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Autophagy as new emerging cellular effect of nanomaterials

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The biosafety issue of nanoscale materials is getting more and more attention with their increasing manufacture and application. In the research of cellular effects and underlying mechanisms related to toxicity of nanomaterials, most emphasis were placed on processes such as apoptosis, metabolic inhibition and oxidative stress. Recent evidence suggests that autophagy is part of the biological effects by nanomaterials and various kinds of nanomaterials are capable of disturbing the autophagic process. This review will highlight the importance of autophagy as an emerging mechanism of nanomaterial toxicity and the implication in the therapy of autophagy-related diseases. We summarize current research status of interaction between nanomaterials and autophagic pathways. It is of note that nanomaterials can either induce or block autophagy, which result in similar phenotype but completely different biological consequence. It is therefore important to perform comprehensive analysis of the whole autophagic flux in the future research.

autophagy, nanomaterials, cytotoxicity, cellular effect

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Nanomaterials refer to materials with the size range between 1 and 100 nanometers in one or more dimensions [1]. Due to their superior physicochemical properties, such as ultra small size, increased ratio of surface area to volume, multiple options for modification and relatively good biocompatibilities, application of nanomaterials in research as well as in industry becomes more and more common. It is predicted that the total amount of nanomaterials' production will increase up to 58000 tons by 2020. Particularly, the utilization of nanomaterials in biomedical research as biosensors, drug-carriers, or imaging agents was extensively studied [2–4]. Large scaled preparation and application of nanomaterials increase their possibilities to be exposed to human beings or enter the environment [5–7]. Therefore the impact of nanomaterials on environment and human health has attracted high attention. Related studies were carried out on the biological effect of various kinds of nanomaterials both *in vitro* and *in vivo* [8]. To date, reported physiological changes of a cell or an organism that exposed to nanomaterials include: reactive oxygen species (ROS)-related pathological responses (such as damaged membrane, mitochondrial damage and necrosis), apoptosis, inflammation and altered differentiation patterns, etc [9–12].

Recently, autophagy as an essential cellular process and a novel biological effect of nanomaterials on eukaryotes, has received much attention. Many nanomaterials were reported to be able to change the basal level of autophagy [13,14]. Autophagy can crosstalk with other cellular process, such as apoptosis and necrosis, and therefore is an important part of the cellular effect of nanomaterials [15,16]. Moreover, nanomaterials-induced autophagy showed potential for the treatment of certain diseases including neurodegenerative diseases and cancer [13,17]. Hence, in this review we summarize the current research on the effect of various kinds of nanomaterials on autophagy. We divide the nanomaterials

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into several categories according to their chemical composition: (1) Carbon-based nanomaterials, (2) metal-based nanomaterials, (3) semiconductor-based nanoparticles, (4) rare earth containing nanomaterials.

1 Autophagy

The homeostasis of cells requires a balance between synthetic and catabolic processes. As one of the catabolic pathways, autophagy mainly regulated the intracellular degradation of long-lived or mis-folded proteins and damaged organelles [18–20]. In most cells, autophagy occurs constitutively at low level and plays an important role in the cellular metabolism, growth and development [21]. Autophagy can be induced by either physiological stress conditions or exogenous stimuli including chemicals and invading particles.

Recently, various kinds of nanomaterials were reported to be able to induce autophgay [17,22–25]. Based on the different mode of intracellular substrates entering lysosome, there are three forms of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. Authophagy induced by nanomaterials often refers to macroautophagy, which was firstly identified by De Duve in 1963 [26]. Macroautophagic process occurs in three stages: (1) The formation of double-membrane-structured vesicles (autophagosome) that containing damaged proteins and organells. At this stage, microtubule-associated protein light chain 3 (LC3) convert from a cytoplasmic form (LC3-I) to a phosphatidylethanolamine (PE)-conjugated form (LC3-II) and localize onto the membrane of autophagosomes. This process is often triggered by drugs (for example, rapamycin), physiological stress (for example, starvation), or invading particles. These double-membrane-structured autophagosomes can be monitored via TEM. Alternatively, the increase of punctuated LC3-II is another sign of autophagosome accumulation. (2) Autophagosomes transfer to and fuse with lysosome to form autolysosome. (3) The wrapped contents are degraded in the autolysosome and the products (for example, amino acid) are released into the cytoplasm and recycled.

