Recent Advances in Molecular Mechanisms of Autophagosome-Lysosome Fusion and Impacts of Exogenous Factors

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Abstract

Autophagy is a highly conserved phenomenon of cell biology, and it is closely related to many major human diseases such as cancer, neurodegenerative diseases, and diabetes. Autophagy is a continuous biological process, including the formation of autophagosomes, the fusion of autophagosomes and lysosomes, the degradation of substrates, and the regeneration of lysosomes. Relative to the stage of autophagosome formation, there are fewer studies on some of the late processes such as the autophagosome-lysosome fusion phase, although some recent advances have been made. Nanoparticles such as silicon and polymeric nanoparticles are being increasingly applied in the electronic and pharmaceutical industries. Recent studies have revealed their role in autophagy and their cytotoxicity. In this review we summarize the current main findings of the molecular mechanisms of the autophagosome-lysosome fusion process and discuss several types of exogenous factors and their impacts on the fusion including protein products of pathogens and nanoparticles. In addition, we indicate remaining questions that need to be addressed in future studies separately in each section.

Keywords: Autophagy; Fusion; Nanoparticles.

Received: 17 April 2020; Accepted: 23 September 2020.

Article type: Review article.

1. Introduction

Cells undergo autophagy to achieve orderly degradation of components that are no longer needed or morphologically abnormal or dysfunctional. Small molecules produced during degradation can be recycled by the cells. Upon autophagy, cytoplasmic content is being surrounded by a bilayer membrane which forms a structure called an autophagosome from which the content is delivered to a lysosome for degradation. In addition, when stress conditions such as external nutrient supply are restricted, cells also produce small molecules by autophagy for synthesis or catabolism to maintain their own survival. In recent years, studies have also

found that cells may resist foreign pathogens through autophagy, termed xenophagy,^[1] so autophagy is also considered to be an important part of natural immunity against exogenous pathogens. A series of structurally and functionally conserved proteins, which are encoded by autophagy-related genes (ATGs) in yeast and their homologs in mammalian cells have been identified.^[2] According to the central events that happen during autophagy, these proteins can be generally divided into the following separate complexes: firstly, the ATG1 complex is responsible for autophagy initiation and directly responds to its upper regulators like mammalian rapamycin complex 1 (mTORC1) through its phosphorylation activity which in turn is controlled by several other upstream intracellular signals such as amino acid deprivation, DNA damage, and low energy or oxygen situations.^[3,4] Second, the Vps34/PI3K complex controls the biogenesis of the phagophore, which starts at a specific area of the cellular membrane called phagophore assembly site (PAS);^[5,6] then, the ATG9 shuttling system and the ATG12 and ATG8 conjugating/deconjugating systems work together to expand the phagophore and ultimately produce an enclosed lumen called autophagosome.^[7] The steps following those mentioned

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above are relatively less understood, such as maturation of autophagosome and its final fusion with endosomes or lysosomes. The whole procedure of autophagy is shown step by step in Fig. 1. Membrane fusion is generally controlled by Rabs, SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors), and tethering complexes. The cytoskeleton is also involved in the fusion process, mainly in conjunction with intracellular trafficking by providing tracks and components for movements of vesicles and correct positioning for fusion.^[8-10] Known components involved in autophagosome and lysosome fusion (hereafter referred as A:L fusion) are listed in Table 1.

Many exogenous factors such as products from pathogens and nanoparticles have shown their impacts on A:L fusion.^[11-13] Prevalent application of nanoparticles nowadays, has arisen associated concerns about global public health and environmental issues. Nanoparticles such as Silica nanoparticles (SiNPs), silver nanoparticles (AgNPs), gold nanoparticles, rare earth oxides, and polystyrene particles, are found able to induce autophagy.^[14-18] However, the details about the

mechanism how these nanoparticles affect autophagy function are still relatively little understood, though one study has claimed that these substances inside the cell will induce autophagy and cause autophagy dysfunction by impairing lysosomal function and autophagic cargo degradation.^[14]

In this review, we summarize recent research about the core mechanisms of autophagosome and lysosome fusion with focus on the several vital proteins and molecules involved in this process, including exogenous proteins from pathogens and nanoparticles as well. Furthermore, several unsolved questions and the future directions in this field are also discussed.

2. The Cytoskeleton in A:L fusion

The cytoskeleton is an important cellular structure of eukaryotic cells. It not only plays important roles in maintaining cell morphology, resisting external mechanical stress and maintaining the order of the internal structure of cells, but also participates in many other important cell biological processes, such as cell division and most importantly here, intracellular

Fig. 1 An overall description of Autophagy process. Autophagy can be roughly divided into following steps; 1) the initiation by upstream signal; 2) the formation of phagophore; 3) the expansion of phagophore and closure of autophagosome membrane; 4) autophagosome maturation and fusion with late lysosome or lysosome to generate amphisome and autolysosome respectively; 5) degradation of autophagic cargo; 6) the reformation of autophagic lysosome. A brief process of endocytic pathway is shown on the right side to indicate the relationship of these two lysosome-dependent pathways.

Table 1. Main known components involved in A:L fusion and their functions.

Component	Identification	Functions	Refs
<i>Cytoskeleton and vesicle transport</i>			
<i>Actin/Filaments system</i>			
Actin Filaments	Cytoskeleton	Tracks for myosin-based motility	[8, 9]
MYO6	Motor protein	Minus end directed motor protein	[19]
TOM1	ESCRT-0 complex subunit	Links MYO6 to endosome/lysosome	[20]
MYO1C	Motor protein	Maintenance of cellular cholesterol homeostasis	[21, 23]
HDAC6	Histone deacetylase	Recruitment of actin remodeling system	[31]
<i>Microtubule system</i>			
Microtubule	Cytoskeleton	Tracks for Dynein-Dynactin and Kinesin based vesicle migration	[10]
Dynein	Motor protein	Subunit of plus end directed motor protein complex	[32]
Dynactin	Motor protein	Subunit of plus end directed motor protein complex	[32]
RILP	Rab7 effector	Links dynactin and Rab7	[34]
Rab7	Rab GTPase	Recruitment of crucial downstream effectors to autophagosome or lysosome	[34-36, 115]
Kinesin	Motor protein	Plus end directed motor protein, lysosomal positioning and signaling	[32, 37]
FYCO1	Rab7 effector	Links autophagosome membrane to kinesin	[36]
LC3	ATG8 family member	Biological marker for autophagosome, protein recruitments	[36, 55, 74]
PI3P	Phosphatidylinositol	Signal for recruitment of proteins, like FYCO1	[36]
Arl8b	Arf-like (Arl) GTPase	Lysosome distribution, positioning and signaling	[37, 115, 116]
<i>SNAREs</i>			
<i>STX17-SNAP29-VAMP8 ternary SNARE complex</i>			
STX17	Qa-SNARE	Qa-SNARE protein localized on autophagosome membrane	[43]
SNAP29	Qbc-SNARE	Cytoplasmic Qbc-SNARE protein	[43]
VAMP8	R-SNARE	R-SNARE protein localized on lysosome membrane	[43]
<i>YKT6-SNAP29-STX7 ternary SNARE complex</i>			
YKT6	R-SNARE	A second R-SNARE protein localized on lysosome membrane	[44]
STX7	Qa-SNARE	Qa-SNARE protein localized on autophagosome membrane	[44]
<i>SNARE recruitment</i>			
ATG14	ATGs	Interaction with STX17, STX17 translocation to autophagic structure	[53, 54]
IRGM	Small GTPase	STX17 recruitment through interacting with LC3	[55]
<i>SNARE disassembly and recycling</i>			
α SNAP & NSF		SNARE disassembly or priming, interacting with GABARAP	[57, 58]
<i>Rabs</i>			
Rab5	RabGTPase	Recruitment of Ccz1-Mon1	[69]
Ccz1-Mon1	GEF for Rab7, Rab5 effector	Rab7 recruitment and activation on autophagosome or lysosome membrane	[69, 70]
Rab2	Rab GTPase	Interacting with HOPS complex	[67]
Rab33b	Rab GTPase	Interacting with ATG16	[71]
OATL1	GAP for Rab33b	Rab33b inactivation	[71]
<i>Tethering factors</i>			
HOPS complex	Multimeric complex	Tethering factors for both the endocytic pathway and A:L fusion	[74]
ATG14	ATGs	Stabilizes the STX17-SNAP29 binary t-SNARE complex	[75]
TECPR1	PI3P binding protein	Interacting with the Atg12-Atg5 conjugate	[76, 190]
EPG5	Rab7 effector	Specificity determination for A:L fusion	[77]
GO-	Golgi stacking protein	Scaffold protein for Golgi multi-flattened structure	[78, 79]
RASP2/GRASP			
55			
<i>PIs & PIs con-versions</i>			
Vps34 Complex	PI3K complex	PI3P generation on autophagosome membrane	[5, 6]
MTMRs	PI3P phosphatases	Liberation of PI3P from autophagosome membrane	[81]
PI4KII α	Lipid kinase	PI4P generation on autophagosome or lysosome membrane	[87, 88]
PI4P5Ks	Lipid kinase	Conversion from PI4P to PI(4,5)P2	[88, 90]
PIKfyve	Lipid kinase	Conversion from PI3P to PI(3,5)P2	[99, 100]
PI(4,5)P2	Phosphatidylinositol	Dissociation of PLEKHM1 from lysosome	[88]
PI(3,5)P2	Phosphatidylinositol	Binding with cortactin on lysosome membrane	[99, 100]
INPP5E	5-phosphatases	Conversion of PI(3,5)P2 back to PI3P	[99, 100]
CTTN	Cortactin	Actin filaments stabilization	[99, 100]
OCRL	5-phosphatases	Conversion from PI(4,5)P2 back to PI4P	[90]
PI5P4Ks	Lipid kinase	Generation of PI(4,5)P2 from PI5P	[97]
<i>Regulation factors</i>			
OGT	OGlcNAc transferase	Post-translation modification and inhibits SNARE activity of SNAP29	[109]
Pacer	UVRAG associated protein	PI3K activity regulation, Intermediate molecule for upstream signals	[107, 108]
ATPV0D2	V-ATPase subunits	Interacting with STX17 and VAMP8	[112]
UVRAG	PI3K complex subunit	Dephosphorylation enhances PI3K complex activity	[102, 104, 191]
PLEKHM1	Rab7 effector	Competing with SKIP, tethering effect	[113, 115]
ORP1L	Cholesterol sensor	Formation of ER-AV site in low cholesterol condition and prevents fusion	[35]

