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REVIEW

The interplay between exosomes and autophagy – partners in crime

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ABSTRACT

The eukaryotic endomembrane system is a complex series of interconnected membranous organelles that play important roles in responding to stress and maintaining cell homeostasis during health and disease. Two components of this system, exosome biogenesis and autophagy, are linked by the endolysosomal pathway. Exosomes are cargo-laden extracellular vesicles that arise from endosome-derived multivesicular bodies, and autophagy is a lysosomal-dependent degradation and recycling pathway. Recent studies have revealed shared molecular machinery between exosome biogenesis and autophagy, as well as substantial crosstalk between these two processes. In this Review, we first describe the classic view of exosome biogenesis and autophagy, including their links to the endolysosomal pathway. We then present the evidence for autophagy-related proteins in exosome biogenesis, the emerging roles of amphisomes and the evolving models of exosome-autophagy pathway interactions. Finally, we discuss the implications of exosome and autophagy interplay in the context of neurodegeneration and cancer.

KEY WORDS: Autophagy, Exosome, Lysosome, Extracellular vesicle

Introduction

The eukaryotic endomembrane system (EMS) is a set of interrelated membrane-bound organelles that include the endoplasmic reticulum (ER), Golgi, lysosome and the plasma membrane, as well as the vesicles trafficking between them. It is responsible for numerous cellular processes, such as endocytosis and exocytosis. The components of the EMS are structurally and functionally intertwined. Delineating these intricate connections will improve our understanding of intracellular vesicular trafficking, the fate of vesicular cargos, and the contributions of membrane compartments to both intracellular and intercellular communication.

This Review will discuss two modules of the EMS: exosome biogenesis, defined as the formation and release of vesicles of endosomal origin into the extracellular space, and macroautophagy, an intracellular lysosome-mediated pathway of self-digestion and recycling. Exosomes were originally identified as means of shedding receptors in reticulocytes (Pan and Johnstone, 1983; Harding et al., 1983), and have since attracted considerable attention due to their novel signaling capabilities and biomarker potential. Similarly, macroautophagy was initially considered to be merely a cellular waste removal program, until subsequent studies revealed additional roles, ranging from unconventional secretion to stress adaptation and cell–cell communication (Claude-Taupin et al., 2017; Deretic et al., 2013; Cadwell and Debnath, 2018).

Recent studies have uncovered the molecular machinery and regulatory mechanisms shared between exosome biogenesis and macroautophagy, suggesting that the two processes are intimately linked. Emerging evidence from studies of normal development, as well as multiple disease contexts is beginning to reveal a coordinated exosome–macroautophagy response that functions to maintain homeostasis through lysosomal degradation and/or release of cellular cargo (Baixauli et al., 2014; Ojha et al., 2017). Here, we begin with a description of the classic view of exosome biogenesis and macroautophagy. We then describe non-canonical roles of macroautophagy and macroautophagy-related proteins, including the discovery that a subset of macroautophagy proteins function in exosome biogenesis. We discuss how the amphisome is emerging as an important organelle linking exosomes and macroautophagy, and also outline how studies of viruses have contributed to our understanding of how these processes interact. We conclude by considering the important implications of coordinated interactions between exosome biogenesis and macroautophagy in the context of disease, with a focus on neurodegeneration and cancer.

Overview of exosomes

Exosomes are nano-sized extracellular vesicles originating from the endocytic pathway. Endocytosis is the process by which cells internalize fluids, macromolecules, membranes and receptors via invaginations of the plasma membrane. These membrane invaginations, sometimes coated with clathrin or caveolin, become intracellular vesicles following membrane scission. Primary endocytic vesicles fuse with early endosomes, where cargo sorting is initiated. Through a process known as endosome maturation, early endosomes undergo a series of biochemical changes that give rise to late endosomes, which ultimately fuse with lysosomes (Huotari and Helenius, 2011; Scott et al., 2014) (Fig. 1).

During maturation, some endosomes undergo another membrane invagination and fission event that produces intermediate organelles characterized by numerous intraluminal vesicles (ILVs). These intermediate organelles are termed multivesicular bodies (MVBs) because of this morphology. MVBs can fuse with the plasma membrane to release the ILVs to the extracellular space, creating exosomes (Théry et al., 2002; Hessvik and Llorente, 2018; Gould et al., 2003) (Fig. 1).