To understand the relationship between nanomaterials and autophagy, we need to monitor the autophagic activity accurately. There are some convenient methods to measure the number of autophagosomes including electron microscopy (EM) image analysis, fluorescent GFP-LC3 dots, or LC3 lipidation on a western blot. These methods are widely applied in current research of nanomaterial-induced autophagy. However, it is important to point out that autophagy is a dynamic, multi-step process that can be modulated at several steps. Accumulation of autophagosomes can reflect either an increase in autophagic activity or a blockage in the turnover of autophagosomes. Therefore, in addtion to above discribed “static measurements”, some “autophagic flux” assays are developed to distinguish these two possibilities, including LC3 turnover assay, GFP-LC3 cleavage assay, and degradation of long-lived proteins.

Autophagy is a tightly regulated process and increasing evidences demonstrate that autophagy may be involved in the progress of many diseases, including cancer, neurodegeneration, liver disease, muscular disorder and pathogen infection [27]. The relationship between autophagy and disease is often complicated. Autophagy can help cells to survive some stress conditions, such as nutrient and oxygen depletion [28,29]; however, autophagy can also promote autophagic cell death after chemotherapies. This is called “double-edged sword” effect of autophagy [27,30]. Therefore, it is crucial to take autophagy into consider in the study of biological effects of nanomaterials, since they may cause uncovered damage to organism through interfering with autophagic process [31]. On the other hand, it also implies that nanomaterials have the potential to be utilized in combination with chemotherapies for the treatment of diseases.

2 Carbon-based nanomaterials

Many forms of carbon-based nanomatreials were manufactured, including fullerenes C_{60}, carbon nanotubes (CNTs), and graphene sheet, carbon dots, nanodiamonds, nano-onions and so on. Out of them, the former three types of nanomaterials are most well developed and widely used because of their unique properties in thermal stability, conductivity, mechanical and optical performance. Application of carbon-based nanomatreials is also a hot spot in biomedicine research [32–35]. There are a large number of reports about the cellular effect of carbon-based nanomatreials. Recently, several studies indicate that carbon-based nanomatreials can induce autophagy in different cell types (Table 1).

2.1 C_{60} and C_{60} derivatives

As early as in 2006, Yamawaki and Iwai [36] has found that the treatment of human umbilical vein endothelial cells (HUVECs) with 100 μg/mL $C_{60}(OH)_{24}$ for 24 h caused extensive internalization of fullerene and vacuolization. They observed aggregated fullerene in autophagosome via TEM. Protein immunoblot analysis of marker protein LC3 verified the occurrence of autophagy. The authors proposed that autophagy could be responsible at least partially for the cell death and injury caused by water-soluble fullerene, which represent a risk for atherosclerosis and ischemic heart disease. Similarly, Stephan et al. [40] showed that cell death upon exposure to $C_{60}(OH)_{24}$ was related with cytoskeleton disruption, loss of mitochondrial capacity and autophagic vacuole accumulation. Indeed, ATP depletion and loss of mitochondrial potential were partially ameliorated when

Table 1 Autophagy induced by carbon-based nanomaterials

Nanomaterials	Average size (nm)	Cell lines	Autophagy marker	Reference
$C_{60}(OH)_{24}$	7.1±2.4	HUVECs	TEM, LC3	[36]
Nano-C_{60}	100	C6 cells	Acidophilic vesicles	[37]
Nano-C_{60}	20–100	GFP-LC3/HeLa cells; Primary MEF cell	Acidic vesicles, LC3, Atg5	[38]
C_{60}(Nd) ,C_{60}	50–100	HeLa cells; MCF-7; MEF cell	LC3, Atg5	[39]
$C_{60}OH_x$	20	LLC-PK1 cells	TEM, LC3	[40]
C_{60}-derived NPs	~78.9	Neuro-2A cells	LC3	[41]
SWNTs	~1	A549	TEM, LC3, Akt–TSC2-mTOR signaling	[42]
Graphene quantum dots	56.6±8.7	U251 cells	TEM, LC3, p62	[43]
Graphene oxide	2.4 μm and 350 nm	RAW264.7 cells	TEM, LC3	[44]

cells were co-treated with $C_{60}(OH)_{24}$ and an autophagy inhibitor (3-methyladenine).