MYO, myosin; TOM1, Target of Myb protein 1; ESCRT, endosomal sorting complexes required for transport; HDAC6, Histone deacetylase 6; RILP, Rab-interacting lysosomal protein; FYCO1, FYVE and coiled-coil domain autophagy adaptor 1; LC3, Microtubule-associated protein light chain 3; ATG, autophagy related gene; PI, Phosphatidylinositol; PI3P, Phosphatidylinositol 3-phosphate; STX17, Syntaxin 17; SNAP29, Synaptosomal-associated protein 29; VAMP8, Vesicle-associated membrane protein 8; IRGM, Immunity related GTPase M; NSF, N-ethylmaleimide-sensitive factor; Ccz1-Mon1, Caffeine, calcium, and zinc 1-monensin sensitivity protein 1; OATL1, Ornithine aminotransferase-like protein 1; HOPS complex, Homotypic fusion and vacuole protein sorting complex; TECPR1, Tectonin Beta-Propeller repeat containing 1; EPG5, Ectopic P-Granules autophagy protein 5; GO-RASP2/GRASP55, Golgi reassembly-stacking protein 2/Golgi reassembly-stacking protein of 55 kDa; MTMRs, Myotubularin-related phospholipid phosphatase proteins; PI4KII α , Phosphatidylinositol 4-kinase II α ; PI4P5K, Phosphatidylinositol 4-Phosphate-5 kinase; PIKfyve, FYVE finger-containing phosphoinositide kinase; PI(4,5)P2, Phosphatidylinositol 4,5-bisphosphate; INPP5E, Inositol Polyphosphate-5-Phosphatase E; OCRL, OculoCerebro Renal of Lowe; PI5P4K, Phosphatidylinositol-5-Phosphate 4-Kinases; PIKfyve, FYVE finger-containing phosphoinositide kinase; Pacer, Protein associated with UVRAG as autophagy enhancer UVRAG, UV radiation resistance associated; PLEKHM1, Pleckstrin homology and RUN domain containing M1; ORP1L, Oxysterol-binding protein (OSBP)-related protein.

trafficking. During the transportation of substances, all kinds of vesicles and organelles are oriented along the cytoskeleton. In mammalian cells, the cytoskeleton also plays an important role in the autophagic pathway.^[8-10]

As an essential part of cellular cytoskeleton, actin filaments, providing tracks for myosin-based motility, are involved in the early stage of autophagy.^[8,9] Two identified proteins of the myosin family are implicated in the late steps. Myosin 6 (MYO6), a minus end directed motor protein, functions in a wide range of intracellular membrane trafficking processes and participates in distinct steps of the endocytosis pathway.^[19] In 2012 Tumbarello, D.A., *et al.* announced that MYO6 interacts with its adaptor protein TOM1 (a component of ESCRT-0 complex) via its WWY motif and binds to autophagic cargo receptor NDP52 via its RRL motif and plays a role in A:L fusion.^[20] Both of these two motifs are present in the C-terminal cargo-binding tail of MYO6.^[20] The other motor protein that is related to A:L fusion is Myosin 1C (MYO1C), a widely expressed motor protein that associates with cholesterol-enriched lipid rafts (plasma membrane microdomains enriched in cholesterol) and is involved in cellular cholesterol homeostasis.^[21] Depletion of MYO1C resulted in intracellular lipid rafts redistribution and enlargement of endocytic compartments without affecting the function of lysosomes and their ability for substrates hydrolyses. However, morphology changes of these compartments were induced,^[22,23] which were distinguishable from increasing cholesterol storage in lysosomes such as in Niemann-Pick Type C disease.^[24-26] The absence of MYO1C also led to deficiency in the clearance of autophagic cargo and accumulation of autophagosomes.^[23] As intracellular organelles, the lipid and cholesterol content of autophagosomes and lysosomes would as well probably be altered by depletion of MYO1C, which may account for the massive accumulation of autophagosomes in the absence of MYO1C, since proper lipid composition of these two enclosed intracellular compartment is required for efficient fusion process.^[27,28]

In addition to actin motility, an actin network is also needed for A:L fusion as many other membrane trafficking events are.^[29,30] Lee *et al.* found that histone deacetylase 6 (HDAC6) promotes A:L fusion by recruiting a cortactin

(CTTN) dependent and actin remodeling system thus produces an F-actin network that is required for the later fusion step.^[31] Notably, this mechanism is not involved in classical starvation-induced autophagy, but in clearance of aggregated proteins.^[31]

It is generally believed that appropriate relative movement of autophagosomes is necessary for their fusion with lysosomes, since the generation sites of autophagosomes are randomly distributed throughout the cytoplasm rather than right beside their fusion target: the late endosomes and lysosomes, which are mainly localized in the vicinity of the nucleus. This migration of autophagosomes is achieved by motor proteins and their physical movement alongside the cytoskeleton. There are two kinds of motor proteins based on the microtubule cytoskeleton system, namely the kinesin and the dynein, both of which bind to the vesicles, while the former generally moves to the plus-end of the microtubule (towards the cell periphery), and the latter towards the minus-end (perinuclear region where the microtubule-organizing center MTOC is located).^[32] In Hela cells, mature autophagosomes are observed accumulating near the nucleus, and the minus end directed dynein-dynactin motor protein complex is as expected found responsible for this phenomenon and is essential for A:L fusion.^[33] Rab7 recruited to these mature autophagosomes plays an important role in recruiting these motor complexes. Rab7 participates in minus-end transport of autophagosomes through binding with RILP (Rab interacting lysosomal protein), dynein and ORP1L which will be discussed later in this review.^[34,35]

For migration towards the microtubulin plus end, FYCO1 (FYVE and coiled-coil domain-containing 1) binds to both Rab7 and ATG8/LC3, and meanwhile, with its N-terminal region binding to kinesin and C-terminal region binding to PIP3.^[36] Thus, FYCO1 acts as a linker between a kinesin protein and LC3 positive autophagosomes.^[36] In addition to autophagosome migration, proper lysosome positioning is necessary for fusion. Kinesin KIF1B- β , KIF2 and GTPase Arl8b have been found responsible for lysosome positioning and lysosome redistribution, which further were related to mTORC1 activity.^[37] Over-expression of KIF1B- β or KIF2, or Arl8b leads to redistribution of lysosomes to the cell periphery and enhanced mTORC1 activity with increased

autophagosome accumulation due to lysosome approaching the plasma membrane where mTORC1 activators such as Protein Kinase B(PKB/AKT) are localized.^[3,37,38] It has been reported that mTORC1 also act as a negative regulator for A:L fusion in addition to its role in autophagy initiation.^[4,39] Consistence with this, Arl8b is basically enriched on peripheral lysosomes with less Rab7 association and lower level of acidification.^[40,41] Actually, Arl8b mediates peripheral positioning of lysosomes by interacting with SKIP (SifA and kinesin-interacting protein, or PLEKHM2), which then recruits Kinesin-1/KIF5B, assembling plus end migrating complex on lysosome.^[42]

3. SNAREs

SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) are protein receptor complexes involved in docking and positioning of vesicles destined for fusion. According to different definitions, people have a variety of classification of SNARE proteins. According to their structure, SNAREs are classified into Q-SNAREs (having a glutamic acid residue in the SNARE domain) and R-SNAREs (having an arginine residue). Q-SNAREs are further divided into Qa, Qb and Qc-SNAREs based on the amino acid sequence of the SNARE domain. The SNARE parallel four-helix bundles formed by the Qa, Qb, Qc and R-SNARE compositions on two approaching membrane structures release the forces of noncovalent interaction between the hydrophobic chains so that the two membrane structures move close enough and eventually fuse with each other.

In starving mammalian cells, Qa-SNARE syntaxin 17 (STX17) is translocated from the cytoplasm to the closed autophagosome, and forms a ternary SNARE complex that mediates A:L fusion through its binding with its ligand R-SNARE VAMP8 on lysosomes and cytoplasmic Qbc-SNARE SNAP29.^[43] The C-terminus of STX17 contains two adjacent transmembrane domains (TMDs) in which the glycine zipper-like motif causes the two to form a hairpin-like structure, and with this structure, localizes itself on the membrane of autophagosomes.^[43]

In 2018 Takahide *et al.* found that autophagy was still occurring to a certain extent even in Hela cells with STX17 knocked out, and subsequently, this group identified a novel human SNARE protein, YKT6, and proposed another SNARE complex that mediates fusion.^[44] YKT6 is a type of R-SNARE, which is highly conserved from yeast to human, and inserts itself into the lipid membrane, such as the Golgi membrane, through the two cysteines modified by palmitoylation or farnesylation (prenylation) of the CCAIM region which is adjacent to the SNARE active region of the C- terminus. YKT6 itself participates in multiple membrane fusion processes and is involved in many other membrane traffic processes.^[36,45-50] Unlike the anchoring manner on the Golgi apparatus, YKT6 is anchored to the autophagosome via the N-terminal Longin domain during autophagy, and forms a ternary complex with the Qbc-SNARE protein SNAP29 and

Qa-SNARE protein STX7 to mediate the fusion process, independently of the STX17 pathway.^[44] Observations from *in vitro* autophagic fusion assays also fit well with the model just mentioned.^[51,52] Along with the identification of this diversity of SNARE complexes there arises questions about the differences and evolutionary relationship between STX17 and YKT6, and whether they function independently in different situations or cooperatively under the same conditions. This remains to be fully explained in future investigations.

