Recent advances in molecular mechanisms of autophagosome-lysosome fusion and impacts of exogenous factors

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 discussed 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.

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, the cytoplasmic contents are surrounded by a bilayer membrane structure called autophagosomes, and delivered to 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 as xenophagy[1], so it 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 responses to its upper regulators like mammalian target of rapamycin complex 1 (mTORC1) through its phosphorylation activity which previously 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 distinguish area of

the celluar 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 above are relatively less understood, such as maturation of autophagosome and its final fusion with endosomes or lysosomes. The hole procedure of autophagy is shown step by step in Figure 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 have 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 were as well discussed in this review.

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 trafficking. During the transportation of substances, all kinds of vesicles and organelles are oriented alongside 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][Figure 2]. Both of these two motifs are present in the 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 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 distinguished from increasing cholesterol storage in lysosome such as Niemann-Pick Type C disease[24-26]. Meanwhile, 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 lysosome 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 do[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][Figure 2]. Notably, this mechanism is not involved in classical starvation-induced autophagy, but in clearance for 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 autophagsomes 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 accumulated near nucleus, and the minus end directed dynein-dynactin motor protein complex is as expected found responsible for this phenomenon and essential for A:L fusion[33]. Rab7 recruited to these mature autophagsomes 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][Figure 2]. 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][Figure 2]. In addition to autophagosome migration, proper lysosome positioning is as well 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 pareneled 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][Figure

2]. 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][Figure 2].

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 for an instance, SNARE is classified into Q-SNARE (having a glutamic acid residue) and R-SNARE (having an arginine residue). Q-SNARE is further divided into Qa, Qb and Qc-SNAREs based on the amino acid sequence of the SNARE domain. The SNARE parallel four-helix bundle formed by the Qa, Qb, Qc and R-SNARE compositions on two closing membrane structures and release the force of noncovalent in-

teraction between the hydrophobic chains so that the two membrane structures moves 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 form 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][Figure 2]. 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, Qa-SNARE protein STX7 to mediate the fusion process, independently of the STX17 pathway [44][Figure 2]. Observations from in vitro autophagic fusion assay also fits well with the model just mentioned[51, 52]. Along with the identification of this diversity of SNARE complex compositions in such a single process are question about the differences and evolutionary relationship between STX17 and YKT6, and whether they function independently in different situations or cooperatively in the same conditions, which remain 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 or how these SNAREs are selectively recruited to specific cellular structures. STX17 are reported to localize at ER-mitochondrial contact sites normally, bind to ATG14 upon starvation and is involved in autophagosome formation at this area, and ultimately increase its localization on autophagosomes[53, 54][Figure 2]. Later, Suresh et al. found that STX17 interacts with mATG8s through its two LIRs (LC3 interacting regions) which lies right separately inside the two SNARE domains, and 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][Figure 2]. These three molecules together form STX17-LC3B-IRGM holocomplex called ARP (autophagosome recognition particle) [Figure 2]. 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, which then may effectively prevent the binding of other SNARE proteins before the mature form autophagic 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][Figure 2].

Meanwhile, proteins like αSNAP and NSF (N-ethylmaleimide-sensitive factor) are found involved in A:L fusion, which are responsible for SNARE disassembly or priming after the fusion is completed, supported by block at autophaic flux in the absence of αSNAP, though interestingly without affecting previous translocation of STX17 to autophagosome[57]. Possible hypothesis is that sustainable usage of SNAREs for the fusion in next round autophagy is impaired by deficiency in SNARE disassembly due to depletion of αSNAP. Interestingly, long before this study, GABARAP are proved to interact with NSF[58], and question arises if members of ATG8 family are involved in A:L fusion in an αSNAP and NSF dependent manner.

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 GTPase, Rab switches 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). Distinguish distribution of Rabs at different vesicles make them perfect biomarkers for various parts among the sophisticated membrane traffic map, and Rab conversions are parallel with compartment maturation events and trafficking process[59-62]. It is in anticipation that some members of the Rab family are involved in the regulation of A:L fusion, since autophagy is as well as a curtail 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][Figure 2].

In mammalian cells, GEFs that mediated the activation of Rab7 and its function of manipulating membrane fusion process has not yet been found. However, in yeast and drosophila, a heterodimer Ccz1-Mon1 has been identified and are required for intact and smooth fusion process, which 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, mutation of Rab5 inactivating did not affect autophagosome and lysosome fusion, indicating that Rab5 is not required for Ccz1-Mon1-dependent Rab7 recruitment here[68]. Later, 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 region) and subsequently activate Ypt7[70][Figure 2].

Other Rab protein, like 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 depends on both its binding with LC3 and GAP activity[71], which may be

results of overwhelm Rab33b inactivation.

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 may somehow 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 [Figure 2]. 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][Figure 2]. 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]. Given that the Atg12-Atg5-Atg16 complex localization at phagophore depends on the exist of PIP3, Chen et al. showed that TECPR1 and ATG16 competitively form a complex with the ATG12-ATG5 conjugate, and TECPR1 binding with PIP3 is dependent on its previous reported interaction with ATG5-ATG12, which then may promote maturation and A:L fusion[76] [Figure 2].

Ectopic P-granule protein 5 (EPG5), a gene that is responsible for the Vici Syndrome and 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 [Figure 2]. Knocking down EPG5 results in fusion abnormalities and various endocytic vesicles 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-acetylamino groups[78]. GORASP2/GRASP55 undergoes de-O-acetylglucosamine modification in the presence of glucose stress. Then, 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[79] [Figure 2].

