring with autophagy. Further, identification of elevated levels of autophagy biomarker, LC3-II, in the tumor cells as opposed to the non-tumor cells showed the selectivity of AgNPs for inducing cytotoxicity [135].

Many studies were carried out that looked at the collective effects of an anticancer drug along with forms of AgNPs. A report depicted a synergistic effect between Cisplatin, a very widely used chemotherapeutic drug) and rGO-AgNPs (a reduced graphene oxide–silver NP nanocomposite) against human cervical cancer (HeLa) cells. This combination led to more pronounced effects on the expression of autophagy genes and the accumulation of autophagosomes and autophagolysosomes, which were associated with the generation of ROS and cell death. These findings depicted the ability of rGO-AgNPs to potentiate cisplatin-induced cytotoxicity, autophagy, and apoptosis in HeLa cells [136]. Another notable study illustrated synergistic interactions between a novel bacterium synthesized AgNPs and salinomycin against ovarian cancer cells. In comparison with their individual anticancer effects, their combination produced a dramatic inhibitory effect on cell viability and morphology. The combination

of salinomycin and AgNPs produced more cytotoxicity and expression of apoptotic genes, along with significantly inducing the accumulation of autophagolysosomes in these ovarian cancer cells. This increased autophagy led to mitochondrial dysfunction, eventually leading to cell death. This proposed a relevant targeted therapeutic strategy for treatment against ovarian cancer [137]. Another study demonstrated that the anti-tumor effect of AgNPs in the B16 mouse melanoma cell model was augmented by wortmannin, a widely used inhibitor of autophagy [138].

The ability of AgNPs was not limited to potentiating the effect of chemotherapeutic drugs, but they were also found to be effective nanoradiosensitizers for the treatment of glioma. In comparison with AuNPs, AgNPs exhibited more powerful radiosensitizing abilities, leading to a higher rate of apoptotic cell death. Furthermore, as compared to AuNPs, AgNPs together with radiotherapy led to a significant upregulation of autophagy as revealed by LC3-II protein level, acridine orange (AO), and monodansylcadaverine (MDC) staining. This study suggested that the modulation of autophagy by treatment with AgNPs may improve glioblastoma therapeutic outcomes [139].

Gold-based nanoparticles

Gold nanoparticles (AuNPs) act as drug delivery scaffolds because their surfaces can act as modifiable sites. These sites can be easily modified with various substances like chemotherapeutics, oligonucleotides, and proteins. AuNPs have great utility in biomedical research due to their fascinating characteristics. These include low toxicity and immunogenicity, good biocompatibility, and excellent stability [140]. In 2011, the mechanism of action of AuNPs on lysosomes was studied. It revealed that AuNPs could be taken up into the lysosomes through endocytosis. Its subsequent accumulation could impair lysosomal degradation and induce autophagosome accumulation. Hence, they play a significant role in interrupting the autophagic pathway [141].

A study reported that 11-mercaptoundecanoic acid-modified AuNPs, when conjugated with chloroquine (GNP-Chl), led to inhibition of cancer cell growth in MCF-7 breast cancer cells. It revealed that cell death was mediated by autophagy [142].

EGFR is found to be overexpressed in 70–80% of TNBC and is a promising target for TNBC treatment [143]. A study demonstrated that near-infrared photothermal therapy (NIR-PTT) using anti-EGFR AuNPs (anti-EGFR-GNs) increases autophagic cell death in breast cancer cells. The researchers validated this idea by investigating autophagic cell death on treatment with anti-EGFR-GNs combined NIR-PTT in TNBC cells and mouse xenograft tumors. Interestingly, on the administration of 3-MA, an autophagy inhibitor, the cytotoxicity of TNBC cells was rescued, implying high levels of autophagy induction by anti-EGFR-GNs-combined NIR-PTT. Many autophagic vesicles and a significant increase in autophagy-specific markers accompanying the inhibition of the AKT-mTOR signaling pathway were responsible for inducing autophagy. Additionally, increased levels of LC3 and beclin-1 in mouse xenograft tumors further validated the result. Hence, the combination of AuNPs with NIR-PTT represents a promising strategy for effectively targeting EGFR in TNBC [144].