Exosomes are a type of extracellular vesicle (EV), a collective term for all membrane-limited vesicles released from cells (Colombo et al., 2014). EVs also include larger vesicles, such as microvesicles and apoptotic bodies, as well as smaller vesicles, such as ectosomes, that originate from the plasma membrane (Yáñez-Mó et al., 2015). In this Review, the term exosomes will be used to indicate what are currently considered bona fide exosomes: EVs that are between 50 and 130 nM in diameter and enriched for a set of

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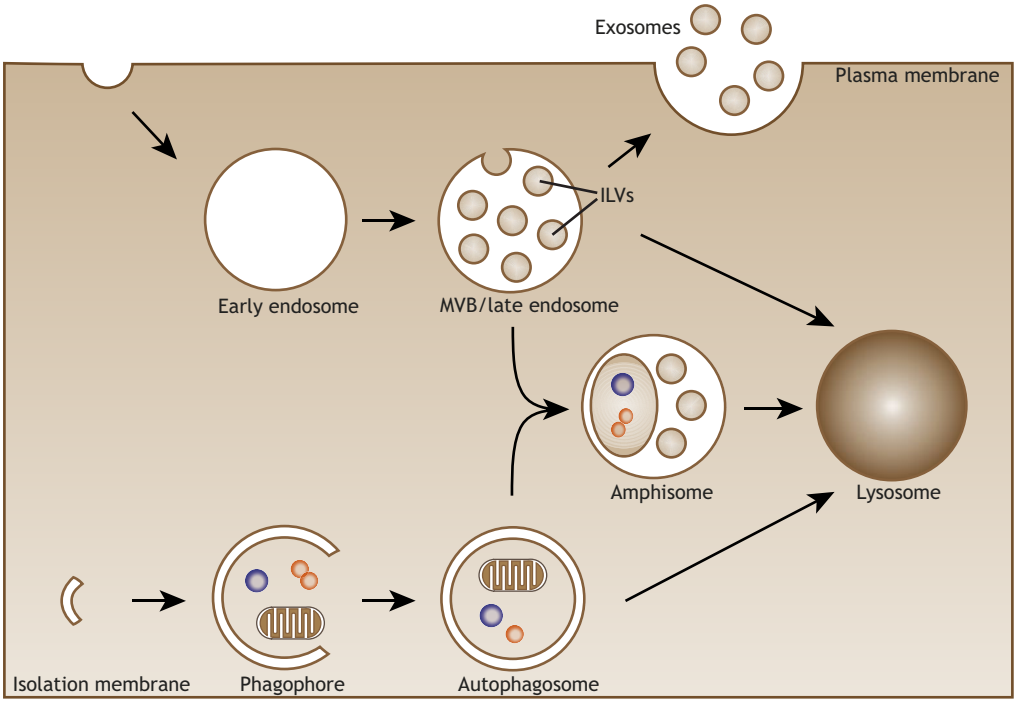

Fig. 1. The classic view of the endocytic pathway, autophagy and exosome biogenesis. The maturation of early endosomes gives rise to multivesicular bodies (MVBs), late endocytic compartments containing many intraluminal vesicles (ILVs). Fusion of MVBs with the plasma membrane results in the release of ILVs into the extracellular space as exosomes. Macroautophagy starts with the nucleation and expansion of phagophores, which engulf cytoplasmic proteins and organelles. Sealing of the double-membraned phagophores results in the formation of autophagosomes, which subsequently fuse with lysosomes to degrade engulfed contents. Alternatively, autophagosomes can fuse with MVBs to form hybrid organelles termed amphisomes, which are believed to eventually fuse with lysosomes.

molecular markers commonly associated with an endosomal origin (Yáñez-Mó et al., 2015). 'Exosome-like' vesicles or small EVs will be used to denote EVs whose size and physical properties are similar to those of exosomes, yet have not demonstrated enrichment of markers associated with classic exosomes (Bobrie et al., 2012; Lötvall et al., 2014). According to the most recent findings, these classical exosome markers include CD63, CD9, CD81, TSG101 and syntenin-1 (Kowal et al., 2016).