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 appears to be loosely related both structurally and functionally. The time and spatial order of re-cruitment and their synergistic effect are still not fully understood.

Phosphoinositides and their conversions in A:L fusion

The major mechanism about 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

were later enriched in a certain subdomain of the membrane, affecting the curvature of the membrane, and enrichment of specific PI would act as a signal or second messenger for downstream proteins binding that contain a special domain, such as the PX domain[80]. Intracellular conversion of PIs has been found involved in both autophagosome generation with recent reseaches focused on their role in the fusion step [81].

Upon autophagy initiation, PI3P was produced by the Vps34/PI3K complex and enriched in membrane subdomains that were prepared for ATG protein recruitment and following phagophore biogenesis, termed as phagophore assembly site (PAS)[5, 6]. The function of PI3P in proper transport of autophagosome has been mentioned above, which in detail is related to the recruitment of FYCO1 and the assembly of motor protein complex. 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] [Figure 3]. In mammalian cells, the amount and distribution of PIP3 were negatively regulated by several PI3P phosphatases of myotubularin protein

family (MTMR), such as MTMR3, MTMR6, MTMR7, MTMR14/Jumpy, and was found involved in the initial part of autophagy [81, 84-86] [Figure 3]. By contrast, MTMR like protein in yeast Ymr1 has been proved 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 KD cells[81]. The functional overlap or some other regulating factors unknown may explain the negative effect of MTMR proteins in higher eukaryotes. More detailed mechanism about MTMR proteins and their role in the late stages of autophagy still remains to be further discovered.

Another PI that generates 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 kinas-α (PI4K Ⅱα) is secreted by trans-Golgi apparatus (TGN) and dispersed into the cytoplasm, some of which are localized to autophagosomes in a palmitoylation-dependent manner to mediate PI4P redistribution[87] [Figure 3]. Interestingly, PI4K Ⅱα interacts with the Atg8 homo-logs GABARAP and GABARAPL2 but not LC3 [Figure 3]. Knockdown of GABARAP inhibits the recruitment of autophagic PI4K Ⅱα, while knockdown of PI4K Ⅱα does not affect the cellular distribution of GABARAP, indicating that GABARAP acts in the upstream of PI4K Ⅱα [87]. However, more details about the mechanism how PI4P itself are involved here are still poorly understood.

PI4P is as well generated by PI4K IIα and have significant functions on membrane of Rab7 positive endocytic compartment like late endosome/lysosome, together with its downstream derivatives[88] [Figure 3]. PI4P was then converted into PI(4,5)P2, which was also found recently 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 PI4P5Kγ on Rab7 positive endosomes, acting as a precursor for PI(4,5)P2 generation[88] [Figure 3]. Knockdown of PI4P5Kγ which impaired the amount of PI(4,5)P2 shows similar effects as PI4K IIα KD cells mentioned above[88]. The increase of endosomal PI(4,5)P2 leads to Rab7 inactivation and thus facilities 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 not, such as RILP or Vps35[88] [Figure 3]. These results suggest that A:L fusion requires cellular cycling of some of its key factors which then is controlled by PIs conversion, while the researchers hypostasis that it is quite possible that there are still other factors related to PI(4,5)P2 and Rab7[88].

On the country, another protein called OCRL (Oculo Cerebro Renal of Lowe) activated on lysosome would converts PI(4,5)P2 back to PI4P

with its PI(4,5)P2 5-phosphatase activity, mutations in which would lead to Oculo Cerebro Renal Lowe syndrome[89] [Figure 3]. Here, upon A:L fusion, an increase of lysosomal PI(4,5)P2 response mediated by PIP5K1α and β was triggered by the release of autophagic cargo to lysosomes like mtDNA (mitochondrial DNA) in the manner of TLR9 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 preciously reported process of mTORC1 inactivation-reactivation in autophagic lysosome reformation (ALR) [90-92] [Figure 3]. OCRL here are proved to be recruited by MCOLN1 (mucolipin-1) with necessary present of AP2[90] [Figure 3]. 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 are required here to maintain the sustainability of autophagic flux[90]. Given that downstream products of TLR9 signaling pathway are proinflammatory cytokines[96], this research indicates that autophagic cargo would potentially induce an immune response leading to inflammation or possibly to resist pathogen evasion, especially in lysosomal storage conditions where cargo like mtDNA will be disable to be degraded due to impairment of the activity of lysosomal hydrolase[90]. Besides, lysosomal PI(4,5)P2 would also be produced by PI5P4Ks from PI5P,

and depletion of PI5P4Ks leads to suppression on A:L fusion and upregulation of lysosomal genes by transcription factor TFEB[97] [Figure 3], which is consistent with the consequence of OCRL knockdown in starvation induced autophagy shown in this research, perhaps somehow due to similar alternation on the amount of PI(4,5)P2 in this regulator circuit. This regulation pathway may as well be involved in nanoparticle treatment conditions since it has been reported that autophagy activation induced by graphene oxide nanoparticle involves the TLR4/9 pathway[98].