Rad6 protein is overexpressed in TNBC cells and is a key player in DNA damage tolerance, post-replication DNA repair mechanism, and mitochondrial stability [145, 146]. Hence, its inhibition (by SMI#9) would provide a new way to combat TNBC. A recent study showed that chemically modified SMI#9-GNP is endocytosed and releases active SMI#9, which selectively induces cytotoxicity only in cancer cells. Increased levels of autophagy and apoptosis were observed in TNBC cells after treatment with SMI#9-GNP. Also, SMI#9-GNP, when combined with Cisplatin, is seen to increase cisplatin sensitivity synergistically. These observations show the essential role of nanotechnology-based Rad6-targeting therapy for TNBCs [147].

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is a highly regarded anti-tumor agent, but its resistance in most cancers led to therapeutic inefficacy. So, in another study, it was found that the combination of AuNPs with TRAIL exhibited greater potency in promoting apoptosis in non-small cell lung cancer (NSCLC) cells compared with TRAIL alone. It was found that the combination was able to promote Drp1-mediated mitochondrial damage leading to apoptosis, autophagy, and mitophagy activation. As a result, the combined administration of TRAIL along with AuNPs may promise a therapeutic strategy to overcome TRAIL resistance in many cancer cells [148].

Metal oxide-based nanoparticles

Metal oxide NPs depict a versatile behavior and provide effective biomedical applications.

Zinc oxide nanoparticles A study revealed that exposure of human ovarian cancer cells (SKOV3) to zinc oxide nanoparticles (ZnO NPs) leads to significant induction of cytotoxicity, apoptosis, and autophagy. Apoptotic features like reactive oxygen species generation and autophagic markers like upregulation of p53 and LC3 indicate that ZnO NPs upregulate apoptosis and autophagy [149, 150]. Another study elucidated the anticancer effect of ZnO NPs on CAL 27 human tongue cancer cells by identifying the role of PINK1/Parkin-mediated mitophagy in this effect. They confirmed that the CAL 27 cell viability decreased upon treatment with ZnO NPs due to reactive oxygen species generation and activation of PINK1/Parkin-mediated mitophagy [151]. The conjugation of ZnO NPs with Meso-Tetra (4-Carboxyphenyl) Porphyrin (MTCP) was seen to augment cytotoxicity in the two human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-468). This was caused by the non-canonical apoptosis and autophagy induction in these tumor cell lines [152]. Hence, ZnO NPs possess interesting characteristics which could be exploited for devising effective tumor therapies.

Iron oxide nanoparticles Iron oxide nanoparticles (IO NPs) have shown their potential in biomedicine applications, such as tumor therapy, specifically by inducing autophagy-mediated cell death of cancer cells. A study revealed that IO NPs selectively induce autophagy in cancer cells (A549) and not in normal cells (IMR-90) [153]. Due to the drug delivery and multi-imaging functions of IO NPs, they are widely used in cancer treatments. Yet, their use is limited due to their therapeutic efficacy and biological safety issues. IO NPs induce autophagosome accumulation through multiple mechanisms like lysosome impairment, mitochondrial dysfunction, and ER stress [154].

IO NPs have multi-functional properties of super-paramagnetism. Combining anti-EGFR with IO NPs suppresses lung tumor growth by abrogating G2/M cell-cycle arrest and inducing DNA damage, autophagy, and apoptosis [155]. In a different study, N-((2-hydroxy-3-trimethylammonium) propyl) chitosan chloride (HTCC)/alginate-encapsulated Fe_3O_4 magnetic nanoparticles (HTCC-MNPs) were developed using an ionic gelation method. These were applied to multi-drug-resistant (MDR) gastric cancer models. They observed that the HTCC–MNPs were more cytotoxic in both the SGC7901 human gastric cancer cell line and MDR variant cell line (SGC7901/ADR) than in normal gastric epithelial cell line (GES). They predicted the reason for cell death as attributed to autophagy induction due to identification of co-localization of LC3 with lysosomal marker LAMP2 and an increased LC3-II/LC3-I ratio [140, 156]. Finally, they concluded that the tumor volume was reduced via the induction of cellular autophagy and apoptosis, which was characterized by ROS generation and mitochondrial damage [156].