On one hand, fullerene-induced autophagy can cause cell death or functional damage, leading to the development of certain diseases [36,40]. On the other hand, fullerene-triggered autophagy was found to play potential role in the treatment of diseases such as cancer and neurodegeneration. For example, Harhaji et al. [37] showed that the anti-glioma effect of low-dose nC_{60} (0.25 μg/mL) was attenuated by pharmacological inhibition of autophagy. Hence, the authors proposed that autophagy might be involved in the anti-proliferative funtion of nC_{60}. Zhang et al. [38] systematically studied the effect of water-dispersed nanocrystal C_{60} and its derivative C_{60}(Nd) nanoparticles on autophagy. Their results indicated that nC_{60} could induce autophagy and sensitize chemotherapeutic killing of both normal and drug-resistant cancer cells at noncytotoxic concentrations (0.5 μg/mL). Nano-C_{60}-induced autophagy was reactive oxygen species (ROS)-dependent and enhanced by photo activation. The chemo-sensitization effect of nano-C_{60} was autophagy-mediated and required functional Atg5, a key player in the autophagic signaling pathway. Compared to nC_{60}, nC_{60}(Nd) is more effective to induce autophagy and to sensitize chemotherapy [39]. These studies imply a novel application of nC_{60}/C_{60}(Nd) nanoparticle in cancer therapy.

Lee et al. [41] reported that fullerene-derived nanomaterials (PTX-C_{60}-2 and PEG-C_{60}-3) could elicit cytoprotective effect partially through autophagy to eliminate the accumulation of β-amyloid (Aβ) 25-35 in Neuro-2A cells. PTX-C_{60}-2 was more effective to induce autophagy compared to PEG-C_{60}-3. It is well known that aggregated β-amyloid is an important contributing factor in neuronal dysfunction and cellular apoptosis. Therefore the above study suggests PTX-C_{60}-2 has a potential in the treatment of neurodegenerative diseases.

2.2 Carbon nanotubes

Carbon nanotubes (CNTs) are mainly consisted of multi-walled carbon nanobutes (MWCNTs) and single-walled carbon nanotubes (SWCNTs). It is commonly accepted that CNTs are toxic to cells and can lead to oxidative stress, inflammation, apoptosis and even necrosis [45–48]. Recently, autophagy was added to the list of CNTs-related cytotoxicity. Liu et al. [42] designed three types of functional single-walled carbon nanotubes (f-SWCNTs), including COOH-CNT, poly-aminobenzene sulfonic acid (PABS-CNT), PEG-CNT and studied the autophagic response and related pathway both *in vitro* and *in vivo*. They demonstrated that only COOH-CNT could trigger formation of autophagosomes and LC3-II up-regulation in A549 cells, indicating that functionalized surface can greatly affect the ability of nanomaterials to induce autophagy. Moreover, they found that COOH-CNT induce autophagic cell death in A549 cells through the AKT-TSC2-mTOR pathway and caused acute lung injury *in vivo*.

2.3 Graphene

Graphene and its oxidized forms (graphene oxide and reduced graphene oxide) refer to a single layer of carbon with a honeycomb structure and have sparked growing interest since 2004. Graphene has been applied in fields such as electrical, mechanical and biomedicine owing to its excellent physicochemical properties and well biocompatibility [49–51]. Therefore, more and more studies started focusing on the biosafety and cytotoxicity of graphene. Recently, Chen et al. [44] demonstrated that graphene oxide (GO) could induce autophagy in macrophage RAW264.7 cells and the effect was concentration-dependent. They observed the appearance of autophagic vacuoles and activation of LC3-II. Additionally, they uncovered that GO can simultaneously induce autophagy and TLR4/TLR9-regulated inflammatory, and the autophagic response was at least partly mediated by the TLR pathways.

