An updated review on implications of autophagy and apoptosis in tumorgenesis; Possible alterations in autophagy through engineered nanomaterials and their importance in cancer therapy

**Running title:** Autophagy and Nanomaterials in Cancer Therapy

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Abstract

Most commonly recognized as a catabolic pathway, autophagy is a perplexing mechanism through which a living cell can free itself of excess cytoplasmic components, i.e., organelles, by means of certain membranous vesicles or lysosomes filled with degrading enzymes. Upon exposure to external insult or internal stimuli, the cell might opt to activate such pathway through which it can gain control over the maintenance of intracellular components, and thus, sustain homeostasis by intercepting the formation of unnecessary structures or elimination of the already present dysfunctional or inutile organelles. Despite such appropriateness, autophagy, might also be considered a frailty for the cell, as it has been said to have a rather complicated role in tumorigenesis. A merit in the early stages of tumor formation, autophagy appears to be salutary due to its tumor-suppressing effects. In fact, several investigations on tumorigenesis have reported diminished levels of autophagic activity in tumor cells, which might result in transition to malignancy. On the contrary, autophagy has been suggested to be a seemingly favorable mechanism to progressed malignancies, as it contributes to survival of such cells. Based on the recent literature, this mechanism might also be activated upon the entry of engineered nanomaterials inside a cell, supposedly protecting the host from foreign materials. Accordingly, there is a good chance that therapeutic interventions for modulating autophagy in malignant cells using nanoparticles may sensitize cancerous cells to certain treatment modalities, e.g., radiotherapy. In this review, we will discuss the signaling pathways involved in autophagy, and the significance of the mechanism itself in apoptosis and tumorigenesis, while shedding light on possible alterations in autophagy through engineered nanomaterials, and the their potential therapeutic applications in cancer.

KEYWORDS: autophagy, apoptosis, nanomaterials, cancer therapy
Significance statement

Autophagy has been said to have a complicated role in tumorigenesis. In the early stages of tumor formation, autophagy appears to be salutary due to its tumor-suppressing effects. On the contrary, autophagy has been suggested to be a favorable mechanism to progressed malignancies. This mechanism might be affected upon the entry of nanomaterials inside a cell. Accordingly, therapeutic interventions for modulating autophagy using nanoparticles may sensitize cancerous cells to certain therapies.
Several types of cell death determine the ultimate fate of a living organism. This phenomenon is an integral part of life as it maintains homeostasis by exterminating redundant cells that may otherwise become a liability. Through the never-ending course of evolution, various mechanisms of cell death have emerged that include apoptosis, necroptosis, and autophagy-dependent cell death (Su et al. 2015; Kang et al. 2011).

A self-digestive process, type II or autophagic cell death (Gozuacik and Kimchi 2004) is one such mechanism that regulates lysosomal degradation of superfluous or erroneous materials, e.g., damaged organelles and misfolded proteins (Choi 2012). Accordingly, autophagy is a regulatory process in which cytoplasmic vesicles with multiple membranes appear inside a cell, and start engulfing bulks of cytoplasmic organelles, only to disintegrate them from the cell. These so-called autophagic bodies are subsequently degraded by the lysosomal system of the very same cell. It is believed that autophagy is fundamentally different from the ordinary turnover cycle of organelles, as it assumes a broader scope in maintenance of cellular activity in conditions, which if not counteracted, might render the organism susceptible. During this process, the cell simply cannibalizes itself from the inside (Gozuacik and Kimchi 2004). On a basal level, autophagy contributes to maintaining homeostasis by mediating the turnover of proteins and organelles; however, it can be accelerated in response to stress as a survival mechanism (Choi 2012).

Based on the molecular pathways associated with the biogenesis of autophagic vesicles or autophagosomes, autophagy can be classified as canonical and non-canonical. In this regard, autophagy-related or ATG genes are also categorized into two eponymous classes, of which more than 30 members have been discovered (Rebecca and Amaravadi 2016).

Once presumed to be a survival mechanism in yeasts under starvation, autophagy has now been recognized as a universal process involved in many cell types, particularly mammalian, that plays a major part in cellular function (Zhang et al. 2009). In fact, the phenomenon is so crucial that if defected, certain ailments may arise as a consequence, e.g., infection, aging, neurodegeneration, myopathy, Crohn's disease, and malignancies (Levine 2008). In spite of all the controversies around the footprint of autophagy in malignancy, it appears that the mechanism assumes an ambivalent approach in development of tumors, as despite being a tumor-suppressive process, autophagy might contribute to the survival of malignant cells (Rosenfeldt and Ryan 2009). Besides, tumor cells can exploit autophagy to gain resistance against several antitumor agents (Chen and Karantza-Wadsworth 2009). Due to rapid proliferation and altered metabolism, cancer cells are subject to more stress and have higher metabolic...
demands (White and DiPaola 2009), that might render them more dependent on autophagy as a survival mechanism (Amaravadi et al. 2011).

As of recent, several studies have reported certain correlations between autophagy and nanotechnological interventions. Pieces of evidence have recently suggested the significance of autophagy in development of adaptive reactions to nanomaterials. However, the nature of such reactions is yet to be elucidated, as they often happen to vary with physicochemical properties of nanomaterials that become up-taken by the cells to which they are introduced. In this regard, it can be asserted that autophagy grants the cell with cytoprotective effects in response to the uptake of foreign materials, which in this case are nanomaterials (Popp and Segatori 2015).

Nanoparticles (NPs) are now recognized as novel materials with a capacity to induce autophagy (Zhang et al. 2009). Different NPs such as Quantum Dots (QD), nanowires, and the more recently studied rare earth oxides can reportedly induce autophagy in cells derived from different tissues, e.g., mesenchymal stem cells, cervical cancer cells, etc. (Zhang et al. 2010b; Stern et al. 2008; Akbarzadeh et al. 2006; Mashayekhi et al. 2020). QDs were first documented to exert size-dependent autophagy-inducing effects on human mesenchymal stem cells in 1999 (Seleverstov et al. 2006). It was only a decade later that an investigation on QDs with different core materials revealed that these particles were able to induce autophagy in porcine kidney cells, further supporting the theory that autophagy might be a common cellular response to nanomaterials. Interestingly, the effects of cellular stress on autophagy determined by cell type and the kind of stimuli (Stern et al. 2008). Another study in 2011 implicated that iron oxide NPs could be utilized for treatment of tumors as they had the potential to mediate autophagy in malignant cells (Khan et al. 2012).

A well-founded understanding of mechanisms involved in the regulation of autophagy in malignancy and their response to nanomaterials might open a new pathway toward developing novel therapeutic interventions that can modulate this pathway either directly or indirectly. The present article will discuss the most recent advancements in understanding of autophagy in malignancy and the potential regulatory role of NPs in it.

2 | Autophagy; Involved Pathways

Autophagy, also known as Type II cell death (Gozuacik and Kimchi 2004), is a conserved catabolic process that can be considered as one of the main degradative pathways of unnecessary or dysfunctional cellular components, old or misfolded proteins, and superfluous or defected organelles in eukaryotic organisms (Kondo and Kondo 2006).
Besides, autophagy has a crucial role in eliminating pathogens and engulfing apoptotic cells (Mathew et al. 2007). Microautophagy, macroautophagy, and chaperone-mediated autophagy are three known types of autophagy, of which macroautophagy is the primary type that occurs most frequently in eukaryotic cells (Li et al. 2017). In *Saccharomyces cerevisiae*, overlapping Atg (autophagy-related) genes, including Apg, Aut, and Cvt, have been found to be involved in the autophagic pathway (Gozuacik and Kimchi 2004). Factors such as nutrient deprivation, reactive oxygen species (ROS), hypoxia, drug stimuli, aggregated proteins, and damaged organelles mainly induce autophagy, causing cells to degrade macromolecules, including proteins, lipids, and carbohydrates, in order to synthesize essential cell components (Mei et al. 2014; Choi et al. 2013).

Basal autophagy brings about protein degradation and organelle turnover, being a vital factor in intracellular quality control and sustaining homeostasis. At the same time, it has been revealed that autophagy is also triggered in stressful conditions to maintain cell survival (Choi 2012). Upon receiving the signal from the cell, a cascade of reactions occur, that result in surrounding of cytoplasmic constituents by intracellular double-membraned structures to form the autophagosomes (Levine 2007; Zhang et al. 2009).

At first, cytoplasmic constituents are enwrapped by a membrane sac to form vesicles (Gozuacik and Kimchi 2004). These vesicles subsequently fuse with lysosomes. Following the release of lysosomal digestive enzymes into the lumen of the resulting autolysosomes, the internal contents are digested by lysosomal hydrolases. The degradation products are then recycled back to the cytosol, and reused by the cell to maintain energetic homeostasis and viability (Levine 2007; Zhang et al. 2009).

In normal cells and tissues, autophagy plays a complex and tissue-dependent role (Mizushima and Komatsu 2011). As a cellular housekeeper, autophagy maintains homeostasis by eliminating inessential proteins and non-functional organelles in normal physiological conditions (Anding and Baehrecke 2017; Mathew et al. 2009). In this regard, aberrant regulation of autophagy can lead to severe conditions, including neurological disease, infection, myopathy, inflammation, aging, and a variety of cancers (Yin et al. 2016; Choi 2012). To our knowledge, the process of autophagy depends on the continuous presence of ATP along with uninterrupted protein synthesis (Gozuacik and Kimchi 2004). Figure 1 represents involved signalling pathways.
2.1 | Phosphatidylinositol 3-kinase (PI 3K) Complex

PI 3-kinase pathway is primarily involved in the autophagy process (Petiot et al. 2000). The pathway is of crucial importance for endocytic and phagocytic trafficking, and formation of autophagic vesicles (Burman and Ktistakis 2010; Mizushima et al. 2001; Simonsen and Tooze 2009). According to several studies, 3-methyladenine (3-MA), an autophagy inhibitor, and Wortmannin, a PI 3-kinase inhibitor, can inhibit the generation of autophagosome precursors in mouse embryonic stem cells (Mizushima et al. 2001).

2.2 | Tor Kinase and Apg Expression

Considered a gatekeeper against the triggering factors of autophagy (Liang 2010), Tor kinase plays a role in Akt signaling pathway by relaying growth factor-induced signals to the main pathway of autophagy. Accordingly, Tor kinase inhibitors, e.g., rapamycin, can induce autophagy in both yeast and mammalian cells (Díaz-Troya et al. 2008). Inhibition of Tor kinase pathway is thought to increase Apg8 expression (Kirisako et al. 1999), which is an important gene in formation and expansion of autophagic vesicles (Gozuacik and Kimchi 2004). Phosphorylation of certain proteins in this pathway coincides with suppression of autophagy in mammalian cells (Blommaart et al. 1997).