With the identification of various SNARE protein combinations involved in membrane fusion, many problems have arisen, such as how to ensure that the fusion process is performed in an orderly and specific manner and how these SNAREs are selectively recruited to specific cellular structures. STX17 is reported to be localized at ER-mitochondrial contact sites normally, binding to ATG14 upon starvation and is involved in autophagosome formation at this area, and ultimately increases its localization on autophagosomes.^[53,54] Later, Suresh *et al.* found that mATG8s interacts with STX17 through the latter's two LIRs (LC3 interacting regions) Its hairpin structure formed by two TMDs interacts with another key molecule, IRGM (immunity-related GTPase), while IRGM interacting with mATG8s in an LIR non-dependent way.^[55] These three molecules together form STX17-LC3B-IRGM holocomplex called ARP (autophagosome recognition particle). The author then reasoned that mATG8s' binding with STX17, which is independent of its previously reported role in autophagy initiation,^[56] effectively blocks the SNARE domain of STX17, and may effectively prevent premature engagement of STX17 in trans-SNARE complex.^[55] When autophagosomes are ready for fusion, mATG8s that bind to STX17 are replaced by other factors to complete SNARE pairing and A:L fusion.^[55]

Meanwhile, proteins like α SNAP and NSF (N-ethylmaleimide-sensitive factor) are found involved in A:L fusion, and are responsible for SNARE disassembly and priming after the fusion is completed, as supported by blocking of at autophagic flux in the absence of α SNAP, though interestingly without affecting previous translocation of STX17 to autophagosome.^[57] A possible hypothesis is that sustainable usage of SNAREs for the fusion in the next round autophagy is impaired by deficiency in SNARE disassembly due to depletion of α SNAP. Interestingly, long before this study, GABARAP (a subfamily of mATG8s) has been shown to interact with NSF,^[58] and question arises if members of ATG8 family are involved in A:L fusion in an α SNAP and NSF dependent manner.

4. Rabs

The Rab family is a class of small GTPases that regulate membrane traffic in eukaryotic cells. They recruit specific effector proteins, such as cargo adaptors, motor proteins, and tethering factors, which are respectively responsible for cargo selection and budding, vesicle movement and vesicle fusion. Like other small GTPases, Rabs switch between two

conformations, an inactive form with GDP binding, and an active form with GTP binding, which is mediated by specific GEFs (guanine nucleotide exchange factors) and GAPs (GTPase activating proteins). Distinct distributions of Rabs at different vesicles make them perfect biomarkers for various parts among the sophisticated membrane traffic, and Rab conversions conform with compartment maturation events and trafficking process.^[59-62] It is anticipated that some members of the Rab family are involved in the regulation of A:L fusion, since autophagy is a curtailed part of membrane traffic.

Rab7 (or Ypt7 in yeast) has been found having multiple functions both in the endocytic pathway and the autophagic pathway.^[60,62-65] Massive accumulation of autophagosomes appeared in Rab7 knockdown cells.^[66] Rab7 cooperates with STX17-SNAP29-VAMP8 and HOPS complex interacting with Rab2 on autophagosomes in mediating A:L fusion.^[67]

In mammalian cells, GEFs that mediate the activation of Rab7 and their function of manipulating the membrane fusion process has not yet been found. However, in yeast and drosophila, a heterodimer Ccz1-Mon1 has been identified and is required for intact and smooth fusion process. It has GEF activity and can recruit Rab7 to autophagic membrane containing PI3P.^[68] The upstream Rab5 recruits the dimer to the endosomal membrane and activates it, thereby recruiting and activating Rab7 and detaching itself, completing the Rab5-Rab7 exchange, marking the conversion from the early endosome to the late endosome and ultimately promoting endocytic fusion with lysosomes.^[69] However, mutations causing Rab5 inactivation did not affect autophagosome and lysosome fusion, indicating that Rab5 is not required for Ccz1-Mon1-dependent Rab7 recruitment here.^[68] Later, the molecular mechanism about Rab7 recruitment by Ccz1-Mon1 was revealed in yeast, which proved that Ccz1 was recruited by autophagic LC3 through its two LIRs (LC3 interacting regions) and subsequently activate Ypt7.^[70]

Another Rab protein, Rab33b, localized to the Golgi complex, also plays a role in autophagosome formation through interaction with ATG16.^[71] Its GAP, OATL1 (ornithine aminotransferase-like protein 1), is recruited to autophagosomes by direct interaction with ATG8/LC3. Itoh *et al.* observed inhibited A:L fusion by over-expression of OATL1, which depended on both its binding with LC3 and GAP activity,^[71] which may be the results of overwhelming Rab33b inactivation.

5. Tethering factors

In addition to the selectivity of A:L fusion partially ensured by different combinations of SNAREs, tethering factors, which are various groups of peripheral membrane associated proteins, bridge the two approaching membrane structures and provide another layer of selectivity for precise docking and fusion.

The HOPS complex is known as a multimeric tethering factor for both the endocytic pathway and autophagic fusion.^[72-74] The HOPS complex, which consists of several subunits

including VPS33A, VPS16, VPS11, VPS18, VPS39 and VPS41, interacts with Qa-SNARE STX17. Knockdown of VPS33A, VPS16 or VPS39 significantly blocked the autophagic flux and led to the accumulation of autophagosomes associated with STX17 and LC3. These results indicate that HOPS greatly manipulates fusion through its interaction with STX17.^[74]

Atg14 (also known as Beclin1-associated autophagy-associated major regulator of Barkor or Atg14L), an essential autophagy-specific regulator of the type III PI3K complex, enhances membrane stability of protein-free liposomes, semi-fusion and complete fusion mediated by SNAREs (t-SNARE) STX17, SNAP29 and vesicle SNARE (v-SNARE) Vamp8.^[75] Atg14 binds to the SNARE core region of STX17 and stabilizes the STX17-SNAP29 binary t-SNARE complex on autophagosomes via its coiled-coil domain.^[75] In cells with this structural defects or mutations in this region, autophagosomes are still effectively formed, but their fusion with lysosomes is inhibited.^[75]

The PI3P binding protein TECPR1 also acts as a tethering factor in A:L fusion.^[76] In TECPR1 knockdown cells, autophagic flux is inhibited.^[76] Chen *et al.* showed that ATG16 and ATG12-ATG5 competitively formed a complex with TECPR1 and that TECPR1 binding to PIP3 was dependent on the association with the ATG5-ATG12 conjugate, which then may promote maturation and A:L fusion.^[76]

Ectopic P-granule protein 5 (EPG5), whose gene is responsible for the Vici Syndrome and which is another effector of Rab7, is also a tethering factor that determines the selectivity of A:L fusion.^[77] EPG5 is recruited to late endosomes/lysosomes by direct interaction with Rab7 and lysosomal R-SNARE VAMP7/VAMP8. EPG5 also contains a LC3-interacting region (LIR) motif to interact with LC3. EPG5 promotes the assembly of the STX17-SNAP29-VAMP8 ternary complex and stabilizes it to promote A:L fusion. Knocking down EPG5 results in fusion abnormalities and various endocytic vesicle accumulation.^[77]

GORASP2/GRASP55 is an important scaffold protein that maintains the Golgi multi-flattened structure.^[78] O-acetylglucosamine modified GORASP2/GRASP55 is localized on the outer peripheral side of the cis-Golgi and oligomerizes between the flat capsules of the Golgi to form a connected structure via the linkage between the O-acetyl amino groups.^[78] GORASP2/GRASP55 undergoes de-O-acetylglucosamine modification in the presence of glucose stress. Thereafter, GORASP2/GRASP55 can interact with the autophagosome marker LC3 and the lysosomal marker Lamp2 to exert its tethering function and mediate the fusion of the two membranes.^[79]

However, since all the molecules mentioned here have been separately proved to involve in the A:L fusion, questions have arisen that if there are any connections among these proteins, though they appear to be loosely related both structurally and functionally. The time and spatial order of recruitment and their synergistic effect are still not fully understood.