Graphene quantum dots (GQD) possess both the unique physicochemical properties of graphene and the optical properties of quantum dots [52,53]. Markovic et al. [43] found that GQD irradiated with blue light (470 nm, 1 W) cuased oxidative stress and and kill U251 human glioma cells. Their results showed that the cell death triggered by photoexcited GQD was associated with both apoptosis and

autophagy. In addition to the accumulation autophagosomes, they further confirmed the up-regulation of autophagic flux by both the increase of LC3B-II and the markedly down-expression of p62, a protein selectively degraded by autophagy [54]. These data suggested potential usefulness of GQD in photodynamic therapy, but also increase more concerns about their possible toxicity.

3 Metal-based nanomaterials

Metal and their oxides have a long history to be applied in basically every aspects of life. With the development of nanotechnology, metal-based nanomaterials have been largely developed and manufactured, and are getting more and more commonly used in bioimaging, drug delivery, biosensors, and photo/thermal therapy. Undesired side effects of these metal-based nanoparticles are always hot topic in nano-related research. Certain metal-based nanomaterials like silver and copper oxide nanopartiels are known to be highly toxic due to oxidative damages. Oxidative stress is a common cause to autophagy [55,56]. Recently, several studies found that metal and metal oxide nanoparticles could cause autophagy *in vitro* and *in vivo* (Table 2).

3.1 Gold nanomaterials

Gold nanoparticles (AuNPs) are well known to be nearly non-cytotoxic and widely used as nanocarriers for many bioactive molecules [65,66]. Li et al. [57] found that gold nanoparticles (AuNPs) induce autophagy in human lung fibroblasts MRC-5 cells. They have observed the formation of autophagosomes, as well as upregulation of autophagy-related proteins (LC3, Atg7). Meanwhile, they found that AuNPs treatment could generate significant oxidative damage, as indicated by lipid hydroperoxides assay, expression levels of stress-response genes and proteins and antioxidants rescue. They hypothesized that AuNPs caused oxidative stress in cells, and autophagy may play an important role in the cellular defense against oxidative toxicity.

In another study, Ma et al. [58] proposed that AuNP actually inhibited the autophagy process *via* block the fusion between autophagosome and lysosome. They have also observed the the accumulation of autophagosomes and the processing of the marker protein LC3-II when cells were incubated with AuNPs. However, they further demonstrated that the substrate protein p62 is also upregulated, suggesting the blockage of autophagy flux rather than induction of autophagy. Accordingly, they found that internalized AuNPs eventually entered lysosomes, caused alkalinization of lysosome pH, and impaired lysosome degradation activity. This finding is important since it point out that nanoparticles can either promote or inhibit the process of autophagy, both resulted in the similar accumulation of autophagosomes and upregulation of LC3-II. Therefore it is important to analyze the autophagy process as a dynamic flux in addition to the classic status assays.

3.2 Iron-related nanomaterials

Wu et al. [59] examined the cytotoxicity of nanoparticles with an iron core and gold shell (Fe@AuO), and found Fe@AuO could cause irreversible loss of mitochondrial membrane-potential (MMP) in cancer cells, but only a transient MMP decrease in healthy cells. The MMP loss was correlated with up-regulation of mitochondria-mediated autophagy (Mitophagy). They also demonstrated that autophagy was the main reason of cytotoxicity. Similarly, Khan et al. [67] found that iron oxide nanoparticles selectively elicited autophagic cell death in cancer cells (A549) but not in normal cells (IMR-90). In this case, autophagy was related with ROS production as well as mitochondrial damage. Their further study suggested that iron oxide-triggered autophagy is partially depended on the classical mTOR pathway. These results imply that Fe@AuO and iron oxide nanoparticles have the potential as therapeutic reagent to specifically induce autophagic cell death in tumor cells.