Some studies revealed the importance of analyzing the size and coating characteristics of the IONPs in their application. An interesting study revealed that Polyethylenimine (PEI)-coated IONPs lead to severe toxicity in both SKOV3 human ovarian cancer cells and macrophages due to multiple mechanisms, including the disruption of cell membrane integrity, ROS generation, and apoptosis. On the other hand, no apparent cytotoxicity was observed for PEGylated IONPs at even much higher concentrations. The authors observed changes in autophagosome formation when SKOV3 cells were exposed to PEGylated IONPs through TEM imaging and by detecting the level of autophagy marker LC3-II [116, 133].

Copper and cuprous oxide nanoparticles Cuprous oxide nanoparticles (CuO NPs) or copper oxide nanoparticles (CO NPs) are also used to selectively induce cytotoxicity in tumor cells, leading to the suppression of tumor proliferation. They adopt mechanisms that include stimulating apoptosis and autophagic cell death, tumor growth and metastasis inhibition, and nephrotoxicity [150, 157–159]. These pharmacological effects make it a potential nanomedicine for cancer therapy.

An excellent study provided evidence of the therapeutic potential of CuO NPs and their effectiveness in comparison with cisplatin in the treatment of cervical carcinoma. According to TEM and autophagic flux, CuO NPs were found to induce autophagosome formation, which increased in a time- and concentration-dependent manner. Also, it was seen that they led to autophagy induction through the AKT/ mTOR pathway by decreasing the phosphorylation of AKT and mTOR [160].

Another study revealed that CuO NPs induce autophagy in MCF3 breast cancer cells in addition to oxidative stress generation and deregulation of normal cellular activities. However, in this case, the authors hypothesized that autophagy is being adopted as a survival strategy by the MCF3 cells, thus representing its pro-survival role. Hence, the inhibition of autophagy by 3-MA led to the induction of apoptosis. Finally, it was concluded that combining CuO NPs with an autophagy inhibitor is essential for apoptosis induction in breast cancer cells [161].

Carbon-based nanoparticles

Many carbon-based nanoparticles (C-NPs) modulate autophagy to target cancer cells effectively. Carboxyl-functionalized carbon nanotubes (COOH-CNTs), a type of functionalized single-walled carbon nanotubes, induced autophagic cell death in human lung adenocarcinoma A549 cells by affecting the mTOR/AKT pathway (by the formation of autophagosomes and LC3-II upregulation). However, other functionalized CNTs (like PABS-CNT and PEG-CNT) did not replicate the same behavior, implicating the impact of surface group characteristics on autophagic modulation. More so, the administration of autophagy inhibitors like 3-MA evaded lung toxicity in mice, potentiated by COOH-CNT [162].

A study on C-NPs demonstrated that derivatized fullerene C60 induces autophagy, augmenting chemotherapeutic killing of both normal and drug-resistant cancer cells by reducing drug resistance [163]. Further, it is also noteworthy that graphene-based nanomaterials (GNM), such as graphene, graphene oxide, and graphene quantum dots also play a role in the induction of autophagy [164]. Several studies have validated this. For instance, co-treatment of graphene oxide (GO) nanomaterial with cisplatin induces significant cell death, by inducing autophagic flux and chemosensitizing colon cancer CT26 cells [165], ovarian cancer Skov-3 cells, cervical cancer HeLa cells, as well as prostate cancer (Tramp-C1) cells [166]. Additionally, GO and Chloroquine co-administration in A549 human lung carcinoma cells caused cellular toxicity by the cytotoxic block of autophagic flux, leading to necroptosis [167].