2.3 | Ubiquitin-like systems

Formation of autophagic vesicles relies on two major ubiquitin-like conjugation systems. In the more predominant systems an E1-like enzyme called “Apg7” is conjugated with Apg12, and then translocated to an E2-like enzyme, Apg10 (Shintani et al. 1999). Next, a covalent linkage is formed between the C-terminal of Apg12 and the central part of the Apg5 protein (Mizushima et al. 1998). Nearly all Apg12 molecules in cells are conjugated with Apg5. In this case, Apg12/Apg5 conjugation is not affected by stimuli, that may otherwise induce autophagy (Gozuacik and Kimchi 2004).

3 | Apoptosis

Type I cell death or apoptosis is a cellular process characterized by the fragmentation of the cell into smaller membraned structures called an “apoptotic body”, that usually succeed alterations in the nucleic material, namely, condensation of chromatin and degradation of the DNA. The remaining components of the cell are then digested by phagocytes after heterophagocytosis (Gozuacik and Kimchi 2004).
Apoptosis is usually mediated via two different cascades, the extrinsic and intrinsic pathways, that result in degradation of cellular organelles (Nagata 2018). The extrinsic apoptotic pathway involves membranous death receptors like CD95 (FAS), TRAIL receptors, and tumor necrosis factor (TNF) receptor (TNFR) family, which bind to specific ligands such as soluble TNF. Upstream to these receptors, there are several caspases, which function to mediate the process. Caspase-8 and caspase-10 activate the effector caspases known as caspase-3, 6, and 7, resulting in final-stage molecular degradation involved in apoptosis (Andreeff 2003). Mitochondria are the central part of the intrinsic pathway of apoptosis. Pro-apoptotic molecules such as Bad, Bax, Bak, Noxa, Bid, and PUMA constitute the intrinsic apoptotic pathways. In this case, Bak and Bax can dimerize, and therefore, permeabilize the outer membrane of mitochondria. As a result, cytochrome C is released into the cytosol and interacts with apoptotic protein activating factor-1 (Apaf-1), leading to the assembly of apoptosome. This multi-protein structure can activate caspase-9 and other effector caspases (Kang and Reynolds 2009).

4 | Autophagy and Apoptosis; Possible Links and Differences

The link between autophagy, apoptosis, and other types of cell death is an area of interest to researchers (Kang et al. 2011), especially in cancer research. Apoptosis one type of programmed cell death, that can be triggered by intra- or extracellular stimuli through activation of a cascade of proteases (Nagata 2018). On the other hand, autophagy or cellular "self-eating" is a mechanism in which a section of the cell is surrounded by an especial intracellular membrane, and its contents are then digested by lysosomal enzymes (Hurley and Young 2017). Autophagy is like a double-edged sword since it is oftenly induced as a response to stress to prevent cell death through ARHI (aplysia ras homolog I) dependent pathway. Nonetheless, in some special occasions, it can serve as a means of cell death (Fulda et al. 2010).

There are contradictive data on the interaction between autophagy and apoptosis. Several stressors can trigger autophagy, e.g., apoptosis-inducing chemotherapeutic agents (Verfaillie et al. 2010), dysfunction of cellular organelles (Anding and Baehrecke 2017), starvation (Li et al. 2013b), etc. Exposure to such stressors might activate autophagy, which can restore the cell to its normal status. But, in the long-term, the cell may undergo apoptosis. It can be concluded that unlike apoptosis, autophagy is a pathway toward survival of the cell, however, should there be prolonged exposure to stress, the cell may die by means of autophagic cell death (Shen et al. 2012). Figure 2 represents the link between autophagy and apoptosis.
4.1 | Implications in cancer

Not only are autophagy and apoptosis independent, but also they have multiplex crosstalk with each other in physiological and pathological incidents like cancer. The tumor-suppressing function of apoptosis is supported by the recent evidence (Chao et al. 2006), however, autophagy is a rather different mechanism that serves as an intricate function in the onset and development of tumors (Sun et al. 2013). Unlike apoptosis, the function of autophagy in tumor cells is partly favorable, and partly unfavorable, hence, it can both instigate and halt tumor development (Eskelinen 2011). It has been argued that cancer cells benefit from autophagy, as it enables them to survive the exposure to several tumor microenvironment stressors such as hypoxia, starvation, and metabolic stresses (Dikic et al. 2010). Besides cancer, other diseases can also be occurred with this mechanism due to the abnormal balance between autophagy and apoptosis or linkage gene concept. For instance, Atg5 deficiency can induce apoptosis as a result of stress to endoplasmic reticulum, and lead to cardiovascular diseases (Nishida et al. 2008).

5 | Can Autophagy Hinder or Aggravate Cancer?

Defects in autophagy contribute to the etiology of many diseases like cancer (Kondo and Kondo 2006). Most studies have indicated the ambivalent nature of autophagy in cancer (Fiaschi and Chiarugi 2012). Likewise, a remarkable body of published studies have pointed to the function of autophagy in tumor suppression (Mei et al. 2014). Accumulating evidence indicates that there might be a link between cancer and autophagy at two levels of cancer progression and cancer prevention. For example, inactivation of some autophagy genes has been shown to lead to increased tumorigenesis in mice (Ni et al. 2014). On the other hand, enforced expression of certain autophagy genes was reported to prevent formation of tumors (Levine 2007). It has also been noted that autophagy can be activated in response to chemotherapeutic drugs in cancer cells (Karantza and White 2007). Table 1 and Figure 3 represent correlation between autophagy and tumorigenesis.

A series of in vitro experiments showed that enhanced activity of beclin 1, an autophagy-inducing protein, might reduce the proliferation of cancer cells (Liang et al. 1999). It was also revealed that down-regulation of beclin 1 might promote the tumorigenicity of Hela cells (Wang et al. 2007b). In another study, scientists were able to show that beclin 1 overexpression by RNA interference methods reduced the proliferation and migration of cancer cells, introducing this protein as a potential target for cancer treatment modalities (Sun et al. 2011b). In 2011, scientists reported that induced autophagy by means of Docosahexaenoic acid could augment the apoptosis rate by affecting
caspase-3 function in cancer cells (Jing et al. 2011). It was only two years later that another investigation confirmed the desirable effects of Kaempferol in treatment of cancer cells, which included arrestment of cell cycle and induction of autophagic cell death (Huang et al. 2013). Several years later, it was reported that a treatment regimen comprising beclin-1-derived protein hindered the proliferation of HER2-positive breast cancer cells (Vega-Rubín-de-Celis et al. 2018). It was also shown that most important autophagy-related genes like beclin1, atg5, bif-1, and atg4c had been lost in the genome of prostate, ovarian, and breast cancer cells (Maes et al. 2013). Allegedly, a combined therapy of autophagy targeting and radiotherapy might prove to be more effective than radiotherapy alone. Accordingly, down-regulation of beclin-1, atg3, atg4b, atg4c, atg5, and atg12 could sensitize cancer cells to radiation (Apel et al. 2008). Recently, cisplatin-induced autophagy in ovarian cancer was inhibited by bortezomib, a proteasome inhibitor, to increase the efficacy of chemotherapy (Kao et al. 2014). Bufalin, in a similar way, causes autophagy-mediated cell death through ROS production and enhanced radiosensitivity in human colon cancer cells (Xie et al. 2011).

Thus, it can clearly be inferred that autophagy should not be considered a definitive solution, but rather, it should be regarded as a doubtful advantage with two sides, each of which have been well supported by several investigations (Jiang et al. 2019). Through the removal of damaged DNA and organelles in the preliminary stages of tumorigenesis, autophagy acts as a protective mechanism to maintain the integrity of the cell, and prevent instigation of malignancy (Hönscheid et al. 2014). A pivotal mechanism for migration and invasion of tumor cells, epithelial-to-mesenchymal transition (EMT) can be counteracted by induction of autophagy, thus, hindering tumorigenesis (Lv et al. 2012; Catalano et al. 2015). However, as the tumors progress in stage, autophagy assumes a seemingly paradoxical role, by delivering essential nutrients to the tumor cells through degradation of unnecessary intracellular structures, resulting in the emergence of resistant tumor cells (Cheong 2015). Therefore, development of an effective autophagy-based cancer therapy for the treatment of malignancies is a rather complicated task for clinicians (Jiang et al. 2019).

For centrally located tumor cells, autophagy can be an excellent option for cancer cells to survive and continue tumorigenesis. In this case, autophagy may function as a big barrier against most routine cancer therapies (Kimmelman and development 2011). Unlike the aforementioned data, dozens of studies have revealed that autophagy is another side of the sword that can help with the maintenance of tumor cells (Gong et al. 2013; Guo et al. 2016), as it contributes to their escape from the immune system (Noman et al. 2011). Table 2 summarizes the

6 | Nanotechnology

A large number of studies have been conducted on wide-ranged applications of nanomaterials (NMs), only to discover their peculiarly unfavorable effects. In terms of cell function and molecular pathway, NMs often cause profound adverse biological effects (Setyawati et al. 2013a; Tay et al. 2013; Afzalipour et al. 2019; Shirvalilou et al. 2020; Kondori et al. 2020). Nanotechnology has multiple applications with a scientific impact; however, the underlying pathways in interaction of NMs with biological systems at a molecular level still remain to be elucidated. These controversies raise concerns for utilizing nanoscale particles in targeted cancer therapies (Warheit 2010; Setyawati et al. 2013b; Sheervalilou et al. 2020; Changizi et al. 2020; Sheervalilou et al. 2021b; Shakeri-Zadeh et al. 2020; Sheervalilou et al. 2021a; Shirvalilou et al. 2021; Kafshdooz et al. 2019).

7 | The Link between Autophagy and Nanotechnology

NPs have been widely used as beneficial research tools for modulating the process of autophagy. Autophagy abnormalities are associated with several disorders, including cancer, and cardiovascular, metabolic, and neurodegenerative diseases (Ghavami et al. 2014). Hence, NP-related autophagy modulations are suggested to be a state-of-the-art therapeutic intervention for treatment of such conditions. Induction of oxidative stress-dependent signaling (ER stress, mitochondrial damage, etc.), inhibition of Akt-mTOR signaling, and alteration of the expression of autophagy-related gene/protein stand amongst the primary mechanisms by which NMs modulate autophagic pathway (Wu et al. 2014). Table 3 and Figure 4 represent the link between autophagy and nanotechnology in cancer.
7.1 | Can NMs Turn On or Turn Off Autophagy? Which One is Preferable for Killing Tumor Cells?

The paradoxical nature of autophagy can be turned into an advantage for development of cancer treatment modalities, as the mechanism is thought to be a driving factor of early survival and late cell death in tumor progression and cancer therapy (Singh et al. 2018). Thus, the role of NMs in cancer therapy enhancement is incontrovertible (Abed et al. 2019; Beik et al. 2019; Mirrahimi et al. 2019; Ghaznavi et al. 2018; Beik et al. 2017).