Another noticeable 5-phosphatase is INPP5E (Phosphoinositide phosphatase inositol polyphosphate-5 phosphatase E), one of the genes that are responsible for Joubert syndrome, is found involved in the late fusion step of autophagy by manipulating conversion from PI(3,5)P2 to PI3P[99, 100] [Figure 3]. Knockdown of INPP5E obviously impaired autophagic flux and significantly increased the number of intracellular autophagosomes labeled with LC3 without affecting the luminal acidity of lysosome and its fusion with endosomes, suggesting a specific role of INPP5E in the autophagic fusion pathway[99]. Once the amount of PI(3,5)P2 on the lysosomal membrane was decreased by INPP5E, PI(3.5)P2 binding CTTN would be released, which then was phosphorylated, bind with and stabilize actin filaments near the approaching lysosome and autophagosome [99, 101][Figure 3]. However, INPP5E exists predominately in neuronal cells, further re-

search is required to elucidate whether there is a similar mechanism manipulating A:L fusion in other cells.

Factors that regulate A:L fusion

The PI3K complex is considered as an essential regulator of autophagy. The core component of the PI3K complex, Beclin1 interacts with VPS34 thus working as a platform for binding with other components, such as ATG14L, Ultraviolet (UV) radiation resistance-related (UVRAG) and Rubicon, and forming diverse complexes respectively with different functions in autophagy[5, 6, 102, 103]. UVRAG binds to HOPS complex subunit VPS16 to stimulate Rab7 activity thus facilitate the A:L fusion[102], while normally, UVRAG binds to Rubicon and inhibits the activity of VPS34/PI3K[104][Figure 2] . In nutrient-rich conditions, UVRAG-Rubicon interaction was enhanced via UVRAG phosphorylation mediated by the upstream mammalian target of rapamycin complex 1 (mTORC1), suggesting a regulation role of mTORC1 in the late step of autophagy[39]. On the contrary, this interaction is reduced when the PPxY motif of UVRAG is ubiquitinated by SMURF1 at lysine residues 517 and 559, resulting in the promotion of autophagosome maturation[105][Figure 2]. 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][Figure 2].

Question arises that how an endosome-resident protein would 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 here antagonizes Rubicon and targets both UVRAG complex and HOPS complex to autophagosomes by interacting 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][Figure 2]. 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] [Figure 2]. 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][Figure 2]. 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 although the mutation of the V0 subunit of V-ATPase can still maintain its acidification function, there are serious vesicle fusion defects in the mutant cells[112]. Here ATP6V0D2 subunit, which was specifically expressed in macrophages, interacts 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 the latter to promote the fusion process[112][Figure 2]. 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 homologs of Rubicon, functions as an effector of Rab7 through its RH-domain interacting with Rab7 and a conserved 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] [Figure 2]. 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 GTPase. Arl8b has been reported to bind with VPS41 of HOPS and are required for endocytic pathway, and VPS39 interacts both with PLEKHM1 and SKIP[115, 116][Figure 2]. Taken together, PLEKHM1 may somehow mediates 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] [Figure 2]. 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 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 furtherly studied in future research.

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. Mainly 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

the 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 infected by *Mycobacterium tuberculosis*, autophagic flux was significantly inhibited, resulting in the accumulation of a large number of autophagosomes failed to fuse with lysosomes[119]. The 6-kDa early secreted 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], 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 resulting in *Mycobacterium tuberculosis* long-term survival and its non-effectively clearance in infected cells[119] [Figure 2]. Other factors like PhoP from H37Rv strain of *M. Tuberculosis*, cause less co-localization 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 as the 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] [Figure 2].

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 positive puncta with 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 author reasoned that increased IsaB may alter the acidification of lysosomes and block A:L fusion indirectly[125] [Figure 2].

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 promotes Rubicon-Beclin 1 interaction inhibiting the kinase activity of the complex, thus blocked 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 autohagosome and lysosome and the cytoskeleton system have also been proved 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 were found binding with STING, a key mediator for IFN signaling[134],

through interaction with Beclin1 and somehow depressing 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 rescue the depressed IFN response and diminished anti-virus ability of the infected cells. Meanwhile, 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 Figure 2.

The impacts of NPs on autophagosome maturation

The prefix “nano” is derivative from the Greek “nanos”, meaning “dwarf” and is becoming increasingly widely mentioned among modern scientific researches. 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 prospect [138]. Following researches have 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. Autoph-

agy 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 corresponding energy and materials [140]. On the other hand, the aggregated protein and other waste products could not be removed immediately due to the weakening of autophagy, which leads to the cell function degradation and causes a series of neurodegenerative diseases, such as Parkinson's disease, 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 could not be ignored. Here in this section, we discussed the impacts of NPs on autophagy, especially the last steps 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 colocalize 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, result in accumulation of NPs in the lysosome and a series of dysfunctions related to the lysosome. However, there are several nanoparticles possess ability to restore (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 mechanism by which different nanoparticles affect the autophagosome maturation allows 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 Au NPs lead to lysosomes swelling and reduce 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, is observed vesicles. 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]. Compared with 10nm and 25nm Au NPs, 50nm Au NPs are readily taken up by cells and cause greater lysosome damage and autophagic flux blockage [16]. Meanwhile, compared with negatively charged Au NPs, positively 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 the internalization of gold nanoparticles varies in a shape-dependent manner [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, in turn damages the trafficking and autophagic flux by lysosomal function damage, including possible probable 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 show the hindrance of autolysosome formation is 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 amass in lysosomes, leading to lysosomes alkalization and greater impairment by regulating dissociation of V1 domain from vacuolar H+-ATPase [151]. Titania- (TiO2-Au) not silica- (SiO2-Au)and poly(ethylene glycol)- (PEG-Au) coated gold nano-bipyramids particles destroy the intracellular distribution of F-actin and inhibit lysosomal proteolytic activity, especially the cathepsin B activity by binding to mature cathepsin B by NPs, result in inhibition of autophagic flux.