Silica-based nanoparticles

Silica-based nanoparticles (Si-NPs) possess many distinctive properties, making them a very suitable drug delivery vehicle and numerous applications in gene transfection,

biosensing, and bioimaging. It has interesting characteristics like biocompatibility, tunable pore size, high surface area, and ease of modification [168–170] and is hence used as a potential approach in cancer therapy.

A study revealed that Si-NPs impart selective toxicity in glioblastoma cells compared to normal skin fibroblasts. On treatment with Si-NPs, the authors noticed the induction of apoptosis and necrosis in LBC3 cells, whereas the induction of necrosis in LN-18 cells. This revealed the anticancer therapeutic potential of Si-NPs for the treatment of glioblastoma [171]. The accumulation of Si-NPs in the human cervix carcinoma cells leads to lysosomal dysfunction and inhibition of autophagy-mediated protein turnover. As a result, the metabolic activity of the cancer cells is hampered [172]. In a recent study, the authors chemically synthesized genistein-PEGylated silica hybrid nanoparticles (Gen-PEG-Si-NPs) by incorporating genistein (Gen) into PEG-Si-NPs. As a result, Gen's antioxidant and anti-proliferative effects on HT29 human colon cancer cells were potentiated, which activated both cell death mechanisms (apoptosis and autophagy), in contrast to Gen, which only triggered apoptosis [173]. The study proposed using Gen-PEG-Si-NPs as an alternative treatment for colorectal cancer in the near future.

In addition to its benefits, it has also been observed that Si-NPs induce ROS and autophagy dysfunction in HCT-116 colon cancer cells, L-02, and HepG2 hepatoma cells, generating an increased concern for the careful study of Si-NPs toxic effects [174–176].

Modulation of autophagy by polymer nanoparticles

Polymeric NPs exhibit various advantages as compared to conventional delivery systems like improved stability, and biocompatibility, non-toxicity, biodegradability, increased accumulation at the cancer site via enhanced permeability and retention (EPR) effect, and controlled drug release [177–180]. Also, the polymeric NPs reduce the multi-drug resistance-associated with many chemotherapeutics [181].

Eudragit RS polymeric NPs were found to be cytotoxic for NR8383 rat macrophages. In continuation with this discovery, a study in 2011 demonstrated the relationship between the cytotoxicity induced by Eudragit RS in NR8383 rat macrophages and autophagy activation. TEM showed internalization of Eudragit RS NPs in the cells leading to mitochondrial damage. The authors proposed that cells adopt the mechanism of autophagy followed by cell death to get rid of the oxidative stress created due to damaged mitochondria [182].

Cationic polyamidoamine dendrimer (PAMAM) triggered autophagic cell death in A549 lung cancer cells by the deregulation of the mTOR pathway [183]. However, it was not specified whether the upregulated LC3, as observed by western blotting and microscopy, was due to the enhanced on rate or decreased off rate of autophagosomes. Hence, the blockage of autophagy cannot be kept aside [184]. Another study demonstrated that PAMAM dendrimers resulted in lysosomal impairment due to the alkalinization of lysosomes [185].