In last decade, inhibition of autophagy introduced as a strategic mechanism in cancer therapy. A growing number of studies are being dedicated to delineating the link between NMs and autophagy to see if NMs are exploitable tools in cancer therapies (Wei and Le 2019). Since then, an expanding number of NMs ranging from soft NMs, liposomes and polymeric NPs to hard NMs such as Cerium dioxide (CeO2 NPs), Zinc oxide, iron oxide (IONPs), silver, gold (AuNPs), and titanium dioxide NPs, QDs, carbon nanotubes (CNTs), graphene oxide (GO), Silica NPs (SNPs), and fullerenes have shown to possess remarkable properties for modulating autophagy (Zheng et al. 2016; Yu et al. 2014b; Hussain et al. 2012). Chemical composition, morphology, and surface chemistry, as well as the size of NMs, determine whether a NP is likely to trigger autophagy under certain conditions. In other words, NPs can be considered as both inducer and inhibitor of autophagy in the target cell based on their size and morphology (Zhang et al. 2018; Popp and Segatori 2015).

Nevertheless, NP-mediated autophagy is associated with nanotoxicity (Sarkar et al. 2014). To boost the therapeutic efficacy and develop safer NMs, scientists investigated the variations of CNTs surface ligand and their impact in modulating the extent to which autophagy is triggered. They reported that the surface modification of CNTs might result in potential pharmaceutical autophagy modulators and biocompatible NMs (Wu et al. 2014).

7.1.1 | Turn-on Effects of NMs

7.1.1.1 | Positive turn-on Effects: Pro-death Nature of Autophagy

Various NMs, including metallic-based NPs (Cordani and Somoza 2019) and light and heavy nanocrystals (Yu et al. 2009), can trigger autophagy. In 2005, scientists showed that nano-sized neodymium oxide (Nano Nd2O3) induced extensive autophagy in NCI-H460 human lung cancer cells (Chen et al. 2005). After that, NMs-related autophagy was generally believed to be a pro-death mechanism. The only way to acquire knowledge on the likelihood of such claims were to evaluate cell death while inhibiting autophagy. As a clarification, it was shown that both molecule inhibitors and Atg5 gene knockdown dramatically reduced the rate of death in HeLa cells incubated with ZnO NPs,
indicating that these NMs triggered pro-death autophagy (Hu et al. 2019). This was suggested to be a positive effect of NMs in cancer therapy through the regulation of oxidative stress and autophagy, which led to cell death. In this case, NMs served as cytotoxics and/or enhanced the efficiency of typical chemotherapies (Sun et al. 2014).

To enhance the efficiency of epidermal growth factor receptor (EGFR)-oriented triple-negative breast cancer (TNBC) therapy, scientists developed EGFR-targeted AuNPs to induce autophagy. In this case, autophagy induction rendered the cancer cells more susceptible to photothermal therapy (PTT) (Zhang et al. 2017b). They discovered that poly(lactic-co-glycolic acid) or PLGA-based NPs were able to trigger autophagy in tumor cells. In this modality, NPs were swallowed by autophagosomes before being delivered to degradative organelles (Zhang et al. 2014b). Modified PLGA-based NPs significantly enhanced the activity of autophagosomes compared with non-modified counterparts. In this study, induction of autophagy via docetaxel-containing NPs contributed to impaired intratumoral drug delivery (Liu et al. 2011).

In another study, redox-responsive nanohybrid GCMSNs were synthesized through GNPs attachment onto amine-functionalized MSNs. Compared to normal 3T3-L1 cells, GCMSNs induced higher oxidative stress-triggered autophagy in A549 lung cancer cells. Synergism, through the combination of chemotherapy and oxidative stress-induced autophagy via camptothecin-loaded nanohybrids, resulted in a superior nanocarrier system for highly effective cancer therapy (Lu et al. 2015). Despite that, autophagy-mediated cell death is still somehow challenging if the normal cells become involved as well. To address this issue, one should ascertain the selectivity of NP-based autophagy, as it must only be triggered in cancer cells.

The best targets for autophagy-mediated therapy are autophagy-deficient cancer cells. Lack of beclin-1 protein required for initiation of autophagosome formation in autophagy is a determining factor. Therefore, designing autophagy-inducing peptides engineered into polymeric NPs (P-Bec1) could significantly enhance autophagy-mediated cell death in these cells (Wang et al. 2015a).

NP-induced autophagy sometimes appears to be useful for cancer therapy, especially against drug-resistant variants, if it were coupled with autophagy-mediated chemosensitization. Fullerene c60, which induces autophagy in tumor cells, was reported to enhance the chemosensitization of both normal and drug-resistant cancer cells. Thus, the subsequent reduction in drug resistance may eventually establish novel therapeutic strategies for cancer treatment (Wei et al. 2010).
7.1.1.2 | Negative turn-on Effect: Pro-survival Nature of Autophagy

To form a verdict on nano-related autophagy-inducing effect in cancer therapy from another perspective, it is appropriate to note the ineffectiveness of Chemo-PTT combination therapy approach in drug-resistant cancer. Turning on the pro-survival autophagy is thought to be a great solution to this issue. With a high absorption in the near-infrared (NIR) region, NMs can also induce pro-survival autophagy. The recent application of custom designed copper (Cu)-palladium (Pd) alloy tetrapod NPs in Chemo-PTT is considered a novel approach, that combines chemotherapy and PPT. Thanks to their unique structure, these NPs elicited an ideal photothermal conversion potential and induced pro-survival autophagic cell death. This achievement paved the way for application of custom-designed NPs as autophagy suppressing agents rather than the conventional therapeutic agents (Zhang et al. 2018).

In contrast to the most noted autophagy-related cell death by NMs, nano-sized paramontroseite VO₂ nanocrystals (P-VO2) were reported to induce cytoprotective autophagy in cultured HeLa cells (Zhou et al. 2013). Furthermore, several NMs were also reported to induce pro-survival autophagy (Zhang et al. 2019).

This increased level of protective autophagy (pro-survival autophagy) could hamper anti-cancer therapies. In such cases, autophagy might function as a cellular protector against NP-induced cytotoxicity in various tumor cell lines. Therefore, autophagy inhibitors have been widely used in company with drug-delivery NMs to improve the treatment efficiency. Hence, when deciding to modulate autophagy for enhancing treatment efficiency, one should consider whether the combined regimen enhances or dampens autophagic activity in tumor cells to accurately determine the modulation method (Høyer-Hansen and Jäättelä 2008; Das et al. 2019).

7.1.2 | Turn-off Effects of NMs

In addition to the above mechanisms, a number of studies suggest that NMs are capable of perturbing autophagic pathways by inhibiting Akt-mTOR signaling or altering the expression of autophagy-associated genes/proteins (Zhang et al. 2009; Liu et al. 2011; Li et al. 2009). Therefore, compared with the well-studied NMs that induce autophagy, inhibitory types are still rare. Citric acid-capped gold, REO, and IONPs have been known as blockers of autophagic activity; however, their mechanism of action and cellular targets are still ill-defined. In a recent study, custom designed titania-coated gold nano-bipyramids (NBP/TiO2) functioned as an innovative autophagy inhibitor for sensitizing U-87 MG brain tumor cells to proteasome inhibitor-induced cell death. Moreover, nanodiamonds (NDs) were recently shown to inhibit autophagy in oxygen-deprived tumors in a synergistic manner (Wan et al. .
In practical terms, high levels of autophagy under hypoxia is an adaptive strategy adopted by cancer cell for survival. Therefore, NDs-related autophagy inhibition, along with oxygen deprivation, may cause significant apoptosis in HeLa cells and MCF-7 cells (Chen et al. 2018b). In a similar study led by Sun, inhibition of autophagy resulted in sensitization of MDA-MB-231 cells to conventional chemotherapeutics (Sun et al. 2016).

NMs have the potential to either induce or inhibit the autophagic pathways. Still, more research on this topic needs to be conducted to delineate the link between NMs and autophagy.

8 | Effects of NMs on Tumor Dormancy; Focusing on Involved Signaling Pathways

NPs can influence the autophagic pathway in different ways; however, their role in the induction of tumor dormancy may hinder their practical applications. Autophagy plays a crucial role in preserving tumor cells in a prolonged state of arrest and senescence, that can be followed by apoptotic cell death (Polewska et al. 2013). That is to say, autophagy may be directly associated with tumor dormancy, as the senescent cells might recover their proliferative capability, giving rise to renewed tumor growth and metastasis (Gewirtz 2009). Nonetheless, PTT therapy has limited capacity for total eradication of tumor cells, as adjacent cells could be very well damaged by mild hyperthermia. In this case, heat shock proteins would naturally be recruited to repair the damaged cells, resulting in tumor relapse, and eventually, escape of tumor from dormancy (You et al. 2019).

Dormant tumor cells often gain drug resistance that protects them against chemotherapy (Aguirre-Ghiso 2007). In 2006, scientists established a link between the activation of the p38 signaling pathway and induction of tumor dormancy. They demonstrated how enhanced activation of PERK, an RNA-dependent protein kinase, compels dormant squamous carcinoma cells to develop drug resistance (Ranganathan et al. 2006). Several newly-designed NMs were reported to activate p38 signaling, therefore, induce drug resistance (Skuland et al. 2014; Eom and Choi 2010). These NMs are conjugated to drugs, and circumvent poor drug retention into the tumor cells for efficient targeting. However, either the induction or inhibition of autophagy could have profound impacts on drug resistance reversal (Panzarini and Dini 2014). One particular investigation, in 2018, adopted hyaluronic acid-based nanoparticles for targeting tumor stem cells, in order to decrease their drug resistance as a result of dormancy. In this work, the previously known antitumor agents (e.g., camptothecin, doxorubicin hydrochloride, or curcumin) were co-delivered to malignant stem cells via four multi-layered core-shell polymeric nanoparticles, that were synthesized from different chitosan-modified polymers (Wang and He 2018).
There is another hypothesis that argues the strict connection between inflammation and senescence, highlighting the role of chronic inflammation in awakening of dormant tumor cells (Manjili 2017). Among cytokines, IFN-\(\gamma\) has been shown to leave anti-tumorigenic effects, that result in arresting of cell cycle and induction of dormancy in indolent tumor cells (Aqbi et al. 2018b). NMs featuring tailored chemical properties have been used for delivering IFN-\(\gamma\) to tumor cells (Mejías et al. 2011). Yet, the beneficial antitumor activity of this pleiotropic lymphokine might be autophagy-independent, since little has been reported regarding this matter. Most recently, scientists developed a novel chemo-Immuno strategy towards targeted delivery of agents with high antitumor and/or anti-fibrotic potency, celastrol and mitoxantrone. In their study, mitoxantrone-responsive nanocarriers successfully curtailed the proliferation of tumor cells and further suppressed tumor invasion. The affected tumor cells remained dormant long after co-treatment with both agents, causing a sustained progression-free survival of the mice affected with desmoplastic melanoma (Liu et al. 2018a).

Nanocarriers were also utilized for the efficient delivery of dormancy-associated miRNAs to tumor cells. To this end, a group scientists opted to prepare aminated polyglycerol dendritic nanocarriers for delivering miR-200c, miR-34a, and miR-93 into MG-63 and Saos-2 osteosarcoma tumor cells. Hence, using nanomaterial-mediated delivery of microRNAs associated with tumor-host interactions might be a useful strategy to induce a dormant-like state (Tiram et al. 2016).