And 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 lysosome leads to the activation of autophagy, while 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 (IO NPs) also cause cell autophagy activation and lysosome damage. Accumulation of LC3-Ⅱ and p62 in the IO NPs-treated THP-1 cells is observed. Other researchers have found that, after IONPs are taken up by cells, the NPs are transported to the lysosome and accumulate in lysosomes, leading to alteration of lysosomes size and shape, abnormal lysosome acidification, cathepsin deregulation, IO 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 IO NPs-treated HepG2, Huh7 and Alexander cells, huh7 is significantly more lysosome damage than the other two cells. Under titanium dioxide nanoparticles (TiO2 NPs) short exposure, autophagy is triggered by the translocation of TFEB from the cytoplasm to the nucleus. However, with TiO2NPs prolonged exposure, in addition to the accumulation of LC3-Ⅱ and p62, a-synuclein, a model substrate of autophagic clearance, did not be 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 serve 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 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 lead to the lysosomal dysfunction, including lysosomal cavity pH increase, hydrolytic proteases inactivation

and aberrant LAMP-2 expression, causing the damage of autophagic flux [157-159]. The released zinc ions also interfere with and destruct the microtubule system, including clustering, fracture and depolymerization of microtubule [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, influence autophagic process and induce 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 indicating lysosome function damage, contributing to accumulation of undegraded autophagosomes and blockage of autophagic flux. and the deposition of CuO NPs in lysosomes leads to the release of copper ions, which may be the real cause of lysosome damage [160]. Studies has been reported that cerium dioxide nanoparticles (CeO2 NPs) promote autophagic clearance by activating TFEB and up-regulating autophagy-lysosomal system-associated genes. Fibroblasts derived from a patient with late infantile neuronal ceroid lipofuscinosis (LINCL) are treated by CeO2 NPs, proteolipid aggregates that accumulated due to the defects of autophagosome lysosomal clearance function, was 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 difmaturation, prominent accumulation of Si NPs in the lysosome cavity through transportation of monolayer or bilayer vesicle, resulting in the enlargement of the lysosome and lysosomal membrane instability, inhibited the expression of cathepsin including active and inactive forms. Overloaded nanoparticles lead to lysosome damage. Si NPs generate reactive oxygen species and cellular oxidative stress also destroy 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 lysosomes 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 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 explore [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 autophagosomes maturation is also inhibited, result in the autophagic flux disorder [170-173]. When exposed to the cells, carbon nanotubes (CNT), including single-wall and multi-wall, accumulate within the lysosome, Fcausing the 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 in microtubule network and actin cytoskeleton [174].

And 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 alkalized 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 lysosome

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 2 different autophagy inhibitor-treated cells, in which chloroquine (CQ) alkalinizes lysosomes and bafilomycin A1 (BafA1) depresses lysosomal V-type ATPase. PLGA NPs also prevent lysosomal membrane permeabilization (LMP) and the destruction of lysosomal structural integrity by 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, X-linked myopathy with excessive autophagy (XMEA) caused by VMA21 gene mutations, the treatment of PLGA NPs restores lysosomal function and enhances 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, autolysosome is able to sprout to form new lysosome [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 hosphatidylinositol 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 as disadvantages, since on the other side, reseachers 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 derivatives 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 until recently clearly elucidated [186]. Recent researches have 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 autophagosome and receive autophagic cargo in Hela cells. Researchers thus hypothesis a reduced metabolic activity in such cancer cells as a result of the effect above [187]. These researches indicate NPs as a novel autophagy monitor in addition to traditional chemical drugs and their potential application in clinical therapies, since new method monitoring 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.

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 spread among the role 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 still worth notice that the safety issues arose with the more and more widely usage of nanoparticles and more details about the mechanisms of their cytotoxicity remains to be further studied. The answers to these questions and future studies will pro-

vide 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.

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

Component	Identification	Functions	Refs
Cytoskeleton and vesicle transport			
Actin Filaments system			
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]
Microtubule system			
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 autophagsome 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]
SNAREs			
STX17-SNAP29-VAMP8 ternary SNARE complex			
STX17	Qa-SNARE	Qa-SNARE protein localized on autophgosome membrane	[43]
SNAP29	Qbc-SNARE	Cytoplasmic Qbc-SNARE protein	[43]
VAMP8	R-SNARE	R-SNARE protein localized on lysosome membrane	[43]

YKT6-SNAP29-STX7 ternary SNARE complex			
YKT6	R-SNARE	A second R-SNARE protein localized on lysosome membrane	[44]
STX7	Qa-SNARE	Qa-SNARE protein localized on autophgosome membrane	[44]
SNARE recruitment			
ATG14	ATGs	Interaction with STX17, STX17 translocation to autophagic structure	[53, 54]
IRGM	Small GTPase	STX17 recruitment through interacting with LC3	[55]
SNARE disassembly and recycling			
αSNAP & NSF		SNARE disassembly or priming, interacting with GABARAP	[57, 58]
Rabs			
Rab5	Rab GTPase	Recruitment of Ccz1-Mon1	[69]
Ccz1-Mon1	GEF for Rab7, Rab5 effctor	Rab7 recruitment and activation on autophagsome 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]
Tethering factors			
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]
GORASP2/GRASP55	Golgi stacking protein	Scaffold protein for Golgi multi-flattened structure	[78, 79]
PIs & PIs con-versions			
Vps34 Complex	PI3K conplex	PI3P generation on autophagosome membrane	[5, 6]
MTMRs	PI3P phosphatases	Liberation of PI3P from autophagosome membrane	[81]
PI4K Ⅱα	Lipid kinase	PI4P generation on autophagosome or lysosome membrane	[87, 88]
PI4P5Ks	Lipid kinase	Conversion from PI4P into PI(4,5)P2	[88, 90]
PIKfyve	Lipid kinase	Conversion from PI3P into 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]
Regulation factors			
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 fuison	[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; GORASP2/GRASP55, Golgi reassembly-stacking protein 2/Golgi reassembly-stacking protein of 55 kDa; MTMRs, Myotubularin-related phospholipid phosphatase proteins; PI4K IIα, Phosphatidylinositol 4-kinase IIα; 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, Oculo Cerebro 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.