Polymer therapeutics or polymer conjugates are observed as one of the most growing first-generation polymeric nanoparticles. A study was performed where polymeric nanomicelles were prepared for the co-delivery of doxorubicin and LY294002 (autophagy inhibitor) for the effective treatment of oral squamous cell carcinoma (OSCC). In this study, the hydrophobic DOX was conjugated with pH-responsive, hydrophilic polyacylhydrazone (HPAH). The amphiphilicity of HPAH – DOX conjugates facilitated the self-assembly of nanomicelles within an aqueous solution, and the pH responsiveness of the conjugates facilitated the release and rapid entry of LY294002 and DOX to an intracellular acidic environment inducing inhibition of proliferation of cancer cells. It was observed that the preferential release of LY294002 inhibited the autophagy of tumor cells, making the cancer cells more sensitive to the subsequent liberation of DOX [186]. Similarly, N-(2-hydroxypropyl) methacrylamide (HPMA)–doxorubicin (Dox) conjugate PK1 (FCE28068), was prepared as a synthetic polymer–drug conjugate. The results showed that the cancer cells treated with FCE28068 exhibited increased levels of LC3II and Beclin 1, the proteins associated with the formation of the autophagosome. Moreover, it was observed that the process of autophagy proceeds via glutamine metabolism, and the treatment with FCE28068 showed a reduction in glutamine concentration, proving the facilitation of autophagy in MCF-7 cells [187]. In this context, polymer–peptide conjugates also play an important role in nanomedicine as cancer therapy due to their longer circulation time and improved therapeutic efficacy for targeted tumor delivery than free peptides. 14-amino acid polycationic peptide (KLAKLAK)2 (abbr. *KLAK*) was conjugated with self-assembled polymers (P-KLAK) which caused autophagosomes accumulation via lysosome impairment facilitating autophagy blockage and mitochondrial damage, inducing U87 cell death [188].

Modulation of autophagy by liposomal nanoparticles

Liposomal NPs also possess many distinctive properties due to their flexible surface characteristics [189–191]. Cationic lipids are a commonly used class of transfection agents. In 2010, a study demonstrated the induction of autophagy by dioleoyl trimethylammonium propane (DOTAP), a cationic

lipid in HeLa cells of cervical cancer. Analysis of autophagy was carried out by observation under TEM and autophagic flux. The results suggested that autophagy induced by cationic lipids was primarily due to increased formation of autophagosomes and not decreased turnover. Also, the process was mTOR independent. The researchers hypothesized that the induction of autophagy might be caused by the triggering of an additional degradative pathway (i.e., autophagy) in response to DOTAP, a synthetic lipid [192]. However, the mechanism of autophagy induction by cationic lipids was not understood. So, another study in 2013 demonstrated that cationic liposomes enter cells in endosomes, activate autophagy, and generate tubulovesicular autophagosomes, which capture autophagic cargoes followed by a slower rate of lysosomal fusion [193].

Naturally, it is known that the administration of transfection agents stimulates autophagy induction, which leads to poor transfection effectiveness. Hence, another possible strategy could be the inhibition of autophagy to improve transfection efficiency. So, the study also showed that gene delivery and expression increased many folds in atg5 (-/-) cells, which were autophagy defective cells [193]. Interestingly, neutral lipid (i.e., dioleoylphosphatidylethanolamine; DOPE) was inefficient in modulating autophagy in HeLa cells [192]. This observation, however, was in stark contrast to another study also carried out in 2013. The authors showed that neutral PEGylated C6-ceramide nanoliposomes activate autophagy in liver HepG2 cells. They further illustrated the synergistic combination therapy combining nanoliposomal C6-ceramide, an autophagy inducer, and vinblastine, the autophagy maturation inhibitor, as being associated with autophagy dysfunction in hepatocarcinoma and colorectal cancer models [194].

Liposomal NPs show several advantages, especially as a modulator of autophagy for the treatment of glioma. For instance, functionalized liposomes can cross the blood–brain barrier in the CNS, which is otherwise impossible for chemotherapeutic drugs and autophagy inhibitors to penetrate. Based on this property, R6dGR peptide-functionalized liposome (R6dGR-Lip) was developed, which encapsulated ZD6474 and hydroxychloroquine (HCQ) inhibitors for the targeting of glioma cells through blockage of autophagic flux [195].

Thus, the versatile liposomal NPs have been identified as the most successful among the others in oncological settings. They can be used as carriers of chemotherapeutics and/or autophagy modulators, due to their useful biological properties including low toxicity and immunogenicity, along with the added advantages of being cheap and biodegradable [195–198].

The various nanoformulations developed in the last decade to modulate the autophagic pathways for the treatment of cancer have been summarized in Table 1.