9 | Autophagy Mediated Multiple Drug Resistance in Chemotherapy of Cancer Cells

Characterized by the gradual development of resistance to multiple chemotherapeutic agents with different mechanisms of action by tumor cells, multidrug resistance (MDR) is an undesirable outcome of chemotherapy that may occur in several instances (Holohan et al. 2013). A major culprit responsible for a significant proportion of cancer-associated mortality, MDR commonly results in the failure of treatment. A strikingly important challenge in cancer therapy, MDR, along with tumorigenesis, were previously thought to be correlated with disruptions in the regulation of autophagy. The idea came to fruition once several investigations reported potential involvement of autophagic pathways in the emergence of MDR (Kumar et al. 2012; Li Liu et al. 2020).

According to the recent findings, autophagy may affect MDR through a number of mechanisms as explained below (Li et al. 2017):

1) Autophagy can prompt MDR as a cytoprotective mechanism (Table 4a):
   - Autophagy is positively correlated with development of MDR
- Inhibition of autophagy may enhance the effectiveness of chemotherapy in cases with MDR

2) Autophagy, when resulting in cell death, can overcome MDR (Table 4b);

3) Autophagy triggers cell death in apoptosis-deficient MDR tumor cells

4) Autophagy accelerates chemosensitization

Induced by many cancer therapies, autophagy has been suggested to improve the survival of tumor cells, and facilitate the development of MDR (Kondo et al. 2005; Amaravadi et al. 2011; Levy et al. 2017; Smith and Macleod 2019). For example, resistance to enzalutamide was counteracted by inhibition of autophagy in an investigation on prostate cancer (Nguyen et al. 2014). Likewise, in one study, inhibition of autophagy in estrogen receptor-positive breast cancer resulted in sensitization of the resistant tumor cells to the cytotoxic effects of tamoxifen (Samaddar et al. 2008; Qadir et al. 2008). Autophagy was also reported to be activated in response to Imanitinib, used for the treatment of gastrointestinal stromal tumor (GIST). In this particular case, chloroquine (CQ) was adopted to overcome autophagy, and trigger apoptosis in tumor cells (Gupta et al. 2010). A growing body of evidence suggests that autophagy is induced in response to many types of cancer therapy, hence, the development of MDR (Galluzzi et al. 2017).

Disinhibition of autophagy is often suggested to be a consequence of low mTOR activity, and is most commonly observed with therapies that target mTOR, PI3K, or AKT (Amaravadi et al. 2011). Nonetheless, one cannot certainly predict the induction of autophagy, since the extent of induction may vary in conventional and non-conventional therapies. An increased p53 activity triggered by DNA damage, due to genotoxic therapeutics such as cisplatin, may partly explain the undesirable induction of autophagy in conventional treatments, that occur as a result of the increased activity of p53-dependent regulators of autophagy, e.g., DRAM1 (Crighton et al. 2006). Nevertheless, the exact role of p53 in this context is debatable, since this tumor-suppressing protein can also inhibit autophagy (Simon et al. 2017). 25. Known to stimulate the activity of autophagy-regulating genes, namely; ATG5, LC3, etc., the induction of ATF4 and FOXO transcription factors due to ER stress response and overproduction of ROS, respectively, may explain the activation of autophagy in these instances (Warr et al. 2013; Ranganathan et al. 2006). The dual pro-apoptotic/anti-apoptotic roles of autophagy largely depends on the characteristics of tumors. In the case of MDR cancer, exerts a protective effect on tumor cells by facilitating resistance to chemotherapeutic agents. Accordingly, inhibition of autophagy might be an effective strategy to sensitize MDR tumor cells to anticancer therapies. Nonetheless, more recent evidence suggests otherwise by pointing to the unappreciated
potential of autophagy at sensitizing MDR tumor cells to anticancer agents, and reversing MDR. Should this be the case, autophagy will inspire development of promising therapeutic modalities to overcome MDR (Li et al. 2017). Table 4 represents the studies on the pro-survival and pro-death role of autophagy in MDR of chemotherapy (Updated from (Das et al. 2019; Li et al. 2017).

10 | Conclusion and Outlook

As of this date, the exact molecular pathways involved in modulation of autophagy, and their significance in tumor formation and progression are not clearly understood. Though, as scientists suggest, autophagy is not an immutable constituent, but a rather dynamic mechanism with quite a various behavior in cell biology. We ought to clarify that due to the double-edged nature of autophagy, this regulatory mechanism can either result in induction or suppression of tumorigenesis, depending on the type and stage of tumor. An increasing number of investigations have pointed to the impact of activated autophagy on the fate of tumor cells. From one point of view, autophagy might serve as an impeccable cellular shield against tumorigenesis, which can be adopted into therapeutic strategies. On the contrary, however, the exact same phenomenon might bring about further formation of tumors, with catastrophic consequences if provoked at will, namely; secondary metastasis and tumor relapse. Nevertheless, pharmacological modulation of autophagy has allegedly led to satisfactory results in limited research areas that could imply the potential of such interventions in development of novel therapeutics for cancer treatment. In this regard, scientists have less frequently discussed the potential effects of nanomaterials in mediation of autophagy.

As of recent, newly emerging technologies have provided us with such convenience for materialization of highly specialized nanoparticles for a variety of therapeutic purposes. Much to our dismay, however, the gross production of nanoparticles has led to an incontrovertible risk of exposure for people, prompting close studies of potentially harmful effects that might be conveyed through these tiny particles. Hopefully, an expansive array of information on the process of cellular uptake of nanoparticles has been gathered, indicating that the rate of cellular internalization is possible to be controlled based on physicochemical properties of nanoparticles, i.e., size, charge, and surface properties. It is anticipated that a thorough understanding of the functional interactions between autophagy and nanoparticles will tremendously impact the design of nanomaterials in such a way that development of tunable and safe nanomaterials will no longer be a far-fetched vision.
As for nanoparticles in this respect, several concerns still remain regarding the effect of different nanoparticles on the activation/suppression of autophagy, since it can very well lead to induction/inhibition of proliferation, differentiation, and invasiveness of tumor cells. According to many studies, nanomaterials can affect autophagy in malignant cells in such a way that can be adopted for development of therapeutic modalities for treatment of this malady. For instance, in a recent investigation (which is currently in press), we demonstrated the prominent role of gold nanoparticles applied through photothermal therapy in determining the destiny of tumor cells by means of regulating autophagy. In a similar fashion, in the present paper, we have sought to advise the scientists investigating in this particular fields of these concerns. That is to say, modulation of autophagy through nanomaterials is thought to be of therapeutic value for suppressing tumorigenesis in normal tissues, and initiation of alternative cell death in compromised cells that struggle to properly kill themselves, on top of which stand malignant cells. This type of intervention can be further complemented by combining with traditional antitumor regimens to achieve a higher level of efficacy.

The complex interaction of autophagy-related pathways with the immune cells is another factor that might determine the fate of a tumor cell. Better understanding of the molecular pathways underlying the immune escape in the recent years has accelerated the development of novel immunotherapies, that aim to target molecules that would otherwise counteract the desirable antitumor immune response. Recent investigations have also highlighted the regulatory effects of autophagy on immunity through modulation of cytokine release and the function of immune cells. In return, a number of cytokines and certain types of immune cells reciprocate by affecting the autophagy itself. Accordingly, autophagy can very well be adopted for development of novel therapeutic approaches when combined with tumor immunotherapy even nanobiotechnology.

An increasing research interest in autophagy and autophagy-related cell death is evidence enough to the significance of this matter. Since the mechanism is of both physiologic and pathologic prominence, it would be best if autophagy were approached from both academic and clinical aspects. One crucial task in this field is to identify new biomarkers and develop novel tests to precisely determine the dynamic processes of autophagy in real-life samples. It is expected that such efforts will help us better understand how autophagy is modulated within tumor cells, and ameliorate the design of clinical approaches aimed at targeting this mechanism. Prospective efforts should focus more on unraveling the genetic and physiologic grounds of autophagy, that would most likely improve the therapeutic value of our knowledge regarding this type of cell death.
AUTHOR CONTRIBUTIONS

All authors contributed in different parts of the review study.

 Participated in research design: Dr. Sheervalilou R, Dr. Ghaznavi H

 Conducted experiments:-

 Contributed new reagents or analytic tools:-

 Performed data analysis:-

 Wrote or contributed to the writing of the manuscript: Dr. Ghaznavi H, Dr. Shirvaliloo M, Dr. Zarebkohan A, Shams Z, Radnia F, Bahmanpour Z, Dr. Sargazi S, Dr. Saravani R, Dr. Shirvalilou S, Dr. Shahraki O, Shahraki S, Dr. Nazarlou Z, Dr. Sheervalilou R
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Kaempferol induces autophagy through AMPK and AKT signaling molecules and causes G2/M arrest via
dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes. *ACS Nano* 6
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between autophagy and the immune system and its applications for tumor immunotherapy. *Mol Cancer* 18
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cells harboring wild-type p53. *Autophagy* 7 (11):1348-1358
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mTOR signaling. Cell Death Dis 2 (5):e159-e159
arrested cancer progression and induced tumor dormancy in desmoplastic melanoma. ACS Nano 12
(8):7812-7825


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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.
### Table 1. Correlation between autophagy and tumorigenesis.

<table>
<thead>
<tr>
<th>In vitro</th>
<th>In vivo</th>
<th>Autophagy related genes/Signaling pathways</th>
<th>Autophagy related gene status in cancer</th>
<th>Techniques</th>
<th>Autophagy interaction with TSG</th>
<th>Autophagy interaction with oncogenes</th>
<th>Correlation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 cells</td>
<td>Nude mice</td>
<td>Mammalian: <em>Bec-1</em> Yeast: <em>apg6/vps 30</em></td>
<td>Monallelically deletion of <em>Bec-1</em> in 40–75% of sporadic human BC</td>
<td>Gene-transfer techniques to induce <em>Bec-1</em></td>
<td><em>Bcl-2</em></td>
<td><em>Bec-1</em> activation: Inhibition of proliferation of MCF7 cells and clonogenicity, Inhibition of tumorigenesis in nude mice</td>
<td>Liang et al. (1999)</td>
<td></td>
</tr>
<tr>
<td>Hela cells</td>
<td>Athymic nude mouse (SPF) bearing human cervical cancer</td>
<td><em>Bec-1</em> (17q21) monoallelically deleted in BC</td>
<td><em>Bec-1</em> silencing using RNA interference</td>
<td><em>Cas-9</em></td>
<td>-</td>
<td>siRNA against <em>Bec-1</em> transfectants: Promoted cell proliferation, Less apoptosis <em>Bec-1</em>-expressing cells: Promoted the autophagy cell death, Regulation of the <em>Cas-9</em> expression, Inhibition of tumorigenesis in nude mice</td>
<td>Wang et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>CaSki Cells</td>
<td>-</td>
<td><em>Bec-1</em> monoallelically deleted in BC</td>
<td>pcDNA3.1-<em>Bec-1</em> and RNA interference vector pSUPER-<em>Bec-1</em></td>
<td>-</td>
<td><em>VEGF and MMP-9</em></td>
<td><em>Bec-1</em> overexpression: Decreased <em>VEGF</em> and <em>MMP-9</em>, Cell cycle arrest in the G0/G1 phase, Inhibited invasion and metastasis</td>
<td>Sun et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>HT-29 cells, Caco-2 cells</td>
<td>-</td>
<td><em>LC3-II, Atg5, Bec-1</em></td>
<td>Bufalin isolated from a TCM, siRNA transfection</td>
<td>-</td>
<td>-</td>
<td>bufalin activated autophagy through: <em>LC3-II</em> accumulation, Stimulation of autophagic flux, ROS generation, JNK activation, Increased expression of <em>ATG5</em> and <em>Bec-1</em>, autophagy-mediated cell death</td>
<td>Xie et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>SiHa, A549</td>
<td>-</td>
<td><em>LC3-I p53/AMPK/mT</em></td>
<td>DHA treatment, <em>p53, Cas-3</em></td>
<td>-</td>
<td>DHA treatment: Cas-3-dependent apoptosis</td>
<td>Jing et al. (2011)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and MCF-7 cells OR GFP-LC3 expression vector and autophagy induction, p53 loss, Increased active form of AMP-activated protein kinase and decreased the activity of mammalian target of rapamycin