Exosome biogenesis and cargo loading

The process of exosome biogenesis and its known participating proteins have been reviewed recently (Colombo et al., 2014). Briefly, the endosome sorting complexes required for transport (ESCRTs) and their accessory proteins have been shown to play a significant role in the formation of ILVs and in exosome biogenesis (Hurley, 2015). Accordingly, silencing selected individual components of the ESCRT machinery leads to changes in exosome size, quantity and protein composition (Colombo et al., 2013). In some cell types, ceramide has been implicated in the inward budding of the MVB membrane and subsequent exosome biogenesis in an ESCRT-independent manner (Trajkovic et al., 2008).

Exosomes contain combinations of membrane-associated and soluble proteins, DNA, mRNAs and species of small RNAs, such as microRNAs (Hessvik and Llorente, 2018). While nonselective bulk loading of exosomes is likely, there is evidence in some instances for selective loading, the mechanisms of which vary depending on cell type and stimulus (Villarroya-Beltri et al., 2014). Oligonucleotides can enter into exosomes by association with RNA-binding proteins or lipid rafts in MVBs (Janas et al., 2015; Villarroya-Beltri et al., 2013). Ubiquitylation also plays a role in the selective incorporation of exosomal proteins (Smith et al., 2015; Katzmann et al., 2001; Buschow et al., 2005). Furthermore, membrane microdomains enriched in tetraspanins also participate in the recruitment of protein and oligonucleotide cargo into exosomes (Andreu and Yáñez-Mó, 2014; Perez-Hernandez et al., 2013). One mechanism of exosomal loading utilizes the electrostatic association of HSC70 (also known as HSPA8) with the MVB membrane to facilitate exosomal loading of proteins possessing KFERQ motifs in mammals (Sahu et al., 2011). The heterogeneity of exosome composition and function suggest that other mechanisms of exosome loading exist; however, the extent of their individual contribution remains to be demonstrated (Villarroya-Beltri et al., 2014).

Exosome targeting and uptake

When an MVB fuses with the plasma membrane, exosomes are released into the extracellular space. Exosomes can then be internalized by the secreting cell itself (autocrine) or by other cells in a paracrine or endocrine fashion. The factors that determine the release and uptake of exosomes are not completely understood. Examples of known components include tetherin, which is a tethering factor utilized by HIV viruses that also attaches exosomes to the surfaces of releasing cells and affects the range of exosome signaling (Edgar et al., 2016). Surface integrins are known to facilitate exosome attachment and intake into their intended recipient cells in mammalian systems (Clayton et al., 2004; Hoshino et al., 2015). Conversely, the presence of CD47 'do not eat me' signals on exosomal membranes protects them from scavenging phagocytes and improves their stability in circulation (Kamerkar et al., 2017).

Uptake of exosomes can occur through various pathways including, but not limited to, membrane receptor-mediated endocytosis, phagocytosis and macropinocytosis (Feng et al., 2010; Fitzner et al., 2011; Christianson et al., 2013), some of which have been reviewed recently (Mulcahy et al., 2014). Interestingly, filopodia have also been found to serve as hotspots for exosome internalization, likely due to an elevated rate of endocytosis during the dynamic construction and deconstruction of these cell protrusions (Heusermann et al., 2016). These observations suggest that exosomes may utilize entry pathways seen for other small extracellular entities such as viral particles.

Macroautophagy

Macroautophagy (hereafter referred to as autophagy) is a process ubiquitous among almost all eukaryotes in which cytosolic proteins

and organelles are captured by double-membraned vesicles, termed autophagosomes, and degraded through fusion with lysosomes (Klionsky, 2000) (Fig. 1). Autophagy serves to remove proteins, protein aggregates and damaged organelles, while the amino acids, lipids and sugars recycled from degradation can be used to sustain cell survival, especially under stress conditions such as starvation (Klionsky, 2000; Kroemer et al., 2010; Levine and Klionsky, 2004). The prefix 'macro' serves to differentiate macroautophagy from other types of cellular self-digestion, namely microautophagy and chaperone-mediated autophagy. Microautophagy facilitates the lysosomal degradation of proteins through inward budding of lysosomes, whereas chaperone-mediated autophagy transports target proteins directly across the lysosomal membrane for degradation; neither process involves autophagosomes, and will not be the focus of this Review (Galluzzi et al., 2017).

Autophagy machinery and regulation

The genes necessary for autophagy, otherwise known as autophagy-related (ATG) genes, were first discovered through genetic screens in yeast (Tsukada and Ohsumi, 1993). More than 30 ATG genes have now been identified that have well-conserved homologs across eukaryotes (Ohsumi, 2014), amidst rapid expansion of the field and a Nobel Prize awarded to Yoshinori Ohsumi in 2016 for his pioneering work in this area.