Future perspective

At present, immunotherapy is rising as a promising treatment approach against cancer. The immune system serves an important role in cancer treatment by recognizing and destroying cancer cells during various stages of cancer development. Various studies showed that autophagy could up-modulate as well as down-modulate the immune response by releasing cytokines and regulating immune cells. Cancer immunotherapy is emerging as a new generation of anticancer therapeutics. Till now, antibody targeting therapy in synergistic with autophagy has been reported. A humanized mAb (farletuzumab) was developed against folate receptor in an ovarian cancer model, which exhibited an anticancer activity via antibody-dependent cellular cytotoxicity by assisting late-stage autophagy. Presently, therapies directed at autophagy to improve the immune responses and anticancer effects of immunotherapy have become an eventual strategy, with increased antigen presentation and higher sensitivity to cytotoxic T cells (CTLs). It was observed that autophagy exhibits a pivotal role in antigen processing for both MHC-I and MHC-II presentation. From a recent study, it was observed that the alpha-tocopheryloxyacetic acid (a-TEA) enhances MHC-I cross-presentation of cancer antigens to antigen-special CD8 + T cells by exhilarating late autophagy. Such an approach was observed as an adjuvant approach for improving cancer immunotherapy. Moreover, it was reported in recent times that DCs-based vaccines have shown promising therapeutic activity in improving cancer immunotherapy by enhancing antigen presentation. For instance, lactosylated N-Alkyl polyethylenimine coated superparamagnetic iron oxide (SPIO) nanoparticles increased the vaccine activity of DCs by inducing autophagy. Further, autophagy can enhance the therapeutic efficacy of DNA vaccines by synthetizing intracellular vaccine-encoded tumor antigens. However, some reports stated that the effects of cancer immunotherapy could be impaired by hypoxia-induced autophagy. Hypoxia-induced autophagy impairs CTLs-mediated cancer cell lysis which is associated with pSTAT3 (hypoxia-dependent phosphorylation of STAT3). Hypoxia-induced autophagy further impairs NK-mediated killing, leading to a decrease in cancer immunotherapy effect. In this context, a study was performed where 3-MA (selective PI3K inhibitor, autophagy inhibitor) was employed which showed a significant increase of IL-24-induced apoptosis in oral squamous cell carcinomas (OSCC), demonstrating the combination of autophagy inhibitors and IL-24 as a promising approach for cancer immunotherapy. In addition to this, many efforts are engrossed on how to modulate autophagy to boost both innate and adaptive immune response and bypass anticancer immune resistance in immunotherapy for cancer [232].

Table 1 Modulation of autophagy by NPs in cancer cells

SL. No	NP	Combination drug therapy	Cancer type	Target cell	Autophagy effect (activation or inhibition)	References
1	Silver	AgNP	Pancreatic Cancer	PANC1	Activation	[135]
2	Silver	AgNP, embedded into exopolysaccharide (AgNP-EPS)	Breast Cancer, Colon Cancer	SKBR3	Activation	[134]
3	Silver	AgNP + Salinomycin (Sal)	Ovarian Cancer	A2780	Activation	[137]
4	Silver	AgNP combined with reduced Graphene Oxide (rGO-AgNP) + CIsplatin (Cis)	Cervical Cancer	HeLa	Activation	[136]
5	Silver	AgNP	Lung Cancer	A549	Activation	[199]
6	Silver	AgNP + Radiation	Glioblastoma (Brain Cancer)	U251	Activation	[139]
7	Silver	AgNP	Cervical Cancer	HeLa	Inhibition	[200]
8	Silver	Biological AgNP (bAgNP) with a fungal-derived outer protein coat	Hepatoma (Liver Cancer), Osteosarcoma (Bone Cancer)	Huh7, HOS CRL-1543	Inhibition	[201]
9	Silver	AgNP	Liver Cancer	HepG2	Inhibition	[202]
10	Silver	AgNP	Prostate Cancer	PC-3	Inhibition	[203]
11	Silver	AgNP	Breast Cancer	MCF-7	Activation	[204]
12	Silver	AgNP	Myeloma	B16 mouse cell	Inhibition	[138]
13	Gold	Au-quercetin into poly(dl-lactide-co-glycolide) NP	Liver Cancer	MHCC97H, Hep3B, HCCLM3, Bel7402	Activation	[205]
14	Gold	Au-NP + TRAIL	Non-Small Cell Lung Cancer (NSCLC)	Calu-1	Activation	[148]
15	Gold	Au-NP conjugated with NKCT1 (purified *Naja kaouthia* protein toxin)	Leukemia	U937, K562	Activation	[206]
16	Gold	Anti-EGFR-AuNP-combined with NIR-PTT	Breast Cancer	MD-MB-231	Activation	[144]
17	Gold	Au-NP + Tmab	Gastric Cancer	MKN7, NCI-N87	Activation	[207]
18	Gold	Au-NP conjugated with chemically modified SMI#9 (SMI#9-AuNP)	Triple-Negative Breast Cancer (TNBC)	SUM1315	Activation	[147]
19	Gold	Au-quercetin into poly(dl-lactide-co-glycolide) NP	Glioma Cancer	U87	Activation	[208]
20	Gold	Au-quercetin into poly(dl-lactide-co-glycolide) NP	Cervical Cancer	HeLa, Caski, Siha	Activation	[209]