SK-HEP-1 cells

- LC3-I, Bec-1, Atg, AMPK and AKT signaling molecules - Kaempferol treatment, GFP-fluorescent LC3 assays Kaempferol treatment: Autophagy induction, Increased protein level of p-AMPK, LC3-II, Atg 5, Atg 7, Atg 12 and Bec-1, Inhibited the levels of CDK1, cyclin B, p-AKT and p-mTOR, G2/M arrest, Long-term cancer prevention

HeLa, BT-474, SKBR3, MDA-MB-361 cells

C57/B6 Becn1 knock-in Becn1F1 21A mice, FVB/N-Tg (MMTV neu) 202 Mul/J strain Bec-1/BECN1, LC3-I Allogenic loss of Bec-1/BECN1 in HER-T positive BC Genetically engineered mutation in Becn1, GFP-LC3 mouse, Tat-Bec-1 treatment (autophagy-inducing peptide) - HER-2 Mice with a genetically engineered mutation in BECN1: Protected from HER2-driven mammary tumorigenesis, HER2 failed to inhibit autophagy Mice under treatment with Tat-Bec-1: Inhibition of tumor growth as effectively as a clinically used HER2 TKI, Disruption of HER2/Beclin-1 binding, Robust induction of autophagy

Cancer promotion

<table>
<thead>
<tr>
<th>In vitro</th>
<th>In vivo</th>
<th>Autophagy related genes/Signaling pathways</th>
<th>Autophagy related gene status in cancer</th>
<th>Techniques</th>
<th>Autophagy interaction TSG</th>
<th>Autophagy interaction with oncogenes</th>
<th>Correlation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231, HTB43, HTB35, A549 and SW707 cells</td>
<td>-</td>
<td>Bec-1, atg3, atg4b, atg4c, atg5, and atg12</td>
<td>Irradiation-induced accumulation of autophagosomes accompanied by strong mRNA induction of the Atg in tumor cells</td>
<td>Blockade of each Atg through specific target siRNAs, Radiation therapy</td>
<td>p53</td>
<td>-</td>
<td>Short-time inhibition of autophagy along with radiotherapy: Strongly diminished accumulation of autophagosomes, Sensitization of resistant carcinoma cells to therapy</td>
<td>Apel et al.</td>
</tr>
</tbody>
</table>

(2011) Jing et al. 2011

(2013) Huang et al. 58

(2013) Huang et al. 2013

(2018) Vega et al. 59

(2018) Vega-Rubín-de-Celis et al. 2018

(2008) Apel et al. 61
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Organism</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGR-Heu cell line, Heu171 cell clone, B16-F10 cells</td>
<td>C57BL/6 mice</td>
<td>With B16-F10-engrafted tumors</td>
<td>Hypoxia-induced autophagy in tumor</td>
</tr>
<tr>
<td>TOV112D, TOV21G, OV90, SKOV3, MDAH2774, and ES2</td>
<td>C57BL/6 mouse</td>
<td>p62</td>
<td>Bortezomib treatment, RNA interference</td>
</tr>
</tbody>
</table>

### Table 2. The autophagic genes involved in cell death, invasion and tumor dormancy.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dormancy activation</strong></td>
<td>P53 overexpression induced by Cdkn1b</td>
<td>Pentose phosphate pathway destruction, Increased ROS</td>
<td>Cell death, Dormancy induction by IFN-b</td>
<td>(Liu et al. 2018b)</td>
</tr>
<tr>
<td><strong>FasL (CD95L or CD178), TRAIL and TNF-α activation</strong></td>
<td>DISC formation</td>
<td>Cas-3, 6 and 7 activation, Bid change into tBid</td>
<td>Directly cell death, Mitochondria dependent apoptotic cell death</td>
<td>(Su et al. 2015)</td>
</tr>
<tr>
<td><strong>Autophagy inhibition</strong></td>
<td>ATG7 depletion</td>
<td>Accumulation of damaged mitochondria, Increase of ROS, Increase of apoptosis</td>
<td>Killing of dormant cells, Has no any effect on cell metastasis and proliferation</td>
<td>(Vera-Ramirez et al. 2018)</td>
</tr>
<tr>
<td><strong>Autophagy activation</strong></td>
<td>TMEM166 overexpression</td>
<td>High LC3II/LC3I Vacuolization Mitochondria membrane permeabilization</td>
<td>Autophagy and apoptosis regulator (autophagic and apoptotic cell death)</td>
<td>(Wang et al. 2007a)</td>
</tr>
<tr>
<td><strong>IRGM</strong></td>
<td>--------</td>
<td>negative regulation of IFN signaling</td>
<td>Autophagic cell death</td>
<td>(King et al. 2011)</td>
</tr>
<tr>
<td><strong>Increase of Bax and Bak1</strong></td>
<td>Inactivation of Bax1 and Bax</td>
<td>Intrinsic pathway (mito), indirectly affect on the autophagy</td>
<td>Increase cancer cell apoptosis</td>
<td>(Karch et al. 2017; Lindqvist and Vaux 2014)</td>
</tr>
<tr>
<td><strong>DAPK1</strong></td>
<td>--------</td>
<td>ARHI dependent</td>
<td>Tumor suppressor, Apoptotic cell death</td>
<td>(Wu et al. 2013; Tong et al. 2018)</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Autophagy activation</td>
<td>PI3K/Akt inhibition, PI3K/AKT/mTORC1 inhibition</td>
<td>Tumor suppressor</td>
<td>(Gundara et al. 2012)</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>PTEN inhibitors</td>
<td>Tsc1 or Tsc2, p27 and Foxo3a</td>
<td>Escape from dormancy</td>
<td>(Richmond et al. 2015; Chen et al. 2018a)</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Apoptosis modulators activation</td>
<td>DRAM, DAPk and DRP-1, PTEN, E93 , Akt/PKB and mTOR, Bcl-2 family proteins , TRAIL and bccl-1</td>
<td>Autophagy act as upstream control of apoptosis death</td>
<td>(Wang et al. 2007a)</td>
</tr>
<tr>
<td><strong>Autophagy abortion</strong></td>
<td>DRAM1 overexpression</td>
<td>By p53</td>
<td>Apoptotic death</td>
<td>(Criollo et al. 2009)</td>
</tr>
</tbody>
</table>

### Autophagic genes involved in invasion (colonization, proliferation, tumor formation, promotion, metastasis)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATG5 and ATG7- RAS</strong></td>
<td>Increased autophagy</td>
<td>Mitochondria activation</td>
<td>Tumor formation</td>
<td>(Li et al.</td>
</tr>
</tbody>
</table>
## Autophagy inhibition

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cas-3/ATG16L1 complex formation</td>
<td>Sustained intracellular stress and pathogen</td>
<td>Disease or tumor promotion</td>
<td>(Murthy et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>ATG4D</td>
<td>Intracellular LC3-B/P62 accumulation, Autophagosome formation abortion</td>
<td>Disease and tumor promotion</td>
<td>(El Andaloussi et al. 2017)</td>
<td></td>
</tr>
<tr>
<td>ATG5/7 increased</td>
<td></td>
<td>Increase in colonization</td>
<td>(Washington et al. 2015)</td>
<td></td>
</tr>
<tr>
<td>p27/Kip1 coaded by CDKN1B</td>
<td>CDK-dependent kinase inhibitor</td>
<td>Tumor promotion</td>
<td>(Cusan et al. 2018)</td>
<td></td>
</tr>
<tr>
<td>ATG3/7/p62 targeting</td>
<td>Pfkfb3 normal expression</td>
<td>Tumor re-proliferation</td>
<td>(Flynn et al. 2019; Mathew et al. 2009)</td>
<td></td>
</tr>
</tbody>
</table>

## STAT1 inhibition

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>p27 (CDKN1B), p21(CDKN1A) upregulation</td>
<td>Increase in IDO1 and Kyn receptors, Rb hypophosphorylation, suppress E2F transcription factor activity</td>
<td>Tumor dormancy, Increase in colony formation, Decrease in proliferation</td>
<td>(Wu et al. 2012)</td>
<td></td>
</tr>
</tbody>
</table>

## ATG9B

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autophagy deregulation</td>
<td></td>
<td>Tumorigenesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Autophagy manipulation

<table>
<thead>
<tr>
<th>Gene Name</th>
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<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>eIF4E/eIF4GI knockdown</td>
<td>Decrease in ERα, SMAD5, NF-kB, CyclinD1, c-MYC, and HIF1α</td>
<td>Decrease in EMT promoter, Increase in EMT inhibitors, Decrease in migration capability</td>
<td>(Attar-Schneider et al. 2016)</td>
<td></td>
</tr>
</tbody>
</table>

## Autophagic genes involved in Dormancy (suppression, inhibition, resistance to anoikis, invasiveness and colonization, recurrence)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation of Nix/BNIP3L+ GABARAPL1+ GABARAP</td>
<td>Autophagy activation and Deletion of damaged Mitochondria</td>
<td>Increase ROS</td>
<td>Tumor suppression</td>
<td>(Poillet-Perez et al. 2015)</td>
</tr>
<tr>
<td>ATG5 and ATG7 deletion</td>
<td>Autophagy inhibition</td>
<td>Oxidative stress, Damaged Mitochondria</td>
<td>Tumor suppression</td>
<td>(Flynn et al. 2019)</td>
</tr>
<tr>
<td>ATG16L1, Bec-1 and LC3-II degradation</td>
<td>Autophagy deficiency</td>
<td>Oxidative stress, Damaged Mitochondria, Inflammation (IL-1β, IL-18)</td>
<td>Tumor suppression</td>
<td>(Fernández and López-Otín 2015; Maruyama and Noda 2018)</td>
</tr>
</tbody>
</table>