6. Phosphoinositides and their conversions in A:L fusion

The major mechanism regarding phosphoinositides' (PIs) involvement in many cellular processes is that different levels of phosphorylation on the third, fourth and fifth positions of the inositol ring, which was manipulated by series of lipid kinases and phosphatases, produce different derivatives that are later enriched in certain subdomains of the membrane and are thereby affecting the curvature of the membrane. Enrichment of specific PIs act as signals or second messengers for downstream protein binding that contain a special domain, such as the PX domain.^[80] Intracellular conversion of PIs has been found to be involved in both autophagosome generation and, with recent research focus, the fusion step.^[81]

Upon autophagy initiation, PI3P is produced by the Vps34/PI3K complex and enriched in membrane subdomains that are prepared for ATG protein recruitment and the following phagophore biogenesis, termed as phagophore assembly site (PAS).^[5,6] The function of PI3P in proper transport of autophagosomes is related to the recruitment of FYCO1 and the assembly of motor protein complexes. However, after the formation of autophagosome is completed, the ATG core complex needs to dissociate from the autophagosome surface and is recycled for new vesicle biogenesis, only after which the autophagosome would become capable for the later fusion step. This means the liberation of both primary protein recruitment factors—PI3P and ATG8/LC3 is necessary, which is considered as the maturation of autophagosome. ATG4, one of the key factors of the previous ATG8 conjugating system is as well responsible for ATG8 deconjugating/recycling.^[82,83] [Fig. 2] In mammalian cells, the amount and distribution of PI3P were negatively regulated by several PI3P phosphatases of the myotubularin protein family (MTMR), such as MTMR3, MTMR6, MTMR7, MTMR14/Jumpy, and was found involved in the initial part of autophagy.^[81,84-86] [Fig. 2] By contrast, the MTMR like protein in yeast Ymr1 has been proven to be a positive regulator of autophagy, since a large amount of autophagosome accumulation was observed and ATG machinery was still associated with the autophagosome surface in Ymr1 knock down cells.^[81] The functional overlap or some other unknown regulating factors may explain the negative effect of MTMR proteins in higher eukaryotes. More detailed mechanism studies about MTMR proteins and their role in the late stages of autophagy still remains to be further discovered.

Another PI that is generated on the autophagosome membrane is PI4P, which is as well involved in A:L fusion in mammalian cells.^[87] Upon starvation, phosphatidylinositol 4-2 type kinase- α (PI4KII α) is secreted by trans-Golgi apparatus (TGN) and dispersed into the cytoplasm, some of which is then localized to autophagosomes in a palmitoylation-dependent manner to mediate PI4P redistribution.^[87]

Interestingly, PI4KII α interacts with the Atg8 homologs GABARAP and GABARAPL2 but not LC3. Knockdown of GABARAP inhibits the recruitment of autophagic PI4KII α , while knockdown of PI4KII α does not affect the cellular distribution of GABARAP, indicating that GABARAP acts upstream of PI4KII α .^[87] However, details about the mechanism how PI4P itself is involved here is still poorly understood.

PI4P is as well generated by PI4KII α and has significant functions on membrane of Rab7 positive endocytic compartment like late endosome/lysosome, together with its downstream derivatives.^[88] PI4P is converted into PI(4,5)P₂, which recently has been found related to autophagic fusion and revealed a potential mechanism about selective control between endocytic pathway and autophagic pathway.^[88] Endosomal PI4P was converted into PI(4,5)P₂ by PI4P5K γ on Rab7 positive endosomes, thus acting as a precursor for PI(4,5)P₂ generation.^[88] [Fig. 2] Knockdown of PI4P5K γ which impaired the amount of PI(4,5)P₂ shows similar effects as PI4KII α KD cells mentioned above.^[88] The increase of endosomal PI(4,5)P₂ leads to Rab7 inactivation and thus facilitates Rab7 cycling. Most importantly, this research found that the production of PI(4,5)P₂ caused the dissociation of PLEKHM1, a Rab7 effector which will be discussed in the following part, from late endosomes//lysosomes, which then tethers the autophagosomes and lysosomes for fusion, while other Rab7 effectors, such as RILP or Vps35, do not.^[88] These results suggest that A:L fusion requires cellular cycling of some of its key factors which then are controlled by PI conversion. The researchers hypothesize that it is quite possible that there are still other factors related to PI(4,5)P₂ and Rab7.^[88]

In contrast, another protein called OCRL (OculoCerebro Renal of Lowe) activated on lysosome may convert PI(4,5)P₂ back to PI4P with its PI(4,5)P₂ 5-phosphatase activity, mutations in which would lead to Oculocerebrorenal Lowe syndrome.^[89] [Fig. 2] Here, upon A:L fusion, an increase of lysosomal PI(4,5)P₂ response mediated by PIP5K1 α and β is triggered by the release of autophagic cargo to lysosomes like mtDNA (mitochondrial DNA) in the manner of TLR9 (Toll-like receptor 9) activation, which led to following recruitments of PI(4,5)P₂ binding proteins AP2 and clathrin even earlier before the end of autophagy and appears to be independent of previously reported process of mTORC1 inactivation-reactivation in autophagic lysosome reformation (ALR).^[90-92] [Fig. 2] OCRL here is proven to be recruited by MCOLN1 (mucolipin-1) with necessary presence of AP2.^[90] [Fig. 2] MCOLN1 is a lysosomal calcium channel with activity to facilitate A:L fusion,^[93] which would be inhibited by PI(4,5)P₂ while promoted by PI(3,5)P₂,^[94,95] thus the PI(4,5)P₂ 5-phosphatase activity of OCRL is required here to maintain the sustainability of autophagic flux and autophagosomes

ubiquitinated by SMURF1 at lysine residues 517 and 559, resulting in the promotion of autophagosome maturation.^[105] Modifications of the core subunit Beclin1 also matters. Phosphorylation of Beclin1 mediated by CK1 and following acetylation controlled by p300 are found to suppress autophagosome maturation and related to tumorigenesis.^[106]

The question arises that how an endosome-resident protein could inhibit autophagosome maturation. In 2017, Sun *et al.* reported for the first time a protein called Pacer (protein associated with UVRAG as autophagy enhancer). Pacer antagonizes Rubicon and targets both UVRAG complex and HOPS complexes to autophagosomes by interacting with autophagosomal STX17, thus promoting maturation and fusion. Pacers homologues are found only in zebrafish and other higher species, indicating that it is a vertebrate-specific gene.^[107] Following studies from this group revealed that Pacer functions as a mediator and post-translation modification substrate in response to upstream metabolic signals such as mTORC1 phosphorylation and GSK3/TIP60 mediated acetylation with negative and positive regulation effects, respectively.^[108]

A:L fusion can also be regulated by direct post-translation modification on SNAREs. The O-linked N-acetylglucosamine (O-GlcNAc) modification of SNAP29 has a negative regulatory effect on SNARE-dependent fusion between autophagosomes and lysosomes.^[109] Thus, knockdown of the O-GlcNAc transferase or mutant SNAP29 O-GlcNAc site promotes the formation of a SNARE complex containing SNAP29 and increases the fusion between autophagosomes and lysosomes.^[109] The acetylation of the SNARE domain of STX17 controlled specifically by the histone acetyltransferase CREBBP/CBP (CREB binding protein) has negative impacts on A:L fusion. Upon autophagy, CREBBP/CBP is inactivated due to mTORC1 inhibition at this time point, and the deacetylation of the SNARE domain of STX17 mediated by HDAC2 is required for fusion promotion and the recruitment of the HOPS complex.^[110] ULK1, another core factor in autophagy initiation, is as well found involved in the fusion process through its physical interaction with STX17.^[111]

In recent years, researchers have found that despite mutations in the V0 subunit of V-ATPase, it can still maintain its acidification function, although there are serious vesicle fusion defects in the mutant cells.^[112] Here, the ATP6V0D2 subunit, which was specifically expressed in macrophages, interacted with the fusion key SNARE proteins Vamp8 and STX17 during the invasion of pathogens such as *Salmonella*, suggesting that the former may form a complex with Vamp8 and STX17 to promote the fusion process.^[112] This subunit does not exhibit the classical acidification function, while the latter is accomplished by another subunit such as ATP6V0D1.^[112]

Another group of proteins that have regulatory roles are Rab specific downstream effectors. PLEKHM1, previously identified as a homologue of Rubicon, functions as an effector of Rab7 through its RH-domain interacting with Rab7 andaconserved LIR (LC3-interacting region) domain binding

with LC3, and is involved in A:L fusion through HOPS complex interactions.^[35,113,114] More importantly, PLEKHM1 competes with SKIP (aka PLEKHM2) to interact with Arl8b through its RUN domain, and ultimately results in perinuclear clustering of lysosomes.^[115] PLEKHM1 acts as a linker between Arl8b and Rab7, and has been, for the first time, identified as part of crosstalk between two different small GTPases. Arl8b has been reported to bind with VPS41 of HOPS and is required for endocytic pathway, and VPS39 interacts both with PLEKHM1 and SKIP.^[115,116] Taken together, PLEKHM1 may somehow mediate selective translocation of the HOPS complex between endocytic and autophagic pathway. Besides, the cholesterol sensor ORP1L, a Rab7 effector molecule, binds to Rab7 in the presence of RILP and has a negative regulatory effect on fusion.^[35,62] ORP1L is recruited to autophagic vesicles by Rab7 and interacts with (VAMP)-associated ER protein A (VAPA) to form an ER-AV (autophagic vesicle, including autophagosomes, amphisomes and autolysosomes) contact site in low cholesterol levels conditions.^[35] This contact site prevents the recruitment of PLEKHM1 to Rab7, which in turn prevents the recruitment of HOPS complexes and leads to defects in autophagosome-late endosomal/lysosome fusion.^[35] The presence of ORP1L and this contact site forced dissociation of dynein-dynactin complex from AVs and thus prevented the negative transport of late autophagosomes.^[35] Meanwhile, ORP1L on lysosome/late endosome plays the same role in lysosomal homeostasis,^[117,118] since abnormal accumulation of cholesterol in late endosomes that occurred in Niemann-Pick C disease has led to lysosome clustering in perinuclear regions.^[24-26] However, how cholesterol is transferred to the leaflet on these vesicles and how the distribution of cholesterol is regulated still remains unclear. These observations indicate that a vital connection exists between cellular cholesterol metabolism and the fusion of autophagosomes with late endosomes/lysosomes and the potential regulation role of cholesterol in this process. More details about the function of cholesterol during the fusion process still needs to be further studied in future research.