Table 1 (continued)

Sl. No	NP	Combination drug therapy	Cancer type	Target cell	Autophagy effect (activation or inhibition)	References
21	Gold	Au-NP combined with Anti-PD-L1 Antibody	Glioma Cancer	C6 (murine glioma)	Inhibition	[210]
22	Zinc Oxide	ZnO-NP	Oral Cancer	CAL27	Activation	[151]
23	Zinc Oxide	ZnO-NP	Ovarian Cancer	SKOV3	Activation	[211]
24	Zinc Oxide	ZnO-NP+Meso-Tetra (4-Carboxyphenyl) Porphyrin (MTCP)	Breast Cancer	MCF-7, MDA-MB-468	Activation	[152]
25	Zinc Oxide	ZnO-NP	Breast Cancer	MCF-7	Inhibition	[212]
26	Iron Oxide	IO-NP	Lung Cancer	A-549	Activation	[153]
27	Iron Oxide	Anti-EGFR Antibody	Lung Cancer	HCC827	Activation	[155]
28	Iron Oxide	Polyethyl-enimine (PEI)-coated IONP	Ovarian Cancer	SKOV3	Activation	[149]
29	Iron Oxide	IONP+Autophagy Inhibitor+Photothermal treatment	Breast Cancer	MCF-7	Inhibition	[213]
30	Iron Oxide	Fe_3O_4- NP	Breast Cancer	MCF-7	Activation	[154]
31	Iron Oxide	IO-NP coated with sodium citrate (IO@citrate)	Cervical Cancer	HeLa	Activation	[214]
32	Iron Oxide	Carboxy-functional iron oxide NP (Fe2O3@DMSA)	Hepatocellular Carcinoma (Liver Cancer)	SK-Hep-1, HepG2	Activation	[215]
33	Iron Oxide	IONP conjugated with quantum dots (IONP-QD)+external magnetic field	B-Cell Lymphoma, Colon Cancer	A20 mouse cell, CT26	Activation	[216]
34	Iron Oxide	Superparamagnetic iron oxide NP (SPION)+Spinning Magnetic Field (SMF)	Osteosarcoma (Bone Cancer)	U2OS, SAOS2	Activation	[217]
35	Iron Oxide	Iron oxide nanocube (IO-cube) & cluster of nanocubes (IO-cluster)	Liver Cancer	HepG2, Huh7, Alexander cells	Activation	[218]
36	Ferro-ferric Oxide	Fe_3O_4-NP+Autophagy Inhibitor (Bortezomib, Doxorubicin)	Leukemia, Multiple myeloma (Blood Cancer)	K562, OCI-AML2; OPM2, RPMI-8226	Inhibition	[219]
37	Ferro-ferric Oxide	N-((2-hydroxy-3-trimethylammonium) propyl) chitosan chloride (HTCC)/alginate-encapsulated Fe_3O_4 magnetic NP (HTCC-MNP)	Gastric Cancer	SGC7901, SGC7901/ADR	Activation	[156]
38	Copper Oxide	Cuo-NP+Autophagy Inhibitor	Breast Cancer	MCF7	Inhibition	[161]
39	Cuprous Oxide	CONP	Cervical Cancer	SiHa, Caski, MS751, HeLa	Activation	[160]
40	Copper Oxide	CuO-NP+X-Ray Irradiation	Breast Cancer	MCF-7	Activation	[220]