## High Atg4B

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autophagy inhibition</td>
<td>LC3-PE degradation, LC3 sequestration in cytosol</td>
<td>Tumor suppressive</td>
<td>(Galluzzi et al. 2017)</td>
<td></td>
</tr>
</tbody>
</table>

## Autophagy activation

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA damage</td>
<td>Atg4a and Atg4c/p53 contribution</td>
<td>Tumor suppression by p53-mediated apoptosis</td>
<td>(Broz et al. 2013)</td>
<td></td>
</tr>
</tbody>
</table>

## Autophagy deficiency

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cellular Pathway</th>
<th>Effector</th>
<th>Consequences</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG16L1 overexpression (non-specific organs)</td>
<td></td>
<td>Tumor formation suppression</td>
<td>(Capparelli et al. 2012)</td>
<td></td>
</tr>
<tr>
<td>UVRAG upregulation</td>
<td></td>
<td>Tumor suppression</td>
<td>(Liang et al. 2020)</td>
<td></td>
</tr>
</tbody>
</table>
Autophagy deficiency

ATG5 and ATG12 deficiency
Decreased of survival capacity to metabolic stress
Tumor suppression (Decrease in colonization and survival capability) (Maes et al. 2014)

Dormancy activation

P53 overexpression induced by Cdkn1b
Pentose phosphate pathway destruction, Increased ROS
Cell death, Dormancy induction by IFN-b (Liu et al. 2018c)

ER stress

K-RAS dependent Eif2ak3/- MEFs
Decrease in VCIP and PDGFRB (angiogenic stabilizer)
Tumor suppression, ECM destruction, Vast hemorrhage (Cubillos-Ruiz et al. 2017)

BBC3/ HSPA8(HSC70) complex formation

CMA
Cargo delivery to lysosome
Tumor protection by autophagy (Xie et al. 2015)

IFN-γ/STAT1 activation

Downregulation of Cyclin E,A, D1,2,3
Downregulation of CDK4 and CDK6
Cell cycle arrest, Cancer cell dormancy (Dimco et al. 2010; Schmitt et al. 2012)

Inherent ATG5 or autophagy KO

Intracellular inherent autophagy
Postpone of recurrence
Recurrence, chemotherapy desensitization, increase of dormancy frequency (Aqbi et al. 2018a)

ER stress

EIf2AK3 suppression
Upregulation of FGF2 -VEGF and IL-6,
Downregulation of THBS1, CXCL14, and CXCL10
Suppression of angiogenesis and tumor promotion (Cubillos-Ruiz et al. 2017)

Tumor suppression

### Table 3. Interaction between nanotechnology and autophagy in cancer.

#### I. Turn on effects of NMs; Pro-death nature of autophagy

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>NPs</th>
<th>Size</th>
<th>Concentration</th>
<th>NPs treatment Effect</th>
<th>Mechanism</th>
<th>Approach</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H460 cells</td>
<td>Nano Nd2O3</td>
<td>Mean diameter: 80 nm</td>
<td>µM equivalent concentration range (40-45 µM)</td>
<td>Cytotoxic effects, Apoptosis induction, Autophagic cell death</td>
<td>Massive vacuolization induction, S-phase cell cycle arrest, Mild disruption of mitochondrial membrane integrity, Inhibition of proteasome activity</td>
<td>Therapy</td>
<td>(Chen et al. 2005)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Induced autophagy and sensitized chemotherapeutic killing of tumor cells in both normal and drug-resistant cancers</td>
<td>Enhancing the cytotoxicity of chemotherapeutic agents and reducing drug resistance</td>
<td>Therapy</td>
<td>(Wei et al. 2010)</td>
</tr>
<tr>
<td>A549 cells, NCI-H1975 cells</td>
<td>-</td>
<td>-</td>
<td>5% COOH-CNT</td>
<td>In vitro: Autophagy-induced cell death, In the presence of autophagy inhibitor (3MA), ATG6 or TSC2 siRNA: Increase in cell viability in vivo: Acute lung injury</td>
<td>Autophagy-blocking reagents as potentia l agents to remedy the ALI induced by NMs</td>
<td>Autophagy-blocking reagents as potentia l agents to remedy the ALI induced by NMs</td>
<td>(Liu et al. 2011)</td>
</tr>
<tr>
<td>A549 cells, IMR-90</td>
<td>IONPs</td>
<td>Size range: 30-65 nm, average size: 51.34 ± 14.71 nm</td>
<td>10-100 µg/ml</td>
<td>Selective autophagy-induced cell death</td>
<td>Autophagy correlated with ROS production and mitochondrial damage, AMPK/mTOR/PI3K/Akt pathway regulation by a significant reduction in phosphorylated mTOR, Akt, and p70S6K levels, Significant increase in phosphorylated APMK</td>
<td>Therapy</td>
<td>(Khan et al. 2012)</td>
</tr>
<tr>
<td>MCF-7 cells</td>
<td>Xenograft PLGA NPs</td>
<td>100-150 nm in</td>
<td>In vitro: DOX: 0.25-25</td>
<td>Significant enhancement of therapy efficacy</td>
<td>Cancer cells captured PLGA NPs and</td>
<td>Clinical application</td>
<td>(Zhang et al. 2011)</td>
</tr>
<tr>
<td>SCID mice model</td>
<td>SCID diameter (mg/mL, 3-MA: 10 mM, CQ: 30 mM, Drug free PLGA NPs: at the same NPs concentration, IC50: PLGA NPs: 38.27 ± 1.23, 3-MA: 6.7 ± 1.05, CQ: 4.78 ± 1.75 mg/mL)</td>
<td>through combined cholic acid conjugated DOX-PLGA NPs with autophagy inhibitors (3-MA and CQ) degraded by autolysosomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>----------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A549 cells, 3T3-L1</strong></td>
<td><strong>A549 lung cancer xenografted nude</strong> A549 GCMSN s: GNP+ MSNs, CPT-loaded GCMSNs GNP s)</td>
<td><strong>In vitro:</strong> 100 mg/mL Growth inhibitory effect, Oxidative stress-triggered mitochondria-mediated autophagy Up-taken GCMSNs: blocking pores and inciting production of ROS, mitochondrial dysfunction, oxidative stress-triggered mitochondria-mediated autophagy, GNP s: as an oxidative stress elicitor Selective and effective cancer combined therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MDA-MB-231 (culture d TNBC cells)</strong></td>
<td><strong>Female BALB/c nude mice with xenograft tumors</strong> MDA GNs, Anti-EGFR-GNs 10 nm × 40 nm 120 pM Anti-EGFR-GNs-combined NIR-PTT: Autophagy-induced cell death in vitro: Formation of a large number of autophagic vesicles, Significant increase in autophagy-specific proteins; LC3, p62, Bec-1, Atg5, Inhibition of AKT-mTOR signaling pathway Combin ed targeted therapy (Zhang et al. 2017b)</td>
<td><strong>In vivo:</strong> Increased LC3 and Bec-1 levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Dox-resistant MCF-7 tumor-bearing female HeLa cells, 4T1 cells

- Average size: 172 nm, = Zetapotential: −5.01 m
- In vitro: 50 μg mL⁻¹, 100 μg mL⁻¹
- In vivo: 2 mg kg⁻¹

Killed tumor cells, Enhanced tumor chemotherapy both normal and drug-resistant cancer cells, Overcame drug resistance

- In vitro: Promoting Atg5-regulated autophagy flux, Accelerating zinc ion release, Accelerating the intracellular dissolution of ZONs, ROS generation
- In vivo: The antitumor therapeutic effect of co-treatment with ZONs or free DOX treatment

Pro-survival nature of autophagy

<table>
<thead>
<tr>
<th>In vitro</th>
<th>In/Ex vivo</th>
<th>NPs</th>
<th>Size</th>
<th>Concentration</th>
<th>NPs treatment Effect</th>
<th>Mechanism</th>
<th>Approach</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa cells</td>
<td>-</td>
<td>P-VO₂</td>
<td>-</td>
<td>-</td>
<td>Induced cyto-protective, rather than death-promoting, autophagy in cultured tumor cells</td>
<td>Up-regulation of HO-1 and protecting cells against death under stressful situations</td>
<td>Therapeutic applications</td>
<td>(Zhou et al. 2013)</td>
</tr>
<tr>
<td>4T1, MCF7/MDR cells</td>
<td>BALB/c mice, NOD/S CID mice</td>
<td>Cu-Pd alloy</td>
<td>TNP-1</td>
<td>~50 nm in length, SNP: 35 nm</td>
<td>Induced pro-survival autophagy in tumor cells, Increased cell viability</td>
<td>Normal autophagy flux without impairment of lysosomal function</td>
<td>Autophagy-inspired chemo-PTT of drug-resistant tumor cells</td>
<td>(Zhang et al. 2018)</td>
</tr>
</tbody>
</table>

II. Turn off effects of NMs;

<p>| HeLa cells, HEK293 cells | Tumor | NDs | Primary particle size: 10 nm, hydrodynamic size: in water: 191nm in cell culture: | 20-50 μg/mL | Suppressed autophagic flux in cultured cells tumors | Selective induction of PCD in hypoxic cancer cells, Minimal impairment of lysosomal functions, Significant accumulation in LC3-II and P62, Significant increase in protein levels of Cas-3 | Targeted anti-angiogenic therapy | (Chen et al. 2018b) |</p>
<table>
<thead>
<tr>
<th>Breast CSCs, ALDH hi, MDA-MB-231, MCF-7 cells</th>
<th>Orthotopic murine model, ICR mice and female NOD/S CID mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPCQ, NPDOX, NPDTX, L, NPDOX/CQ, NPDTX L/CQ</td>
<td>Similar diameter of about 110 nm</td>
</tr>
<tr>
<td>289 nm</td>
<td>1-15 μg/mL, 6.5, 1 and 2 mg/kg</td>
</tr>
<tr>
<td>In vitro</td>
<td>In vivo</td>
</tr>
<tr>
<td>Inhibited nanoparticle-mediated autophagy, Reduced &quot;stemness&quot; and increased susceptibility to chemotherapy drugs</td>
<td>Impairment of endosomal acidification, blocking endosome and autophagosome fusion with lysosomes, Increasing accumulation of LC3-II and p62</td>
</tr>
<tr>
<td>Effective therapy (Sun et al. 2016)</td>
<td></td>
</tr>
</tbody>
</table>

**U-87 MG cells**

<table>
<thead>
<tr>
<th>NBP/TiO2 nanostructures, CTAB-Capped, Capped</th>
<th>Lengths: 47 ±4, 95 ±5, 142 ±8 nm, Widths: 20 ±2, 33 ±2, 42 ±3 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 or 60 μg Au mL-1</td>
<td>Inhibited Autophagic flux leading to cancer cell death</td>
</tr>
<tr>
<td>Significant autophagosome accumulation in cancer cells via blocking the autophagosome-lysosome fusion process, inhibiting lysosomal degradation, Reducing the mature cathepsin B, Inhibiting the proteolytic activity of cathepsin B, Inhibiting trypsin-like proteolytic activity, UPS inhibition, Synergistic loss of brain cancer cell viability by combination of Bortezomib and PTT</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Anticancer agent (Wan et al. 2018)</td>
<td>30 or 60 μg Au mL-1</td>
</tr>
</tbody>
</table>