Table 2 Autophagy induced by metal-based nanomaterials

Nanomaterials	Average size (nm)	Cell lines	Autophagy marker	Reference
AuNPs	20	MRC-5 cells	TEM, LC3, Atg5, Atg 7, Atg 12	[57]
AuNPs	10, 25 and 50	NRK cells	TEM, LC3, p62	[58]
Fe@Au NPs	10	hNOK cells; OECM1 cells	TEM, LC3	[59]
Iron oxide NPs	51.34±14.71	A549 cells; IMR-90 cells	LC3, mTOR	[31]
Superparamagnetic iron oxide	8	HCECs cells	TEM, LC3	[60]
TiO_2 NPs	<25	H4-LC3-GFP cells	LC3	[61]
TiO_2 NPs	21	HCECs cells	TEM, LC3	[60]
α-Al_2O_3 NPs	60; 200	Dendritic cells (DCs)	TEM, LC3, Atg5-Atg12	[62]
Nanoalumina	8–12	HCMECs/D3 cells; C57BL/6 mice	LC3, p62	[63]
MnNPs	25	N27 dopaminergic neuronal cells	TEM, LC3	[64]
AgNW	3 μm (length)	THP-1 cells	TEM, LC3	[24]

3.3 Alumina and its oxide nanoparticles

It has been reported that aluminum can cause neurodegenerative diseases, which are resulted from the presence of oxidative stress, inflammatory and the breakdown of the blood-brain barrier (BBB) [63,68,69]. Chen et al. [63] studied the toxicity and related mechanism of nanoalumina at both cellular and animal levels. They found that nanoalumina could pass through BBB, accumulate in the mice brain and locate into mitochondria in brain cells. Nanoalumina-induced dysfunction of mitochondria elicited autophagy, decreased tight-junction protein expression, and elevated BBB permeability. Therefore, autophagy may be a primary mechanism involved in nanoalumina-induced neurovascular toxicity in the central nervous system.

In another study, Li et al. [62] showed that α-Al_2O_3 nanoparticles at two sizes (60 and 200 nm) could both induce autophagy effectively and exhibited potent antitumor capability. α-Al_2O_3 nanoparticles function as an antigen carrier to T cells through the autophagy pathway, which decreased the needed amount of antigen to stimulate production of enough T-cells. Administration of α-Al_2O_3 NPs incubated with the autophagic vacuoles collected from the tumor cells greatly enhanced the effect of cancer therapy.

3.4 Others metal-based nanomaterials

Titanium dioxide (TiO_2) is widely applied as a supplement in cosmetics, coating and paint due to its high refractive index and bold white coloration [61]. Therefore, the cytotoxicity of titanium dioxide attracted much attention. In a parallel study including different metal oxide NPs like ZnO, FeO and TiO_2, Yu et al. [61] found that only TiO_2 can effectively induce autophagy, as measured by the activation of LC3 and the increase of autophagic flux. Similarly, Blanka et al. [60] demonstrated that TiO_2-induced autophagy was mainly related with NPs aggregation and oxidative stress. They also found that nanomaterials-mediated autophagy was size-dependent.

In addition, it has been reported that silver nanowire (AgNW), manganese nanoparticles (MnNPs) could effectively elicit autophagy in epithelial, endothelial, gastric, phagocytic cells [24] and dopaminergic neuronal cells [64], respectively.

4 Rare earth oxides nanomaterials

Rare earth elements refer to a set of seventeen chemical elements including fifteen lanthanides plus scandium and yttrium in the periodic table. Rare earth oxides naturally exist in the earth crust. Owing to their unique chemical and physical properties, rare earth oxides materials are widely applied in metallurgy, ceramics, magnets laser industries and biological field [70]. With the growing application, the biosafety issue of rare earth oxides materials has caused great concerns, especially in the field of nanoscale research.