8. Factors that disturb fusion exist in pathogens

Xenophagy refers to autophagy triggered by cells recognizing intracellular and extracellular bacteria or virus in order to wipe out these uninvited guests.^[1] In this case, autophagy acts as a natural immune mechanism against exogenous pathogens. Main known exogenous pathogen factors and their host targets together with specific functions are summarized in Table 2.

Although a variety of bacterial microorganisms can be directly targeted by autophagy, in the long-term survival struggle against the host cell, these bacteria or pathogens have evolved a number of molecular mechanisms to manipulate the autophagy process of host cell, thereby escaping clearance of themselves. Among them, some proteins secreted by pathogens enable themselves to survive in host cells by interfering A:L fusion. In ACM cells and the Raw264.7 cells

Table 2. Factors that disturb fusion exist in pathogens.

Pathogen	Factors	Host targets and possible mechanism	Refs
<i>Bacteria factors</i>			
<i>M. Tuberculosis</i> str. H37Rv/BCG	ESAT-6	Enhancing mTORC1 activity, interrupting Rab7 recruitment	[119, 121]
<i>M. Tuberculosis</i> str. H37Rv	PhoP	Interrupting Rab7 recruitment, ESAT-6 transcription	[121, 122]
Methicillin-resistant <i>S. aureus</i>	IsaB	Preventing lysosomal acidification	[124, 125]
<i>Virus factors</i>			
Human parainfluenza virus (HPIV3)	PhosphoproteinP	Binding with SNAP29 and inhibiting STX17-SNAP29 interaction	[126]
Kaposi's sarcoma-associated herpesvirus (KSHV)	K7	Promoting Rubicon-Beclin1 interaction;	[127]
Human immunodeficiency viruses (HIV)	Nef	Interacting with Beclin1; Disturbing IRGM-STX17 interaction and STX17 recruitment	[55, 128]
Influenza A Virus (IAV)	M2	Interacting with Beclin1; Altering lysosomal acidification?	[130, 131]
Hepatitis C virus (HCV)	Unknown	Targeting Arl8b, affecting lysosomal positioning	[133]
Coronavirus	PLP2/NSP3	Interacting with Beclin1-STING	[135]

ESAT-6, Early secretory antigenic target of 6KD; M2, Matrix protein 2; PLP2, Papain like protein 2; NSP3, Non-structural protein 3.

infected by *Mycobacterium tuberculosis*, autophagic flux was significantly inhibited, resulting in the accumulation of a large number of autophagosomes failing to fuse with lysosomes.^[119] The 6-kDa early antigenic target ESAT-6 and the 10-kDa culture filtrate protein CFP-10 of *Mycobacterium tuberculosis*, which are secreted by the ESX-1 system, one of the five type VII secretion systems,^[120] are secreted into the host cell in the form of a combination. In cells expressing ESAT-6, activity of mTORC1 was significantly enhanced. Given that mTORC1 and its downstream molecules have inhibition activity on the fusion step,^[39,108] the results above suggest that ESAT-6 inhibits fusion in a mTORC1-dependent manner, which then results in *Mycobacterium tuberculosis* long-term survival and non-effective clearance in infected cells.^[119] Other factors like PhoP from H37Rv strain of *M. Tuberculosis*, cause less colocalization between the bacteria and LC3-Lysotracker double positive autolysosome by interrupting Rab7 recruitment. However, this virulence effect of PhoP and autophagic flux blockage would be reverted by depletion of ESAT-6.^[121] Here, PhoP may act upstream of ESAT-6, since the former is a crucial transcriptional factor regulating the RD1 region, of which ESAT-6 is the major products.^[122]

Lysosomal acidification is another available target for bacteria components to manipulate. Cells treated with IsaB from methicillin-resistant *Staphylococcus aureus* (MRSA), showed similar dose-dependent accumulation of LC3-II as cells treated with Bafilomycin A1, an inhibitor of lysosomal hydrogen pumps to prevent lysosomal acidification resulting in a blockage of A:L fusion.^[123] Since the expression of IsaB in *S. aureus* has been reported facilitated in acidic environment (pH 4-5),^[124] the authors reasoned that increased IsaB may alter the acidification of lysosomes and block A:L fusion indirectly^[125] and that acidification of the luminal space of autophagosomes or lysosomes is important for the autophagosome and lysosome fusion.

Viruses, as well in the long journey of evolution race against host cells, have obtained the ability to exploit the components of autophagy and benefit their own survivals partially by altering lysosomal fusion, while the mechanisms involved varies individually among different species. Firstly, human parainfluenza virus (HPIV3) phosphoprotein (P) just directly binds to the SNARE domain of SNAP29, thus inhibiting STX17-SNAP29 interaction and inducing the accumulation of autophagosomes.^[126] K7 protein of Kaposi's sarcoma-associated herpesvirus (KSHV) targeted the VPS34/PI3K complex and promoted Rubicon-Beclin 1 interaction inhibiting the kinase activity of the complex, thus blocking A:L fusion.^[127] Other factors like Nef in human immunodeficiency viruses (HIV) were also found interacting with Beclin1, inducing translocation of VPS34 and ultimately affecting autophagosome maturation.^[128] Besides, Nef targets the recruitment of STX17 mentioned above and diminished IRGM-STX17 interaction.^[55] When it comes to lysosomal acidification in virus infection situations, a proton channel, matrix 2 (M2) protein itself in Influenza A Virus (IAV) would block A:L fusion, supported by observation of accumulation of mRFP and GFP double positive compartments in cells expressing mRFP-GFP-LC3 tandem construct, surprisingly independent of its previous identified proton channel activity,^[129] and in other words, without altering the lysosomal acidification, about which controversial conclusion is drawn.^[130,131] However, a highly conserved LC3-interacting region (LIR) of M2 was later confirmed related to GFP-LC3 translocation to plasma membrane in cells with IAV infection and seemed to work independently of suppression on A:L fusion.^[132] M2 was also found interacting with Beclin1, which may impair A:L fusion by affecting UVRAG-Beclin1 interaction.^[130] Interestingly, relative movement of autophagosome and lysosome and the cytoskeleton system has also been proven to be affected by virus like Hepatitis C virus (HCV) in a Arl8b-dependent manner, which has been

previously mentioned in this review related to lysosomal positioning.^[133] Finally, Chen *et al.* claimed that papain like protease PLP2 or non-structural protein 3 (NSP3) of coronavirus would induce incomplete autophagy with late phase like A:L fusion impaired. PLP2 here was found binding with STING, a key mediator for IFN signaling,^[134] through interaction with Beclin1 and somehow suppressing the IFN response,^[135] consistent with previous reports about relatively lower IFN response in coronavirus infection.^[136,137] Depletion of Beclin1 would interrupt this interaction and counteract the suppressed IFN response and diminished anti-virus ability of the infected cells. This feature of PLP2 is conserved among several types of coronavirus such as HCoV-NL63 and SARS-CoV.^[135] Virus factors and their host targets are also summarized in.

9. The impacts of Nanoparticles on autophagosome maturation

The prefix “nano” is derivative from the Greek “nanos”, meaning “dwarf” and is becoming increasingly widely mentioned in modern scientific research. A nanoparticle (NP), one of the novel nano conceptions, is generally defined as particle with at least one dimension less than 100 nm. Due to its unique physicochemical characteristics or high surface-to-volume ratio specifically, NPs exhibit unlimited artificial modification space and application prospects.^[138] Following research has revealed that NPs have different effects on physiological functions of cells due to several vital characteristics of themselves, such as NP composition, concentration, size, and surface charge. Even distinguish specific cell lines exposed to the investigated NP and the different exposure time scales still matter. Autophagy plays a crucial role in maintaining the homeostasis of the cellular environment and cell survival, differentiation, development, and so on. Meanwhile, autophagy dysfunction is associated with diverse pathologies, including cancer, neurodegeneration, aging, and heart disease.^[139] In tumors with enhanced autophagy, its inhibition can reduce the proliferation and survival of tumor cells due to the inability to provide energy and materials.^[140] On the other hand, the aggregated protein and other waste products which can not be removed immediately due to the weakening of autophagy, may lead to the cell function degradation and may cause a series of neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease. Though many nanomedicines have entered the clinical evaluation by combining with therapeutic drugs, their inherent risks to immune cells, nervous system and cardiovascular system should not be ignored. In this section, we discuss the impacts of NPs on autophagy, especially the last steps of the maturation process, in order to provide

comprehensive insights into their actual effects on human cells related to safety concerns and potential therapeutic values.

Nanoparticles enter cells through phagocytic and non-phagocytic pathways, and finally end with lysosome internalization.^[13] Free nanoparticles in the cytoplasm could also be recognized by the autophagy mechanism, researches both *in vitro* and *in vivo* showed that intracellular NPs are able to be ubiquitinated or co-localize with ubiquitinated protein aggregates, binding to autophagy receptors like p62/SQSTM1, introduce themselves into selective autophagy pathway and facilitate their delivery to lysosome.^[141,142] It is worth noting that the autophagic degradation process triggered by NPs does not necessarily strengthen the clearance capacity of cells. On the contrary, on the one hand, the accumulation of nanoparticles in lysosomes through endo-lysosomal pathways may cause impairment of lysosome and lysosome-related functions, leading to inhibition of autophagosome maturation. On the other hand, the NPs in autolysosomes cannot be hydrolyzed due to lack of corresponding enzymes, resulting in accumulation of NPs in the lysosome and a series of dysfunctions related to the lysosome. However, there are several nanoparticles possessing the ability to restore (or at least partially restore) the lysosome function in pathological cells, promoting the maturation of autophagosomes and removing unnecessary intracellular substances.