### Table 4. The studies on the pro-survival and pro-death role of autophagy in MDR of chemotherapy

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Cell line</th>
<th>Intervention/Drug</th>
<th>Methods or Molecular Mechanism to study autophagy</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer</td>
<td>SGC7901 (Vincristine-resistant)</td>
<td>miR-23b-3p</td>
<td>siRNAs (Atg12, HMGB2), CQ</td>
<td>(An et al. 2015)</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Esophageal cancer cells (Drug-resistant)</td>
<td>5-FU</td>
<td>siRNAs (Beclin1, Atg7), 3-MA</td>
<td>(O’Donovan et al. 2011)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>MDA-MB-231 cells (Epirubicin-resistant)</td>
<td>Epirubicin</td>
<td>CQ</td>
<td>(Zhang et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells (Adriamycin-resistant)</td>
<td>Docetaxel</td>
<td>CQ</td>
<td>(Shi et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells (Adriamycin-resistant)</td>
<td>DOX</td>
<td>CQ</td>
<td>(Gao et al. 2017)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells MDA-MB-231 cells</td>
<td>Capsaicin</td>
<td>P38 and ERK</td>
<td>(Choi et al. 2010)</td>
</tr>
<tr>
<td></td>
<td>SKBR3 cells</td>
<td>Trastuzumab</td>
<td>↑autophagic flux</td>
<td>(Vazquez-Martin et al. 2009)</td>
</tr>
<tr>
<td></td>
<td>JIMT1 cells</td>
<td>Lapatinib, Gefitinib, Erlotinib, Trastuzumab</td>
<td>↑Atg12 transcripts</td>
<td>(Cufí et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells</td>
<td>Epirubicin</td>
<td>↑Bec1</td>
<td>(Sun et al. 2011a)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>Anthracycline</td>
<td>↑LAPTM4B</td>
<td>(Li et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells T47D cells MCF-7-HER2 cells</td>
<td>Tamoxifen</td>
<td>↑Atg5, Atg-7, and Bec1</td>
<td>(Qadir et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>Taxol group</td>
<td>mTOR pathway inhibition</td>
<td>(Notte et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells MDA-MB-436 cells</td>
<td>Carboplatin</td>
<td>↑ATG7 by HSF1</td>
<td>(Desai et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells</td>
<td>Bortezomib</td>
<td>autophagy induction through ATF4</td>
<td>(Milani et al. 2009)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>DOX</td>
<td>eEF-2K induction</td>
<td>(Tekedereli et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 CSCs</td>
<td>Ginsenoside F2</td>
<td>Atg7 elevation</td>
<td>(Mai et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>Paclitaxel</td>
<td>↑autophagy, ↑clearance of damaged mitochondria</td>
<td>(Wen et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>Radiation</td>
<td>TAK1 activation</td>
<td>(Han et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells</td>
<td>CSTS203</td>
<td>↑Bec1 expression</td>
<td>(Wang et al. 2014a)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PC3 cells (PTEN-deficient)</td>
<td>Ursolic acid</td>
<td>siRNAs (Atg5, Bec1), 3-MA</td>
<td>(Shin et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>PC3 cells Rv1 cells</td>
<td>BITC</td>
<td>mTOR signalling inhibition</td>
<td>(Lin et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>LNCaP cells, PC-3 cells</td>
<td>Sulphoraphane</td>
<td>Mitochondria-derived ROS</td>
<td>(Naponelli et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>PC-3 cells</td>
<td>Ursolic acid</td>
<td>Inhibition of Akt/mTOR pathway</td>
<td>(Shin et al. 2012)</td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Cell Line</td>
<td>Treatment</td>
<td>Pathway/Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------</td>
<td>----------------------------</td>
<td>------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>Ovarian Cancer</strong></td>
<td>PC-3 cells</td>
<td>Piperlongumine</td>
<td>inhibition of Akt/mTOR pathway through ROS</td>
<td>(Makhov et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer cells (Cisplatin-resistant)</td>
<td>FTY720</td>
<td>Baf A1, siRNAs (Bec1, LC3)</td>
<td>(Zhang et al. 2010a)</td>
</tr>
<tr>
<td></td>
<td>Ovarian carcinoma SKVCR (VCR-resistant)</td>
<td>Vincristine</td>
<td>CQ, 3-MA</td>
<td>(Liang et al. 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2780</td>
<td>↓AKT/mTOR pathway</td>
<td>(Brasseur et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>RMG-1 cells, OV90 cells, OV433 cells, OVCA420 cells, CAOV3 cells</td>
<td>Cisplatin</td>
<td>ERK pathway activation</td>
<td>(Wang and Wu 2014)</td>
</tr>
<tr>
<td><strong>Cervical Cancer</strong></td>
<td>HeLa cells</td>
<td>TAW</td>
<td>↑Bec1</td>
<td>(Zhang et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>Hela cells</td>
<td>Paclitaxel</td>
<td>ER stress-mediated</td>
<td>(Xu et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>Hela cells</td>
<td>Paclitaxel</td>
<td>Warburg effect-activated HIF1-α-mediated</td>
<td>(Peng et al. 2014)</td>
</tr>
<tr>
<td><strong>Glioblastoma</strong></td>
<td>glioma cell lines (PTEN-deficient)</td>
<td>PI-103</td>
<td>Baf A1, 3-MA, siRNA (Atg5)</td>
<td>(Fan et al. 2010)</td>
</tr>
<tr>
<td></td>
<td>U251MG cells U87MG cells</td>
<td>Gambogic acid</td>
<td>Atg5, ↑Bec1</td>
<td>(Luo et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>U251 cells</td>
<td>ZD6474</td>
<td>PI3K/Akt/mTOR signalling inhibition</td>
<td>(Shen et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>T98G cells U251 cells</td>
<td>Cucurbitacin I</td>
<td>AMPK activation, ↓PI3K/Akt pathway</td>
<td>(Yuan et al. 2014)</td>
</tr>
<tr>
<td></td>
<td>U87MG cells T98G cells U373 cells</td>
<td>Bevacizumab</td>
<td>↑(HIF-1α)/AMPK pathway</td>
<td>(Hu et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>C6 glioma cells</td>
<td>Caffeic acid phenethyl ester</td>
<td>AMPK and MAPKs pathway phosphorylation</td>
<td>(Yu et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>U251 cells</td>
<td>Cisplatin</td>
<td>↑autophagy through Chloride channel-3</td>
<td>(Su et al. 2013)</td>
</tr>
<tr>
<td><strong>Leukemia</strong></td>
<td>CML cells (Imatinib-resistant)</td>
<td>SAHA</td>
<td>CQ</td>
<td>(Carew et al. 2007)</td>
</tr>
<tr>
<td></td>
<td>K562 cells</td>
<td>Perifosine</td>
<td>↑Atg5</td>
<td>(Tong et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>K562 cells</td>
<td>Daunorubicin</td>
<td>ERK activation</td>
<td>(Han et al. 2011)</td>
</tr>
<tr>
<td></td>
<td>K562 cells, KU812 cells</td>
<td>Asparaginase</td>
<td>Akt/mTOR and ERK</td>
<td>(Song et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>Nalm-6 cells, KU812 cells</td>
<td>Bortezomib</td>
<td>Bcl-2/Bec1 complex disruption</td>
<td>(Wang et al. 2015b)</td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td>melanoma cells (B-Raf inhibitor-resistant)</td>
<td>B-raf inhibitors</td>
<td>HCQ</td>
<td>(Ma et al. 2014)</td>
</tr>
<tr>
<td><strong>Lung cancer</strong></td>
<td>A549/DDP cells (Cisplatin-resistant)</td>
<td>Cisplatin</td>
<td>3-MA</td>
<td>(Ren et al. 2010)</td>
</tr>
<tr>
<td></td>
<td>HCC827 cells, HCC4006 cells</td>
<td>Gefitinib</td>
<td>↑autophagic flux</td>
<td>(Sakuma et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>H23 cells, H1975 cells</td>
<td>Erlotinib</td>
<td>LC3A activation</td>
<td>(Nihira et al. 2014)</td>
</tr>
<tr>
<td>Cell Line</td>
<td>Treatment</td>
<td>Effect</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>--------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>Pterostilbene</td>
<td>ERK activation and AKT/mTOR pathways inhibition</td>
<td>(Hsieh et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>H3122 cells</td>
<td>Crizotinib</td>
<td>AKT/mTOR pathways alteration</td>
<td>(Ji et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>Paclitaxel</td>
<td>↓miR-17-5p</td>
<td>(Chatterjee et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>Green tea extract</td>
<td>↑autophagic flux</td>
<td>(Izdebska et al. 2015)</td>
<td></td>
</tr>
<tr>
<td>95D cells</td>
<td>Cucurbitacin E</td>
<td>AKT/mTOR pathway regulation through ROS</td>
<td>(Ma et al. 2016)</td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>Radiation</td>
<td>↓ROS under hypoxia</td>
<td>(Chen et al. 2017)</td>
<td></td>
</tr>
<tr>
<td>A549 cells</td>
<td>Cisplatin</td>
<td>Hypoxia induction</td>
<td>(Wu et al. 2015; Lee et al. 2015)</td>
<td></td>
</tr>
</tbody>
</table>

**Colorectal cancer**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Treatment</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLD-1 cells, HT-29 cells, Colon26 cells</td>
<td>Adiponectin</td>
<td>AMPKα and PPARα activation and inhibition of IGF-1/PI3K/Akt/mTOR pathway inhibition through Glucose deprivation</td>
<td>(Habeeb et al. 2011)</td>
</tr>
<tr>
<td>HCT116 cells, SW620 cells, C26 cells</td>
<td>Gambogic acid</td>
<td>inhibition of Akt-mTOR signalling by ROS</td>
<td>(Zhang et al. 2014a)</td>
</tr>
<tr>
<td>SW480 cells</td>
<td>NVP-BEZ235</td>
<td>PI3K/mTOR signalling inhibition</td>
<td>(Yang et al. 2016)</td>
</tr>
<tr>
<td>HT-29 cells, RKO cells, Caco2 cells</td>
<td>PLX4032</td>
<td>AMPK activation</td>
<td>(Sueda et al. 2016)</td>
</tr>
<tr>
<td>HT-29 cells</td>
<td>Huh7, HCT116</td>
<td>AMPK/Ulk1 signaling activation</td>
<td>(Min et al. 2014)</td>
</tr>
<tr>
<td>RKO cells, HCT116 cells, SW480 cells</td>
<td>Aspirin</td>
<td>AMPK activation and mTOR inhibition independently</td>
<td>(Din et al. 2012)</td>
</tr>
<tr>
<td>Huh7 cells, HCT116 cells</td>
<td>Atorvastatin</td>
<td>activation of p21 and ER stress via AMPK</td>
<td>(Makhov et al. 2014)</td>
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</tbody>
</table>