Table 2. Factors that disturb fusion exist in pathogens

Pathogen	Factors	Host targets and possible mechanism	Refs
Bacteria factors			
M. Tuberculosis str. H37Rv/BCG	ESAT-6	Enhancing mTORC1 activity, interrupting Rab7 recruitment	[119, 121]
M. Tuberculosis str. H37Rv	PhoP	Interrupting Rab7 recruitment, ESAT-6 transcription	[121, 122]
Methicillin-resistant *S. aureus*	IsaB	Preventing lysosomal acidification	[124, 125]
Virus factors			
Human parainfluenza virus (HPIV3)	Phosphoprotein P	Binding with SNAP29 and inhibiting STX17-SNAP29 interaction	[126]
Kaposi's sarcoma-associated herpes-virus (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.

Table 3. Nanoparticles and their impacts on autophagosome maturation

Types of NPs	Nanoparticles	Average core size (nm)	zeta-potential(mV) (medium)	Dose (μg/ml)	Exposure time (h)	Cell lines	Impacts on A:L maturation and mechanism	Refs
Metal-based NPs	Au NPs	10,25,50	negative	1nM	24	NRK	Maturation blockage, lysosome alkalinization，lysosomal enzyme activity decline, size-dependent manner	[16]
	Au NPs	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 NPs	25	-5.43±0.59	2,4,8	24	HEK293T, A498	Maturation blockage, lysosome degradative activity repression，lysosome impairment	[144]
	Ag NPs	~30	-4.3(water)	5,10	24,48	THP-1	Maturation blockage, lysosome alkalinization, lysosomal membrane permeabilization	[148]
	Ag NPs	9±2.2,19±2	-31, -29.8	10,25,100μM	6	MCF-7, MDA-MB-468, HaCaT	Maturation blockage, lysosomal membrane permeabilization, time-dependent manner	[147]
	Ag NPs		-5.98±0.73	2,4,6	24	PC-3	Maturation blockage, lysosomal membrane integrity decline, lysosomal quantity decrease, lysosomal protease activity attenuation	[145]
	Ag NPs	~20		2.5	6	HepG2	Maturation blockage, actin cytoskeleton morphologic change	[150]

	Ag NPs	69.8 ± 0.55	−30.5	50,100,200	24	A549	Maturation blockage, lysosome alkalization	[149]
	Pd NPs	6.3±1.9, 11.9±2.3, 20.4±1.7	-2.09±0.32, -2.51±0.45, -3.48±0.66	0.1,10	24	Hela, HEK293, HepG2	Maturation blockage, lysosome alkalization, size-dependent manner	[151]
	Au@Ag NPs	length,width: 72.7±5.8, 33.7±2.4	−8.4 ± 0.7	0~40(Ag)	6,12,24	HepG2	possible maturation blockage, lysosomal degradation inhibition	[153]
	TiO2-Au NPs	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 NPs	15~20		100	24	MCF-7	Maturation blockage, lysosomal membrane disruption	[192]
	IO NPs	IO-cubes:36 IO-clusters: 38		10,50,100	24	HepG2, Huh7, Alexander cells	Maturation blockage, lysosomal membrane permeabilization	[193]
	IO NPs	200(with fluorescent label)	-2	10,50,100	12	HepG2, Huh7, Alexander cells	Maturation blockage, lysosome acidification impairment, F-actin and tubulin remodeling, lysosome dysfunction	[194]
	TiO2 NPs	15,50,100	-30~-15	10,50,100,200,500	24,48,72	Hela, H4 neuroglioma cells	Maturation blockage, lysosomal membrane permeabilization	[154]
	TiO2 NPs	TiO2-A: 27.3±5.8 TiO2-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]

	TiO2 NPs	~25	-9.57 ± 1.68	1,10	24,24*7	A549	Possible maturation blockage, cytoskeleton irreversible disruption	[156]
	ZnO NPs	50,200	-10.3, -10.0	10,30,100	12,24	A549	Maturation blockage, lysosome dysfunction	[158]
	ZnO NPs	length,width: 57.8, 25.6	15.6 ± 7.2	20	0~12	PC12	Maturation blockage, lysosome dysfunction, microtubule system impairment	[157]
	ZnO NPs	~50	-11.5	3,10,30	0~24	HEK293T, A549	Maturation blockage, lysosome dysfunction	[159]
	ZnO NPs	100		1.25	6	HepG2	Maturation blockgae, actin cytoskeleton morphologic change	[150]
	CeO2 NPs	4.3±0.5	CeO2-GlcNAc:-13.6±1.5; CeO2-PEG200:-2.6±1.8; CeO2-PEG1K:-2.0±1.5; CeO2-PEG10K:-4.4±2.4; CeO2-PVP10K:-5.3±1.1; CeO2-PVP40K:-6.8±3.0 （water）	100ppm	24	Hela, Fibroblasts derived from patients with LINCL	Maturation promotion, TFEB up-regulation,	[195]
	CuO NPs	~50	-13.17	1,2,5,10,20, 50	24	HUVEC	Maturation blockage, lysosome dysfunction	[160]
Silica-based NPs	Si NPs	63.88±10.35, 46.15 ± 5.53	-29.8, -27.8	50	24	L-02	Maturation blockage, lysosome disruption	[161]