Different nanoparticles have various effects on the maturation of autophagosomes. The mechanisms by which different nanoparticles affect the autophagosome maturation allow us to apply nanoparticles more accurately in the future as complementary therapies for cancer and other diseases. For this reason, we summarized most recent advances about modulation of autophagosome maturation by various nanoparticles through various mechanisms.

Metal-based NPs: In the case of gold nanoparticles (Au NPs)-treated normal rat kidney (NRK) cells, the accumulation of autophagosomes, the up-regulation of LC3-II and p62 is caused by the impairment of autophagic flux rather than the induction of autophagy.^[16] Further studies have shown that Au NPs lead to lysosome swelling and reduced lysosomal degradation by affecting the activity of lysosomal enzymes activities in a size-, dose-, and shape-dependent manner, in which lysosomal alkalization is caused by down-regulating the activity of V-ATPase activity.^[16,143] Multiple Au NPs are devoured by vesicles with double-layer membrane, either autophagosomes or autolysosomes. In addition, remnants of organelles, possibly partially degraded mitochondria, were also observed within these vesicles, indicating that Au NPs did not completely block autophagosomes maturation.^[143] Ten nm 25 nm Au NPs, and 50 nm Au NPs are readily taken up by cells and cause great lysosome damage and autophagic flux

Table 3. Nanoparticles and their impacts on autophagosome maturation.

Types of NPs	NPs	Average core size (nm)	zeta-potential (mV) (medium)	Dose ($\mu\text{g/mL}$)	Exposure time (h)	Cell lines	Impacts on A:L maturation and mechanism	Refs
Metal-based NPs	Au	10, 25, 50	negative	1nM	24	NRK	Maturation blockage, lysosome alkalinization, lysosomal enzyme activity decline, size-dependent manner	[16]
	Au	nanosphere diameter:20,50 nanorod length:40,90		30,90,180	24	HeLa, SMMC-7721, HepG2, HUVEC	Maturation blockage, lysosome swelling, lysosomal impairment, dose- and shape-dependent manner	[143]
	Ag	25	-5.43 $\pm$ 0.59	2,4,8	24	HEK293T, A498	Maturation blockage, lysosome degradative activity repression, lysosome impairment	[144]
	Ag	~30	-4.3(water)	5,10	24,48	THP-1	Maturation blockage, lysosome alkalinization, lysosomal membrane permeabilization	[148]
	Ag	9 $\pm$ 2.2, 19 $\pm$ 2	-31, -29.8	10,25,100 μM	6	MCF-7, MDA-MB-468, Ha-CaT	Maturation blockage, lysosomal membrane permeabilization, time-dependent manner	[147]
	Ag		-5.98 $\pm$ 0.73	2,4,6	24	PC-3	Maturation blockage, lysosomal membrane integrity decline, lysosomal quantity decrease, lysosomal protease activity attenuation	[145]
	Ag	~20		2.5	6	HepG2	Maturation blockage, actin cytoskeleton morphologic change	[150]
	Ag	69.8 $\pm$ 0.55	-30.5	50,100,200	24	A549	Maturation blockage, lysosome alkalization	[149]
	Pd	6.3 $\pm$ 1.9, 11.9 $\pm$ 2.3, 20.4 $\pm$ 1.7	-2.09 $\pm$ 0.32, -2.51 $\pm$ 0.45, -3.48 $\pm$ 0.66	0.1,10	24	Hela, HEK293, HepG2	Maturation blockage, lysosome alkalization, size-dependent manner	[151]
	Au@Ag	length,width : 72.7 $\pm$ 5.8, 33.7 $\pm$ 2.4	-8.4 $\pm$ 0.7	0~40(Ag)	6,12,24	HepG2	possible maturation blockage, lysosomal degradation inhibition	[153]
	TiO ₂ -Au	length,width : 47,20; 95,33; 142,42		0~160(Au)	6,12,24,36,48	U-87 MG	Maturation blockage, F-actin distribution disruption, cathepsin B activity inhibition	[152]

Metal oxide-based NPs	IO	15~20		100	24	MCF-7	Maturation blockage, lysosomal membrane disruption	[192]
	IO	IO-cubes:36 IO-clusters: 38		10,50,100	24	HepG2, Huh7, Al-exander cells	Maturation blockage, lysosomal membrane permeabilization	[193]
	IO	200(with fluorescent label)	-2	10,50,100	12	HepG2, Huh7, Al-exander cells	Maturation blockage, lysosome acidification impairment, F-actin and tubulin remodeling, lysosome dysfunction	[194]
	TiO ₂	15,50,100	-30~-15	10,50,100,200,500	24,48,72	Hela, H4 neuroglioma cells	Maturation blockage, lysosomal membrane permeabilization	[154]
	TiO ₂	TiO ₂ -A: 27.3±5.8 TiO ₂ -R: 27.9 ± 5.7	-13.41 ± 1.79, -13.70 ± 4.23	12.5,25,50,100,200	24	RAW264.7	Maturation blockage, lysosomal membrane permeabilization	[155]
	TiO ₂	~25	-9.57 ± 1.68	1,10	24,24*7	A549	Possible maturation blockage, cytoskeleton irreversible disruption	[156]
	ZnO	50,200	-10.3, -10.0	10,30,100	12,24	A549	Maturation blockage, lysosome dysfunction	[158]
	ZnO	length, width: 57.8, 25.6	15.6 ± 7.2	20	0~12	PC12	Maturation blockage, lysosome dysfunction, microtubule system impairment	[157]
	ZnO	~50	-11.5	3,10,30	0~24	HEK293T, A549	Maturation blockage, lysosome dysfunction	[159]
	ZnO	100		1.25	6	HepG2	Maturation blockage, actin cytoskeleton morphologic change	[150]
	CeO ₂	4.3±0.5	CeO ₂ -Glc-NAc:- 13.6±1.5; CeO ₂ -PEG200:- 2.6±1.8; CeO ₂ -PEG1K:- 2.0±1.5; CeO ₂ -PEG10K:- 4.4±2.4; CeO ₂ -PVP10K:- 5.3±1.1; CeO ₂ -PVP40K:-	100ppm	24	Hela, Fibroblasts derived from patients with LINCL	Maturation promotion, TFEB up-regulation,	[195]

			6.8±3.0 (water)					
	CuO	~50	-13.17	1,2,5,10,20,50	24	HUVEC	Maturation blockage, lysosome dysfunction	[160]
Silica-based NPs	Si	63.88±10.35, 46.15 ± 5.53	-29.8, -27.8	50	24	L-02	Maturation blockage, lysosome disruption	[161]
	Si	58.4±7.4	-34.83±1.78, -36.28±1.99, -36.36±1.35, -35.20±0.98, -36.66±2.35	6.25,12.5,25,50,100	3,6,12,24	L-02, HepG2	Maturation blockage, lysosomal membrane destabilization, cathepsin expression down-regulation	[162]
	Si	16.75±3.38	-16.81 ± 0.5	25,50,100	24	BEAS-2B	Maturation blockage, ROS, lysosomal alkalization, lysosome degradation defect	[163]
QD-based NPs	GO QDs	3.28±1.16	1.78±0.94	1,10,100	24	GD-2, TM4	Maturation blockage, lysosome degradation defect	[164]
	MoS ₂ QDs	3,4	-25.4 ± 0.8	10,25,50	6,12,24	HUVEC, HCAEC	Maturation promotion, TFEB up-regulation,	[165]
	CdTe/CdS QDs	~9	-29	32nM	0~48	HL-7702, HepG2	Possible maturation promotion, lysosomal enzyme activity enhancement	[166]
carbon-based NPs	Fullerene	15.7	-49.1 ± 2.0	0.01~10 mM	6,24,48	LLC-PK1	Possible maturation blockage, cytoskeleton disruption.	[168]
	Fullerene	3.92		40	48	NIH-3T3, MDA-MB-231	Possible maturation blockage, interference with actin filaments self-assembly, possible maturation blockage	[169]
	Fullerene derivative C60(OH) ₂₀	~1.0, 5.7		0~100		in vitro	Possible maturation blockage, Inhibits microtubule polymerization by interacting with tubulin heterodimers	[167]
	GO	Thickness:1 lateral size: 500~800	-9.82 ± 1.06	40,50,60	1,3,6,12,24	PC12	Maturation blockage, lysosome alkalization, lysosomal membrane permeabilization	[171]
	GO	Thickness:1 lateral size: 50~800	-11.90 ± 1.06	40,60,80	24	F98	Maturation blockage, lysosome alkalization, lysosomal membrane destabilization	[173]
	GO	Thickness:1		5,10,20	24	mESCs	Maturation blockage, lysosome alkalization, lysosomal membrane permeabilization	[172]

	GO	Thickness:1	-7.5	10	50	Mouse peritoneal macrophages	Maturation blockage, lysosomal membrane destabilization	[170]
	GCNF	outer,inner diameter: 79 ± 6.6, 7 ± 0.8	-29.7	1,10,25,50,100	24,48	A549	maturation blockage, Lysosomal destabilization, actin cytoskeleton disruption	[176]
	MWCNT	diameter:25		50	6	RAW 264.7 macrophages	maturation blockage, maturation-related protein expression decrease, shape-dependent manner	[174]
	SWCNT	diameter:1–2	-5	10,50	24	Mouse peritoneal macrophages	Maturation blockage, lysosomal membrane destabilization	[170]
	MWCNT	Diameter,Length:20.4 ± 6.0,1054.0 ± 573.2(pristine); 17.1 ± 4.2, 907.0 ± 414.9(carboxylated)	-8.31 ± 0.57, -8.83 ± 0.37	8,16,32,64,128	24	HepG2	maturation blockage, Lysosomal destabilization	[175]
rare earth oxide-based NPs	La ₂ O ₃ ,Gd ₂ O ₃ ,Sm ₂ O ₃ ,Yb ₂ O ₃	26 ± 7.47 ± 10, 186 ± 34.71 ± 13	+27.95±0.51, +23.64±1.64, +35.13±2.48, +18.76±1.4 (DI Water)	50,100,150,200	4,8,12,16,20,24	THP-1, BMDM	maturation blockage, Lysosome Acidification, lysosomal structural phosphate disruption	[17]
Other NPs	NH ₂ -P	50	-13.9 ± 0.3	25,50,75,100,125	24	HeLa, PC12, Fibroblasts derived from LINCL patients	Maturation blockage, lysosome disruption	[179]
	poly(β-amino ester)			50, 100, 200	4, 12, 24	MCF-7	Maturation blockage, Abnormal cathepsin expression, lysosomal alkalization	[180]