Table 1 (continued)

Sl. No	NP	Combination drug therapy	Cancer type	Target cell	Autophagy effect (activation or inhibition)	References
41	Cuprous Oxide	CONP & CONP+Gemcitabine	Bladder Cancer	T24, J82, 5637, UMUC3	Activation	[221]
42	Copper Oxide	Zinc doped copper oxide nanocomposite (Zn-CuO NP)	Pancreatic Cancer	MIA PaCa-2, AsPC-1	Activation	[222]
43	Silica	Genistein incorporated into PEGylated silica nanoparticle (Gen-PEG-SiHNM)	Colon Cancer	HT29	Activation	[173]
44	Silica	SNP	Colon Cancer	HCT-116	Activation	[176]
45	Silica	Si-NP	Cervical Cancer	HeLa	Inhibition	[172]
46	Silica	SNP	Liver Cancer	HepG2	Activation	[175]
47	Silica	SNP	Lung Cancer	A549	Inhibition	[223]
48	Silica	Si-NP	Liver Cancer	L-02, HepG2	Activation	[174]
49	Silica	Si-NP	Glioblastoma (Brain Cancer)	LBC3, LN-18	Activation	[171]
50	Silica	Hydroxychloroquine -loaded hollow mesoporous silica NP (HCQ-HMSN)+Radiotherapy	Colon Cancer	HCT116	Inhibition	[224]
51	Silica	Bismuth crystals embedded silica NP, loaded with chloroquine (Bi@SiO$_2$-CQ)+Photothermal Therapy (PTT)	Breast Cancer	4T1 mouse cell	Inhibition	[225]
52	Polymer	Poly (lactide-co-glycolide) (PLGA)+Autophagy inhibitor	Breast Cancer	MCF-7	Inhibition	[226]
53	Polymer	Docetaxel loaded dendritic copolymer H40-poly(D,L-lactide) NP (DTX-H40-PLA NP)++Autophagy inhibitor	Breast Cancer	MCF-7	Inhibition	[227]
54	Polymer	Doxorubicin and shAkt1 co-delivery by Poly(ethylenimine)–Glycyrrhetinic Acid NPs (PEI–GA/DOX/shAkt1)	Hepatocellular Carcinoma (Liver Cancer)	HepG2	Activation	[228]
55	Liposome	5-fluorouracil (5-FU) and LY294002 (LY)-loaded PEGylated nanoliposome (FLNP)	Oesophageal squamous cell carcinoma	EC 9706	Inhibition	[229]
56	Liposome	Ropivacaine-loaded liposomes (Rop-DPRL)	Cervical Cancer	B16 mouse cell, HeLa	Inhibition	[230]
57	Liposome	188Re-liposome	Ovarian Cancer	ES2, SKOV-3	Activation	[231]

Conclusion

In conclusion, from the historical evidence to modern-day research, autophagy is one of the most compelling mechanisms, with its relevance in cancer. Though its exact mechanism, leading to tumorigenesis or tumor suppression, is ambivalent, empirical evidence and research show a strong correlation between autophagy modulation and tumor death. Hence, the findings summarized in this review bring into light the optimistic hope of a clinically relevant, effective complementary therapy by exploiting nanoparticle-based systems to modulate autophagy in cancer.

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Declarations

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