	Si NPs	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 NPs	16.75±3.38	-16.81 ± 0.5	25,50,100	24	BEAS-2B	Maturation blockage, ROS, lysosomal alkalinization, 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]
	MoS2 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 NPs	15.7	-49.1 ±2.0	0.01~10mM	6,24,48	LLC-PK1	Possible maturation blockage, cytoskeleton disruption.	[168]
	Fullerene NPs	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 NPs	Thickness:1 lateral size: 500~800	-9.82 ± 1.06	40,50,60	1,3,6,12,24	PC12	Maturation blockage, lysosome alkalinization, lysosomal membrane permeabilization	[171]

	GO NPs	Thickness:1 lateral size: 50~800	-11.90 ± 1.06	40,60,80	24	F98	Maturation blockage, lysosome alkalization, lysosomal membrane destabilization	[173]
	GO NPs	Thickness:1		5,10,20	24	mESCs	Maturation blockage, lysosome alkalinization, lysosomal membrane permeabilization	[172]
	GO NPs	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	La2O3,Gd2O3,Sm2O3, Yb2O3	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	NH2-P NPs	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) NPs			50, 100, 200	4, 12, 24	MCF-7	Maturation blockage, Abnormal cathepsin expression, lysosomal alkalization	[180]
	PLGA NPs	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.N370Sand p.G377S), XMEA fibroblasts (c6A to G mutation)	maturation promotion, lysosomal pH restoration, cathepsin D activity restoration	[181]
	PLGA NPs	~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

Figure 1.

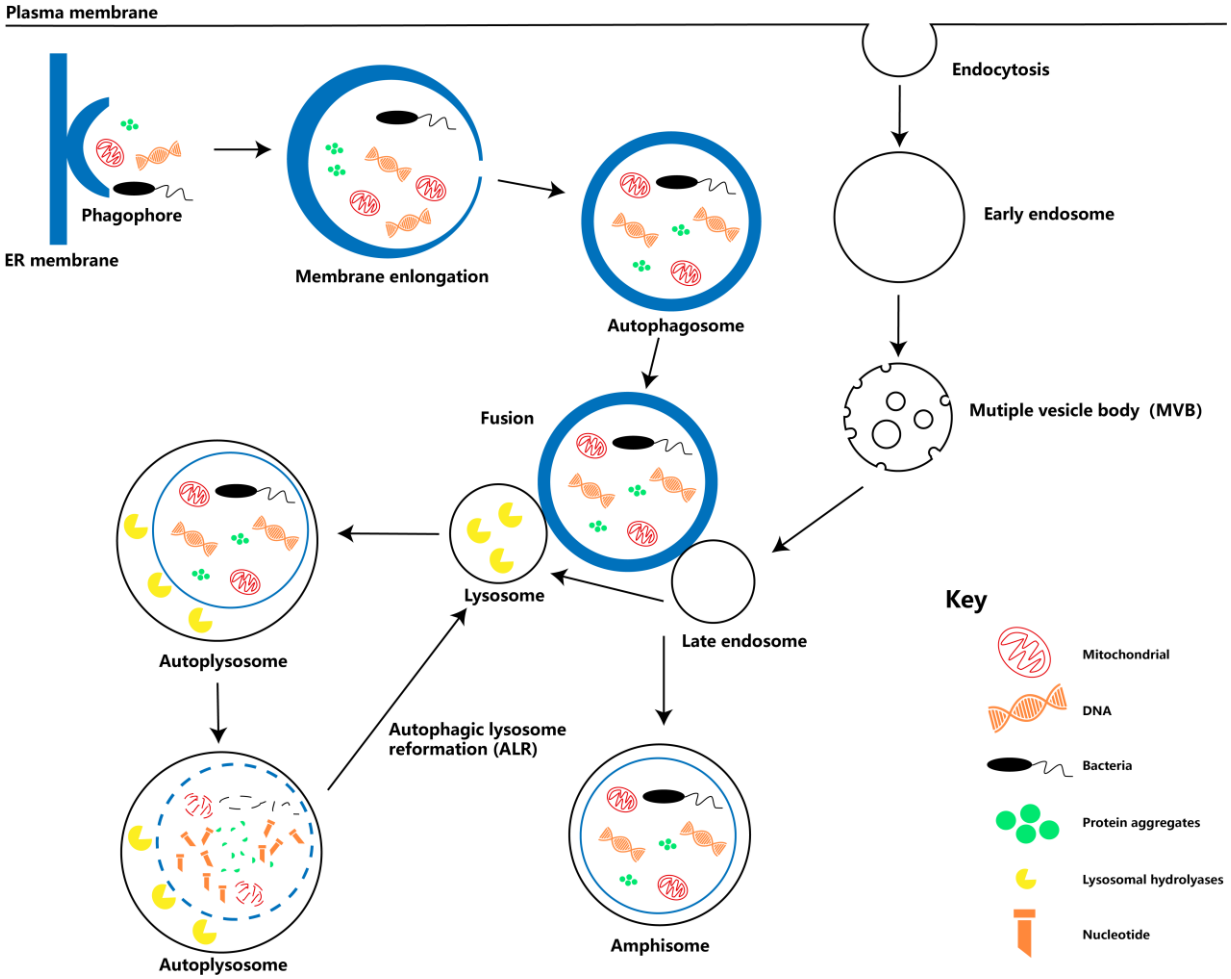

Fig 1. An overall description of autophagy process.