PLGA	50~100		180	0.25, 0.5, 1, 24	BE-M17, fibroblasts from one PD patient harboring the ATP13A2 mutation (L3292), fibroblasts from 2 PD patients with GBA mutations (p.N370S and p.G377S), XMEA fibroblasts (c6A to G mutation)	maturation promotion, lysosomal pH restoration, cathepsin D activity restoration	[181]
PLGA	~100	-35~-30	100	24	PC-12	Maturation promotion, lysosomal pH restoration	[182]

Au NPs: gold nanoparticles;
 Ag NPs: silver nanoparticles;
 Pd NPs: Palladium nanoparticles;
 Au@Ag NPs: gold nanorod core/silver shell nanoparticles;
 TiO₂-Au NPs: Titania-coated gold nano-bipyramids particles;
 IO NPs: iron oxide nanoparticles;
 TiO₂ NPs: titanium dioxide nanoparticles;
 ZnO NPs: zinc oxide nanoparticles;
 CuO NPs: copper oxide nanoparticles;
 CeO₂ NPs: cerium dioxide nanoparticles;
 Si NPs: silica nanoparticles;

GO QDs: graphene oxide quantum dots;
 MoS₂ QDs: Molybdenum Disulfide Quantum Dots;
 CdTe/CdS QDs: CdTe/CdS core/shell quantum dots;
 GO NPs: graphene oxide nanoparticles;
 SWCNT: single-walled carbon nanotubes;
 MWCNT: multi-walled carbon nanotubes;
 GCNF: graphite carbon nanofibers;
 NH₂-P NPs: cationic surface-modified polystyrene nanoparticles;
 PLGA NPs: poly-(lactic-co-glycolic acid) nanoparticles;
 LINCL: late infantile neuronal ceroid lipofuscinosis type 2

blockage.^[16] Meanwhile, compared with negatively charged AuNPs give rise to the more accumulation of autophagosomes and conspicuous lysosome enlargement.^[16] Au nanospheres induce more autophagosomes than Au nanorods by reducing autophagic flux, which may be due to shape dependent variations in the internalization of gold nanoparticles.^[143] Silver nanoparticles (Ag NPs) induce initial autophagy by AMPK/mTOR signaling pathway under low dose exposure in renal cells (HEK293T, A498) and prostate cancer cells (PC-3). However, autophagic flux was inhibited by reducing lysosome quantity, lysosomal hydrolase activity as well as the integrity and stability of the lysosomal membrane with the passage of time.^[144,145] Studies in other cells also showed similar results.^[146-148] Short-term exposure (at 1h or 6h) of Ag NPs enhanced autophagic flux through MAPK/JNK pathway, as evidenced by the increase in LC3-II and the decrease in p62. Meanwhile, autophagy facilitates Ag NPs uptake over time,

will in turn damage the trafficking and autophagic flux by lysosomal function damage, including possibly probably lysosomal membrane permeabilization (LMP) or destruction of lysosomal integrity and lysosomal alkalization.^[149] Impairment of autophagic flux causes more reactive oxygen species generation^[147] Ali *et al.* also showed that the hindrance of autolysosome formation coincided with the actin cytoskeleton morphologic alteration in Ag NPs-treated HepG2 cells.^[150] Palladium nanoparticles (Pd NPs) induce autophagy at low concentrations, and cause blockage of autophagic flux at high concentrations, which may be due to the higher concentration of palladium nanoparticles mass in lysosomes, leading to lysosomes alkalization and greater impairment by regulating dissociation of V1 domain from vacuolar H⁺-ATPase.^[151] Titanium- (TiO₂-Au), but not silica- (SiO₂-Au) and poly(ethylene glycol)- (PEG-Au) coated gold nano-bipyramids particles, destroys the intracellular

distribution of F-actin and inhibit lysosomal proteolytic activity, especially the cathepsin B activity by binding to mature cathepsin B by NPs, resulting in inhibition of autophagic flux. The smallest TiO₂-Au NPs possess the strongest capacity to accumulate autophagosomes, and induce more LC3-II conversion and p62 accumulation in TiO₂-Au NPs-treated U-87 MG cells.^[152] When low-dose gold nanorod core/silver shell nanoparticles (Au@Ag NPs) are exposed to human hepatocellular carcinomas HepG2 cells, most of Au@Ag NPs aggregate and accumulate within the lysosomes, result in lysosomes swelling and acid phosphatase activity reduction. However, Au@Ag NPs and dissolved silver ion from the NPs contribute to the production of reactive oxygen species (ROS) and autophagy activation by ROS-mediated AKT/mTOR pathway, in accordance with increase of LC3-II and the decrease of p62. It may be that the slight damage of the lysosomes lead to the activation of autophagy, while the effect of Au@Ag NPs high-doses exposure on autophagic flux needs further exploration.^[153]

Metal oxide-based NPs: Like many nanoparticles, iron oxide nanoparticles (Fe₂O₃ NPs) also cause cell autophagy activation and lysosome damage. Accumulation of LC3-II and p62 in the Fe₂O₃ NPs-treated THP-1 cells is observed. Other researchers have found that, after Fe₂O₃ NPs are taken up by cells, the NPs are transported to and accumulate in lysosomes, leading to alteration of lysosome size and shape, abnormal lysosome acidification, and cathepsin deregulation. Fe₂O₃ NPs also result in remodeling of the cytoskeleton (F-actin and tubulin), which may cause blockage of autophagosome maturation. It is well recognized that the NPs-cell interaction is greatly different even in different cell lines of the same lineage, for example, in the Fe₂O₃ NPs-treated HepG2, Huh7 and Alexander cells. Huh7 shows significantly more lysosome damage than the other two cell lines. Under titanium dioxide nanoparticles (TiO₂ NPs) short exposure, autophagy is triggered by the translocation of TFEB from the cytoplasm to the nucleus. However, with TiO₂ NPs prolonged exposure, in addition to the accumulation of LC3-II and p62, α -synuclein, a model substrate of autophagic clearance, was not eliminated, indicating blockage of autophagic flux.^[154] Interestingly, two main crystalline phases of TiO₂ NPs, Anatase (TiO₂-A) and rutile (TiO₂-R), present different toxicity due to distinct electrical and optical properties. TiO₂-R NPs are prone to adhere to the lysosomal inner surfaces and cause more lysosomal membrane permeabilization on account of greater affinity to phospholipids.^[155] In addition, TiO₂ NPs-treated cells show irreversible cytoskeleton destruction and reduced polymerization of actin and tubulin.^[156] With the prolonged exposure time of zinc oxide nanoparticles (ZnO NPs), the cells showed the opposite tendency of autophagic flux from promotion to blockage. Autophagy is triggered by ZnO NPs, accompanied by the activation of TFEB, this process accelerates trafficking of ZnO NPs to the lysosome and dissolution of ZnO NPs to release of zinc ions in the lysosome. The excessive accumulation of zinc ions in the lysosome in

turn leads to lysosomal dysfunction, including lysosomal cavity pH increase, hydrolytic protease inactivation and aberrant LAMP-2 expression, causing damage of autophagic flux.^[157-159] The released zinc ions also interfere with and destruct the microtubule system, including clustering, fracturing and depolymerizing of microtubules.^[157] In addition, the blockade of autophagic flux related to the actin cytoskeleton has also been mentioned.^[150] 50nm ZnO NPs, but not 200nm ZnO NPs, influences autophagic processes and induces autophagic dysfunction, which may be responsible for the size-related cellular endocytosis level.^[158] In copper oxide nanoparticles (CuO NPs)-treated cell, CuO NPs was found to be deposited within lysosomes, resulting in the enlargement and abnormal accumulation of lysosomes. At the same time, abnormal distribution and accumulation of LAMP-1 and LAMP-2 were found in the CuO NPs-treated cell. These results indicated lysosome function damage, contributing to accumulation of undegraded autophagosomes and blockage of autophagic flux. Also, the deposition of CuO NPs in lysosomes lead to the release of copper ions, which may be the real cause of lysosome damage.^[160] Studies have been reported that Cerium dioxide nanoparticles (CeO₂ NPs) promote autophagic clearance by activating TFEB and up-regulating autophagy-lysosomal system-associated genes. In fibroblasts derived from a patient with late infantile neuronal ceroid lipofuscinosis (LINCL) treated by CeO₂ NPs, proteolipid aggregates that accumulated due to the defects of autophagosome lysosomal clearance function, were eliminated, and there was no lysosome damage. It proved that CeO₂ NPs promote autophagy activity, and the up-regulation of TFEB also promotes autophagic flux.