**Hepatocellular carcinoma**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Treatment</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHCC97-L cells, PLC/PRF/5 cells, HepG2 cells</td>
<td>Sorafenib</td>
<td>↑IRE1 pathway through ER stress signalling</td>
<td>(Shi et al. 2011)</td>
</tr>
<tr>
<td>Huh7 cells, SMMC-7721 cells</td>
<td>Oxaliplatin</td>
<td>ROS modulation</td>
<td>(Ding et al. 2011)</td>
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<tr>
<td>HepG2 cells</td>
<td>Capsaicin</td>
<td>ROS-STAT3-mediated</td>
<td>(Chen et al. 2016)</td>
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</table>

**Pancreatic cancer**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Treatment</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANC-1 cells, PaCa3 cells</td>
<td>Gemcitabine</td>
<td>mutant p53 nuclear stabilization</td>
<td>(Fiorini et al. 2015)</td>
</tr>
<tr>
<td>PANC-1 cells</td>
<td>Bortezomib</td>
<td>AMPK-Ulk1 signalling activation</td>
<td>(Min et al. 2014)</td>
</tr>
<tr>
<td>PANC-1 CSCs</td>
<td>Gemcitabine</td>
<td>OPN/NF-κB signalling induction</td>
<td>(Yang et al. 2015)</td>
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</tbody>
</table>

**Head and neck cancer**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Treatment</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE19 cells, OE21 cells, OE33 cells, KYSE450 cells</td>
<td>Cisplatin, 5-Fu</td>
<td>Bec1, Atg7-mediated</td>
<td>(O’Donovan et al. 2011)</td>
</tr>
</tbody>
</table>
### b. The pro-death role of autophagy in MDR of cancer

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Cell line</th>
<th>Intervention/Drug</th>
<th>Methods or Molecular Mechanism to study autophagy</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>MCF-7 cells (Tamoxifen-resistant)</td>
<td>SAHA</td>
<td>3-MA</td>
<td>(Lee et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>MCF-7 cells (Adriamycin-resistant)</td>
<td>Isoliquiritigenin</td>
<td>CQ, 3-MA</td>
<td>(Wang et al. 2014b)</td>
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<tr>
<td></td>
<td>MCF-7 cell lines (Apoptosis-resistant)</td>
<td>Hernandezin</td>
<td>3-MA, Atg7-knockout</td>
<td>(Law et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>MDA-MB-231 cells</td>
<td>LYN-1604</td>
<td>cell death modulated by ULK1</td>
<td>(Zhang et al. 2017a)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>SW620 cells (Apoptosis-resistant)</td>
<td>Tanshinones</td>
<td>3-MA</td>
<td>(Hu et al. 2015)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>leukemic K562 cells (Edelfosine-resistant)</td>
<td>Edelfosine lipid NPs</td>
<td>Starvation, Staurosporine</td>
<td>(Aznar et al. 2014)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>lung cancer cells (Multidrug-resistant)</td>
<td>GMI protein</td>
<td>CQ</td>
<td>(Chiu et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>A549 cell lines (Apoptosis-resistant)</td>
<td>Hernandezin</td>
<td>3-MA, Atg7-knockout</td>
<td>(Law et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>H460 cells (Cisplatin-resistant)</td>
<td>Cisplatin</td>
<td>Trifluoperazine, 3-MA</td>
<td>(Sirichanchuen et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>H460 cells (Apoptotic deficient)</td>
<td>RAD001</td>
<td>3-MA, siRNAs (Atg5, Bec1)</td>
<td>(Kim et al. 2008)</td>
</tr>
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<td></td>
<td>H1299 cell lines (Apoptosis-resistant)</td>
<td>Hernandezin</td>
<td>Atg7-knockout, 3-MA</td>
<td>(Law et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>A549 cells, H1299 cells, H292 cells, H460 cells, HCC827 cells BEAS-2B</td>
<td>SBI0206965</td>
<td>Ulk1 inhibition</td>
<td>(Tang et al. 2017)</td>
</tr>
<tr>
<td></td>
<td>NCI-H1299 cells, NCI-H460 cells</td>
<td>PS VII</td>
<td>autophagy blocking by 3-MA via AMPK-ULK1</td>
<td>(Qian et al. 2020)</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>urothelial cancer cells (Cisplatin-resistant)</td>
<td>NVP-BEZ235</td>
<td>3-MA</td>
<td>(Li et al. 2013a)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>SKVCR cells (Multidrug-resistant)</td>
<td>p53 plasmids</td>
<td>3-MA</td>
<td>(Kong et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>ovarian cancer cells (Chemoresistant)</td>
<td>Quinacrine</td>
<td>Baf A1</td>
<td>(Khurana et al. 2015)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Hela cells (Apoptosis-resistant)</td>
<td>Hernandezin</td>
<td>Atg7-knockout, 3-MA</td>
<td>(Law et al. 2016)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PC3 cells (Apoptosis-resistant)</td>
<td>Hernandezin</td>
<td>Atg7-knockout, 3-MA</td>
<td>(Law et al. 2016)</td>
</tr>
<tr>
<td>(Hepatocellular carcinoma) HepG2 cells (Apoptosis-resistant)</td>
<td>Hernandizine</td>
<td>Atg7-knockout, 3-MA</td>
<td>(Law et al. 2016)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(Hep3B cells (Apoptosis-resistant))</td>
<td>Hernandizine</td>
<td>Atg7-knockout, 3-MA</td>
<td>(Law et al. 2016)</td>
<td></td>
</tr>
<tr>
<td>(Gastric cancer) SGC7901 (Drug-resistant)</td>
<td>HTCC-MNPs</td>
<td>3-MA</td>
<td>(Li et al. 2016)</td>
<td></td>
</tr>
</tbody>
</table>

Legends

Figure 1. Mechanisms involved in autophagy. Stressors such as starvation and infection can induce autophagy. Upon nutrient deficiency in the environment, autophagy is usually triggered in *S. cerevisiae* to provide the prerequisite amino acids and macromolecules for the survival of the microorganism. A nutrient sensor, TOR protein kinase is the primary molecule responsible for the regulation of autophagy inside the cell, which has been mostly investigated in fungal cells, particularly yeasts (Gozuacik and Kimchi 2004). When nutrients are amply available, TOR protein kinase prompts phosphorylation of Apg13, the affinity of which for Apg1 significantly decreases upon hyperphosphorylation, resulting in reduced Apg1 kinase activity (Kamada et al. 2000; Scott et al. 2000) and concomitant activation of the Cvt pathway. Administration of rapamycin or inaccessibility to nutrients, in contrast, lead to inhibition of TOR protein kinase, and subsequent dephosphorylation of Apg13. The resulting increase in the activity of Apg1, thus, promotes autophagy (Gozuacik and Kimchi 2004). In this circumstance, autophagy is mediated through macroautophagy and mitophagy (Popp and Segatori 2015). In the former, the PI3K/Akt and MAPK/ERK1/2 pathways become initiated, and are then succeeded by inhibition of mTOR and activation of beclin-1/Atg6. Mitophagy, however, is triggered following mitochondrial damage and the activity of ubiquitin-like conjugation systems. Further down the cascade, an interaction between p62 and LC3-II results in cargo delivery to autophagic membranes or phagophores. Fusion of the phagophores surrounding the cargo material results in formation of autophagosomes, structures that subsequently bud into lysosomes to form autophagolysosomes or degradation center. ROS can induce autophagy through several different mechanisms; nuclear PTEN, the PI3K pathway and LKB1/AMPK. Rapamycin acts as an mTOR inhibitor (Popp and Segatori 2015; Díaz-Troya et al. 2008). Rubicon, 3-MA and chloroquine function to inhibit autophagy (Zhang et al. 2014b; Rebecca and Amaravadi 2016). Activation of p53 leads to up-regulation of DRAM and sestrin 1/2, that ultimately accelerates autophagy. p70S6 kinase (p70S6K) might be a good option for controlling of autophagy downstream to mTOR (Gozuacik and Kimchi 2004).

(Abbreviations; 3-MA: 3-Methyladenine, ALFY: Autophagy-linked FYVE Protein, AMPK: AMP-activated Protein Kinase, Atg5/6/7/10/12/13/16L1: Autophagy-related Gene5/6/7/10/12/13/16L1, Ambra: Activating Molecule in Beclin 1-regulated Autophagy, Beclin1: a Mammalian Homolog of Yeast Atg6 Encoded by the BECN1 Gene, Bnip3: BCL2 and Adenovirus E1B 19 kDa-interacting Protein 3, BNIP3L/NIX: BNIP3-like, CVT: Cytoplasm Vacuole Targeting, DRAM: Damage-regulated Autophagy Modulator, FIP200: Focal Adhesion Kinase Family Interacting Protein of 200 kDa, GBL: Gamma-Butyrolactone, HMGB1: High Mobility Group Box Protein 1, LKB1:}

**Figure 2.** Correlation of apoptosis and autophagy. Oxidative stress, pathogens and cell death signals can induce autophagy or apoptosis through either dependent or independent pathways. (modified from (Gozuacik and Kimchi 2004))

**Figure 3.** Autophagy in tumor cells. Oncogene products (blue) inhibit autophagy, whereas tumor suppressors (green) accelerate autophagy, except for cytoplasmic p53, however. Growth factor signaling through activation of the PI3K/Akt/mTOR axis leads to inhibition of autophagy. On the contrary, class III PI3K activates autophagy. Low levels of cellular energy with an increased AMP/ATP ratio activate the LKB1-AMPK-mTOR pathway, that ultimately results in up-regulation of autophagy. p53 is a complex regulatory factor in the process of autophagy, as nuclear p53 activated by genotoxic or oncogenic stress positively regulates autophagy by inhibiting mTOR in an activated AMPK- and TSC1/TSC2-dependent manner. In contrast, cytoplasmic p53 has a negative regulatory effect on autophagy. Autophagy can also be induced by the cell death-associated protein kinase (DAPK) and the death associated related protein kinase 1 (DRP1) 51. (Modified from (Choi 2012))


**Figure 4.** Mechanisms involved in nanomaterial-regulated activation of autophagy. Nanomaterials may induce autophagy through different mechanisms, including; enhanced formation of autophagosomes, induction of oxidative
stress, and instigation of lysosomal damage. Based on the nature of the nanomaterial, activation of autophagy might result in enhanced clearance or blockage of autophagic flux. Upon their release into the cytoplasm, nanomaterials may also impair the cytoskeleton integrity and function, leading to autophagosome dysfunction and blockage of autophagic flux. Else, nanomaterials can also enhance the formation and functioning of lysosomes and autophagosomes and induce autophagy.

Autophagy and Apoptosis connection

Cell death signals, Oxidative stress, Pathogens

Independent pathway

Autophagy

Apoptosis

Cell death type I

Cell death type II

Dependent pathway

Apoptosis induction

Apoptosis inhibition

Cell death type I

Cell survival

Figure 2