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.

Figure 2.

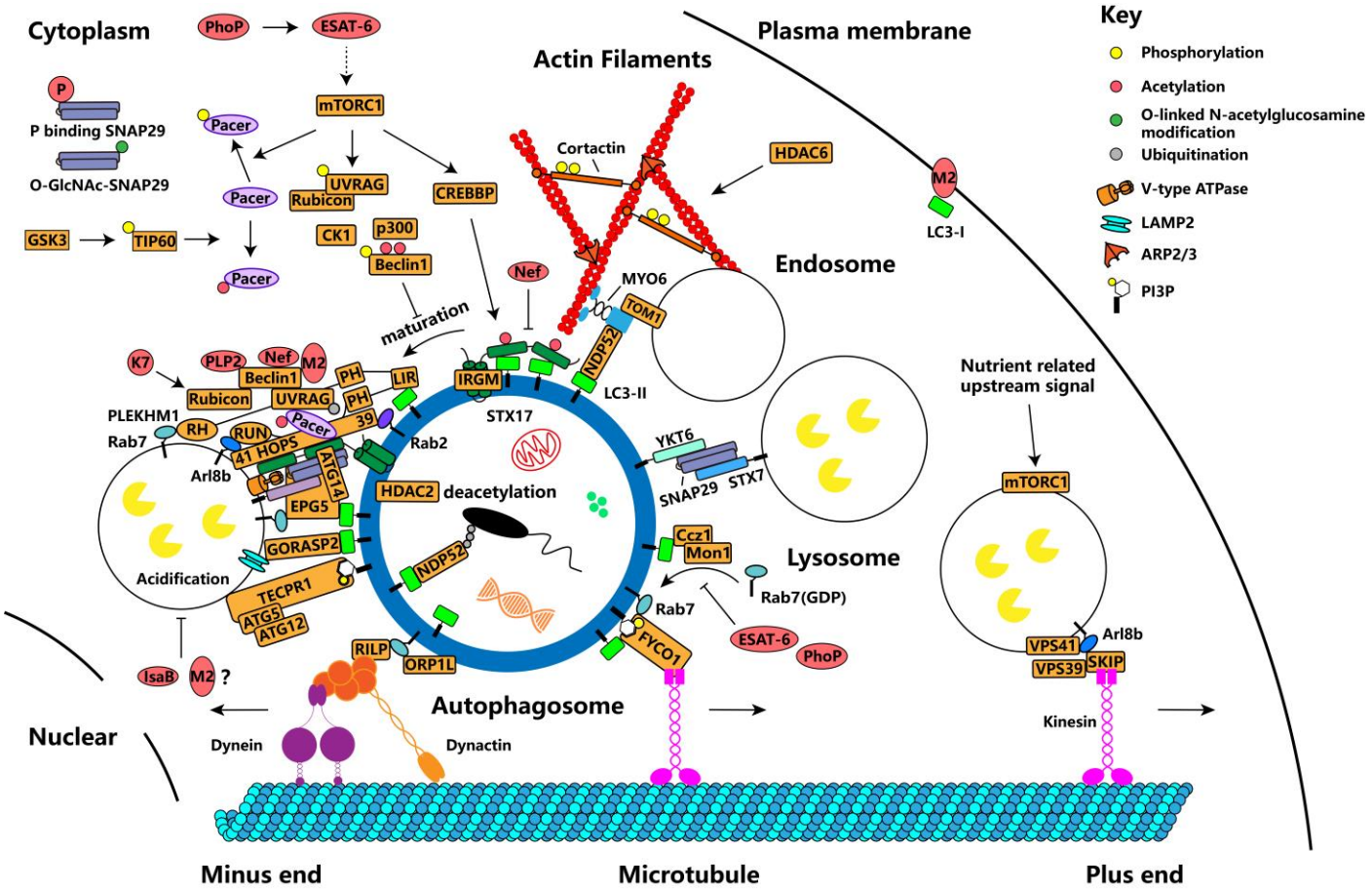

Fig 2. The cytoskeleton, SNAREs, tethers, pathogen factors and regulations involved in A:L fusion.