Wen group [71–74] in University of Science and Technology of China made a series of excellent study on the toxicity of rare earth oxides nanomaterials, mainly focused on their impact on cellular autophagy (Table 3). They found that micromolar equivalent concentration of nano-sized neodymium oxide (Nano Nd_2O_3) could induce autophagy and cell death in non-small cell lung cancer NCI-H460 cells. They have observed the massive vacuolization, S-phase cell cycle arrest, loss of mitochondrial membrane potential (MMP) and inhibition of proteasome activity, suggesting that cell death was resulted from autophagy [71]. Subsequently, they demonstrated that all of four different kinds of rare earth oxides nanomaterials, including samarium oxide (Nano Sm_2O_3), europium oxide (Nano Eu_2O_3), gadolinium oxide (Nano Gd_2O_3) and terbium oxide (Nano Tb_2O_3), could induce accumulation of GFP-LC3 punctate in Hela cells stable expressing GFP-LC3 funsion proteins. And the autophagic response was dose- and time-dependent [72]. In another study, they investigated three rare earth oxides nanomaterials, Yttrium oxide (Y_2O_3), Ytterbium (Yb_2O_3) and Lanthanum (La_2O_3). Their results demonstrated that all of

Table 3 Autophagy induced by rare earth oxides nanomaterials

Nanomaterials	Average size (nm)	Cell lines	Autophagy marker	Reference
Nano Nd_2O_3	80	Human NCI-H460 cell line	Vacuoles	[71]
Nano Eu_2O_3	~50	GFP-LC3/HeLa cells	TEM, LC3	[72]
Nano Sm_2O_3	~20	GFP-LC3/HeLa cells	TEM, LC3	[72]
Nano Gd_2O_3	~100	GFP-LC3/HeLa cells	TEM, LC3	[72]
Nano Tb_2O_3	~80	GFP-LC3/HeLa cells	TEM, LC3	[72]
Y_2O_3	<50	GFP-LC3/HeLa cells $Atg5^{-/-}$ MEF cells Normal MEF cells	Vacuoles, LC3, p62, Atg5	[73]
Yb_2O_3	<50	GFP-LC3/HeLa cells $Atg5^{-/-}$ MEF cells Normal MEF cells	Vacuoles, LC3, p62, Atg5	[73]
LN-based nanocrystals	~20	GFP-LC3/HeLa cells	TEM, LC3	[74]
CeO_2 NPs	10–30	$CD14^+$ cells	TEM, LC3B	[15]

them could trigger autophagy but only Y_2O_3 and Yb_2O_3 cause vacuolization, suggesting that commonly observed vacuolization differs from autophagic pathway [73]. More recently, Zhang et al. [74] achieved to control the ability of Ln_2O_3 nanocrystals to induce autophagy via surface-coating peptides. In a screening assay they identified a short peptide RE-1 that could reduce Ln_2O_3 induced autophagy. Furthermore, adding an RGD motif to RE-1 could effectively target the nanocrystals to tumors and increase autophagic cell death *in vivo*. This work discovered a powerful tool to regulate the nanomaterial-cell interactions and to achieve the desired level of autophagy, which may promote the application of rare earth oxides nanomaterials in cancer diagnose and therapy.

Besides, Hussain et al. [75] reported cerium dioxide (CeO_2) nanoparticles could simultaneously induce apoposis and autophagy, resulting in cell death of human monocytes. The autophagy effect induced by CeO_2 is time- and dose-dependent, and modulated by p53. However, the detailed mechanism is unclear.

5 Semiconductor quantum dots

Quantum dots (QDs) are semiconductor nanocrystals with a typical diameter ranging from 2 to 10 nm. QDs possess superior optical properties, such as high photoluminescence quantum yield (PLQY), broad absorption coupled with narrow emission, and strong photostability [76]. Consequently, several kinds of QDs have been fabricated and utilized for applications including biosensing, bioimaging and disease diagnosis [77]. However, Most QDs are made of heavy metal ions, which may result in potential toxicity that hampers their practical applications. And some heavy metal ions can induce autophagic cell death. Therefore, in the study of QDs-related cellular effects, it is necessary to inspect the change of autophagy levels [78].