Silica-based NPs: Exposure of silica nanoparticles (Si NPs) also caused disorder in the autophagic flux. When Si NPs were internalized into the cell by the active endocytotic pathway and passive diffusion, prominent accumulation of Si NPs in the lysosome cavity through transportation by monolayer or bilayer vesicles was observed. This resulted in the enlargement of the lysosome and enhanced lysosomal membrane instability, and inhibited the expression of cathepsin including active and inactive forms. Overload of nanoparticles lead to lysosome damage. Si NPs generate reactive oxygen species and cellular oxidative stress and also destroy the lysosome. Lysosome dysfunction leads to the inhibition of autophagic flux, leading to the aberrant increase of LC3-II and p62.^[161-163]

QD-based NPs: Exposure of graphene oxide quantum dots (GO QDs) to the cell leads to the hindrance of autophagic flux through the reduction of the quantity and activity of cathepsin B and the defect of lysosomal degradation capacity.^[164] Molybdenum Disulfide Quantum Dots (MoS₂ QDs) affect lysosome biogenesis, as evidenced by the increased amount of acidic vesicles and the expression of cathepsin B and LAMP-1. MoS₂ QDs cause the nuclear localization of TFEB, and an increase in autophagic flux, accompanied by an increase in LC3-II and p62 degradation. In TGF- β -induced Endothelial-to-Mesenchymal Transition by

HUVECs, MoS₂ QDs rescue the autophagic flux damage caused by induction.^[165] When cells are treated by Cadmium Telluride and Cadmium sulphide, CdTe/CdS core/shell quantum dots (CdTe/CdS QDs), the intracellular lysosomal enzyme activity is abnormally enhanced. Although quantum dots induce autophagy, the effect on the autophagosome maturation needs further exploration.^[166]

Carbon-based NPs: Many studies have proved that fullerenes and their derivatives interfere with the self-assembly of actin filaments and microtubule polymerization, change the morphology, destruct cytoskeleton.^[167-169] Although studies have suggested that fullerenes can cause autophagosome accumulation, the mechanism that causes this phenomenon still needs to be explored.^[168] In different types of cells, graphene oxide nanoparticles (GO NPs) exhibit similar toxicity. After exposure in the cell, the nanoparticles will accumulate in the lysosome, affecting lysosome acidification and membrane stabilization. The autophagosome maturation is also inhibited, resulting in the autophagic flux disorder.^[170-173] When exposed to the cells, carbon nanotubes (CNT), including single-walled and multi-walled, accumulate within the lysosome, causing lysosomal membrane destabilization.^[170,174,175] Zhou *et al.* found that carbon nanotubes, but not spherical nanoparticles impair autophagic flux by inhibiting the expression of SNAPIN (SNAP-associated protein), important to the autophagosome-maturation and lysosomal acidification in macrophages, without disruption of the microtubule network and actin cytoskeleton.^[174] Graphite carbon nanofibers (GCNF) hinder the autophagic flux by actin cytoskeleton disruption and lysosomal membrane destabilization.^[176]

Rare earth oxide-based NPs: Giant cellular vacuoles were observed in the cells exposed to rare earth oxide nanoparticles (REO NPs), including La₂O₃, Gd₂O₃, Sm₂O₃, and Yb₂O₃. Following research found that these cellular vacuoles were abnormally enlarged and had alkalinized lysosomes. Meanwhile, studies found rare earth oxide ion shedding in acid lysosomes produced a biotic phosphate complexation with phosphates from the surrounding lysosomal lipid bilayer, causing lysosomal organelle damage and autophagosome maturation block.^[17,177,178]

Other NPs: The internalization of all polystyrene nanoparticles (P NPs) can activate autophagy, but the effect on autophagic flux depends on the surface charge of P NPs. Studies have shown that cationic surface-modified polystyrene nanoparticles (NH₂-P NPs) cause lysosome dysfunction and hinder autophagic flux, while neutral (P NPs) and anionic surface-modified nanoparticles (COOH-P NPs) promote the clearance of autophagic substrates.^[179] Poly-(β -amino ester) polymeric nanoparticles, as pH-sensitive polymeric nanoparticles, were reported to activate autophagy through mTOR-dependent pathways at low concentration. Long periods stimulation and high concentrations exposure of NPs blocked autophagic flux by affecting lysosomal acidification and abnormal cathepsins expression as well as damaging lysosomal V-ATPase complex via dissociating V1 protein from the lysosome-binding V0 protein.^[180] It has been

demonstrated that biodegradable poly-(lactic-co-glycolic acid) nanoparticles (PLGA NPs) tend to localize to lysosomes when exposed to different types of cells, and it can reduce the lysosomal pH and increase the activity of lysosomal proteolytic enzymes by releasing their acidic components.^[181,182] PLGA NPs rescue lysosomal pH in two different autophagy inhibitor-treated cells, in which chloroquine (CQ) alkalinizes lysosomes and bafilomycin A1 (BafA1) suppresses lysosomal V-type ATPase. PLGA NPs also prevented lysosomal membrane permeabilization (LMP) and the destruction of lysosomal structural integrity by acting against ROS induced by mitochondrial parkinsonian neurotoxin 1-methyl-4-phenyl-pyridinium ion (MPP⁺). In lysosomal-associated *in vitro* genetic models, for example Parkinson disease (PD) harboring ATP13A2 mutations, Gaucher disease (GD) caused by GBA gene homozygous mutations encoding GBA protein, and X-linked myopathy with excessive autophagy (XMEA) caused by VMA21 gene mutations, treatment with PLGA NPs restore lysosomal function and enhance autophagosome clearance.^[181] Notably, PLGA NPs (GA:LA 50:50) with a higher glycolic acid (GA) to lactic acid (LA) ratio content possess stronger ability of lysosomal pH modulation and autophagic flux modulation than PLGA NPs (GA:LA 100:0, 75:25).^[182]

Autophagic lysosome reformation (ALR) is the ultimate step in autophagy process and plays a vital role in maintaining lysosome homeostasis. During autophagy, autophagosomes will fuse with lysosomes to form autolysosomes, in which cargo be hydrolyzed by diverse lysosomal enzymes, numerous lysosomes are consumed during this period, in order to maintain the lysosome homeostasis, autolysosomes are able to sprout to form new lysosomes.^[183] Studies have shown that NPs can damage ALR. When primary hepatocytes are exposed to NPs, including upconversion NPs (UCNs) and SiO₂ NPs, lysosomal organelles enlarge persistently over time, but the lysosomal cavity is acidic and not alkalinized. Further research found that phosphatidylinositol 4-phosphate (PI(4)P) relocate to autolysosomes but fail to turn into phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂), which resulted in the failure of clathrin recruitment and tubule budding and autophagy blockage.^[184] This finding may partly account for earlier reported hepatotoxicity of UCNs *in vivo*.

The impacts of NPs on autophagic pathway, lysosome dysfunction and their cytotoxicity is not fully considered disadvantageous, since on the other side, researchers have been developing their therapeutic values based on investigation on the mechanism of their interaction with cell cultures. Many traditional chemical compounds have been applied to modulate different phases of autophagy and autophagy inhibition appears to be beneficial for the treatment of several types of tumor and inflammatory diseases.^[185] Among them, chloroquine (CQ) and its derivative HCQ, profoundly different from other autophagy inhibitor like BafA1, are the only Food and Drug Administration (FDA) approved drugs and currently used for tumor treatment based on decreased autophagosome

maturation, which was only recently clearly elucidated.^[186] Recent research has shown that AgNPs treatment will induce p62 and LC3-II-II accumulation, suggesting a blockage in autophagic flux, together with cellular damage in A549 cells.^[149] Lysosomes with high level of SiNPs fail to fuse with autophagosomes and receive autophagic cargo in Hela cells. Researchers thus hypothesize a reduced metabolic activity in such cancer cells as a result of the effect above.^[187] This research indicates NPs as a novel autophagy treatment in addition to traditional chemical drugs and their potential application in clinical therapies, since new methods of regulating autophagy has been developed using peptide conjugated polymeric NPs.^[188] In addition to tumor treatment applications, lysosomal dysfunction induced via LMP by dendrimers and NPs has been applied to ensure lysosomal escape for intracellular drug delivery in gene therapy protocols.^[189] In addition, nanoparticles that promote maturation, such as PLGA NPs, can be served as a treatment for neurodegenerative diseases. However, it is always worth notice that the balance between cellular toxicity and therapy values needs to be maintained and the mechanism behind the cell selectivity of NPs treatment should be further investigated in order to ensure the safety of the application of NPs in clinical situations.

10. Conclusion

In the past nearly 3 decades, we have witnessed more and more intensive investigations on autophagy, from the primary identification of autophagy related genes to specific molecular mechanisms mediating each single phase and their time and space regulation with following research directions spreading along the roles of autophagy in pathogen invading situations and nanoparticle cytotoxicity. These advances provide us new insights and more comprehensive understanding about the basic biological pattern. Although many of these molecular mechanisms have been discovered, we have also summarized some unknown problems that still exist in this field. How all these different molecules involved in fusion are recruited to mature autophagosomes in appropriate time and space order, and how the selectivity of some shared proteins between the endocytic pathway and the autophagic pathway are precisely regulated are still not fully understood. More interestingly, details need to be discovered about pathogens and their effectors' function on A:L fusion, which may provide novel research ideas for basic biological mechanisms. In addition, it is worth noticing that safety issues arise with the more and more widely usage of nanoparticles. More details about the mechanisms of their cytotoxicity remains to be studied. The answers to these questions and future studies will provide a theoretical basis for the development of new treatments and the research for new drug design targets and the improvement of the safety items for the applications of nanoparticles in the future.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Supporting Information

Not Available.

Acknowledgments

The work was supported by grants to R.L. the National Key Research and Development Program of China (Grant No.2017YFD0400200), from the National Natural Science Foundation of China (Grant No. 31771532), the “Shuangchuang”, “Six talent peaks” and “333”projects in Jiangsu province.

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