Boxes presenting exogenous pathogen factors are filled with red color and their relationship with host targets are listed. The stabilization of an F-actin network required for fusion is ensured by HDAC6. MYO6 links endosome and autophagosome in this network by interacting with TOM1 and NDP52. GDP-binding Rab7 is activated on autophagosome membrane by its GEF Ccz1-Mon1, which is previously recruited by LC3. Rab7 effector RILP and FYCO1 recruit different components to form two distinguish motor complexes. SKIP/PLEKHM2, recruited by Arl8b, links kinesin to lysosome and leads to cell periphery positioning of lysosome, where mTORC1 will be activated by upstream signals. The Canonical SNAREs STX17-SNAP29-VAMP8 ternary complex, following post translation modifications are shown here. STX17 binds to ATG14 and translocates to autophagy related structure in the early phase of autophagy. Selective recruitment of STX17 depends on its binding with IRGM and LC3. YKT6-SNAP29-STX7 ternary complex is recently identified as a second SNARE complex that mediates A:L fusion. Rab2 is found directly binding with VPS39 and cooperates with Rab7 mediating A:L fusion. Tethering factors like the HOPS complex, TECPR1,

EPG5 and GORASP2/GRASP55 together with their interacting proteins are also shown here. Regulation factors like PLEKHM1 is described with specific functional domains. Three components of PI3K complex, Belcin1, UVRAG and Rubicon, have opposite function in A:L fusion and post-translation modification are shown. Promoting fusion function of UVRAG will be inhibited when phosphorylated by mTORC1 with Rubicon interacting enhanced or facilitated when ubiquitinated by SMURF1. Pacer, here function as a vital intermediator for upstream signal. Specific expressed ATP6V0D2 in macrophage interacts with STX17 and VAMP8.

Figure 3.

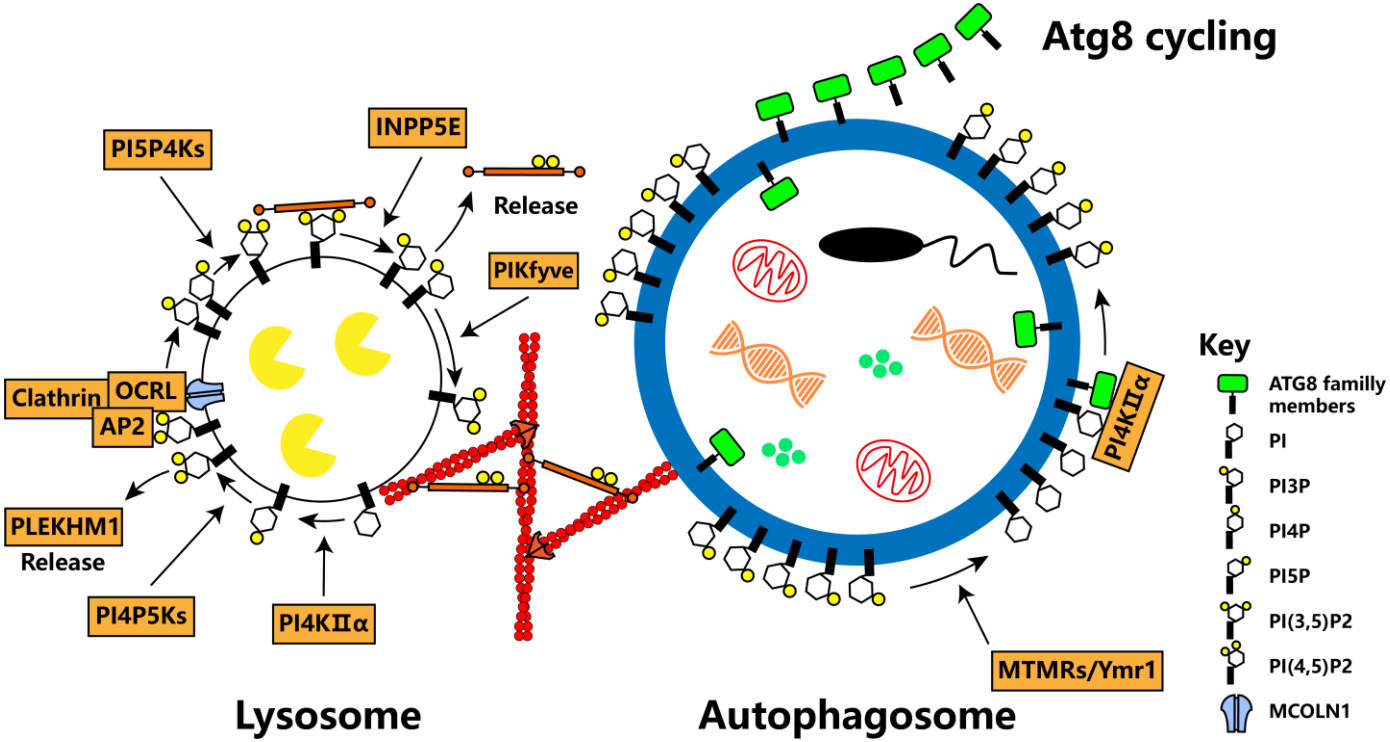

Fig 3. PIs and PIs conversions in A:L fusion.

PI3P down regulation by MTMRs with 3-phosphotase activity and ATG8 family members deconjugating make autophagosome capable for fusion. PI4K IIα recruited by GABARAP generates PI4P on autophagosome membrane. PI4P is also produced on lysosome membrane by PI4K IIα and further converted into PI(4,5)P2 by PI4P5Ks (α, β, γ), leading to dissociation of vital Rab7 effector PLEKHM1. PI(4,5)P2 recruits AP2 and clathrin earlier before ALR (autophagic lysosome reformation) is going to happen, which then are required for OCRL recruitment. OCRL with 5-phospotase activity is also recruited by MCOLN1 to diminish PI(4,5)P2 amount and facilitate its role in fusion. PI(4,5)P2 can also be product from PI5P by PI5P4Ks. Conversion from PI(3,5)P2 to PI3P mediated by INPP5E are required for the release of cortactin (CTTN), which is further phosphorylated to stabilize actin filaments network. PI3P will be converted back to PI(3,5)P2 by PIKfyve (FYVE finger-containing phosphoinositide kinase).