Seleverstov et al. [79] studied the process of internalization and compared the cytotoxicity of two different-sized QDs (605 and 525 nm) in the human mesenchymal stem cells (hMSC). In the cells treated with QDs of smaller size (QD525), they observed autophagic cell death and the massive formation of autophagosomes containing damaged organelles and aggregated QDs after 72 h. In contrast, little autophagic vacuoles or damaged mitochondria were found in hMSC cells labeled with QDs of bigger size (QD605). These results for the first time suggested that the ability of nanoparticles to induce autophagy is size-dependent.

In another study, Stern et al. [78] compared the cytotoxicity on LLC-PK1 porcine kidney cells of two QDs species, which have similar size but are made of different core materials (CdSe and InGaP). The core size of CdSe and InGaP was 5.1 and 3.7 nm, respectively. Both QDs can induce autophagy as demonstrated by LC3 immunobloting, TEM pictures and Lysotracker staining, suggesting that autophagy is not caused directly by certain metal element in the composition. Besides, they also found that CdSe QD elicited stronger autophagic response than InGaP QD at equal molar concentrations, which is positively correlated to their cytotoxicity [78].

6 Summary and outlook

Autophagy was originally defined as type II programmed cell death. With the detailed study, it is more and more clear that autophagy plays fundamental roles in multiple cellular processes and is closely associated with cancer and neurodegenerative disease. Particularly, rapamycine, a classic drug in the treatment of cancer and metabolic disease, is the central regulator of autophagy. Therefore, manipulation of autophagy levels with nanomaterials provides novel potential opportunities for cancer therapy.

Lately, a variety of nanomaterials have been reported as autophagy inducers. However, in many studies, the accumulation of autophagosome protein LC3II was commonly used as a marker of up-regulated autophagy. However, the process of autophagy is a dynamic flux (Figure 1). It is essential to carefully examine and distinguish between the "upstream induction" of autophagosome formation and the

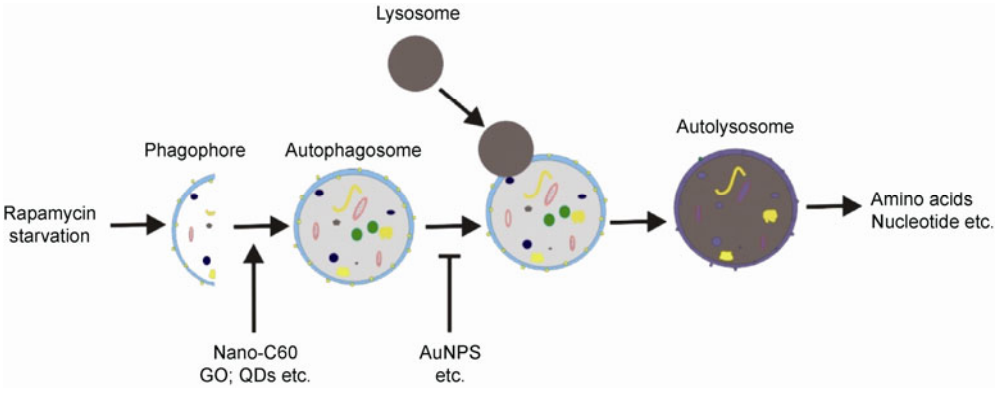

Figure 1 Nanomaterials interfere with the process of autophagy.

"downstream blockage" of fusion between autophagosome and lysosome, which result in similar phenotype but completely different biological consequence. Gold nanoparticles, for example, were firstly reported to induce autophagy, which turned out to be inhibitor of the acidification of lysosome and turnover of autophagosomes by "autophagic flux" assays. Such incompleteness also exist in current study of other nanomaterials and autophagic acitivity. Therefore it is essential to perform multiple assays to verify an autophagic response. In future studies, we suggest performing studies to better understand detailed interactions between nanomaterials and autophagic pathways and to uncover the underlying mechanism.

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