The rate of autophagy turnover, or autophagy flux, is regulated by various signaling pathways. Stressors, such as starvation, reactive oxygen species (ROS) and hypoxia, are known to induce autophagy (He and Klionsky, 2009). The best-known regulator of autophagy is nutrient availability, which is mediated through the mechanistic target of -rapamycin (mTOR) pathway. In the presence of abundant nutrients and growth factors, the mTOR complex 1 (mTORC1) phosphorylates and inactivates the autophagy-initiating kinase ULK1, thereby inhibiting autophagy. Conversely, inactivating mTORC1 through nutrient starvation induces autophagy (Park et al., 2016; Kim et al., 2011). Upon induction of autophagy, a complex comprising ATG1 and ULK1 initiates nucleation of the nascent phagophore and recruits the ATG6 (Beclin1 in mammals)-containing PI3K complex, which synthesizes phosphatidylinositol 3-phosphate [PI(3)P] to promote phagophore expansion (Matsuura et al., 1997). Two ubiquitin-like conjugation systems consisting of ATG5–ATG12 and ATG7–ATG3 complexes enable the covalent linkage of ATG8 [microtubule-associated protein 1 light chain 3B (MAP1LC3B) or LC3B in mammals] to phosphatidylethanolamine (PE) on the growing autophagosomal membrane (Mizushima et al., 1998; Ichimura et al., 2000). The completion of autophagy is achieved when the completed autophagosomes fuse with lysosomes, where cargos are degraded by acid hydrolases and their components recycled (Fig. 1).

Non-canonical functions of autophagy

In addition to its degradative functions, the autophagy machinery participates in the secretion of cytosolic proteins, in a manner that is distinct from the conventional secretion pathway from the ER to the Golgi and then the plasma membrane (PM), which requires signal peptide sequences. This autophagy-dependent, unconventional secretion pathway is gaining increasing interest. For example, an LC3B-positive carrier is thought to sequester the cytokine interleukin 1β (IL-1β) from the cytosol and subsequently fuse with the plasma membrane to release the IL-1β contained through a process that is sometimes referred to as secretory autophagy (Ponpuak et al., 2015; Kimura et al., 2017; Zhang et al., 2015). Autophagy has also been shown to facilitate conventional and regulated secretion, as well as the movement of membrane proteins to the plasma membrane (Deretic et al., 2012), demonstrating its versatility in cellular functions and potential roles in intercellular communication.

LC3B has long served as a marker of autophagy flux owing to its incorporation into autophagosome membranes. LC3B can also be recruited to single-membrane phagosomes and macropinosomes in a process termed LC3-associated phagocytosis (LAP), which requires the LC3 lipidation machinery, but not the formation of double-membrane autophagosomes (Florey et al., 2011; Martinez et al., 2011). Here, the ATG5–ATG12–ATG16L1 complex plays a significant role in targeting LC3B to the phagosome membrane (Fletcher et al., 2018; Fujita et al., 2008). Often referred to as a type of non-canonical autophagy (Codogno et al., 2012), LAP has been speculated to expedite the degradation of phagosome content by mediating fusion with lysosomes. Recent observations of LC3B lipidation occurring at single-membrane endosomes, even in the presence of lysosomal inhibition, raises exciting possibilities of non-degradative functions of a LAP-like machinery (Jacquin et al., 2017), and these involves exosomes as discussed below.

Crosstalk between autophagy and exosome biogenesis

Beyond known interactions between autophagy and endocytosis previously reviewed (Tooze et al., 2014), emerging evidence suggests additional direct links between autophagy and exosome biogenesis through shared molecular machinery or organelles, with important implications for normal physiology and disease states.

Autophagy-related proteins in exosome biogenesis

Subsets of the autophagy machinery have been shown to contribute to exosome biogenesis (i.e. the formation and release of vesicles of endosomal origin into the extracellular space), while the completion of the autophagic process itself appears dispensable (Guo et al., 2017; Murrow et al., 2015). A recent report highlighted crucial non-autophagic functions of ATG5 and ATG16L1 in exosome biogenesis (Guo et al., 2017) (Fig. 2A). ATG5 has been shown mediate the dissociation of vacuolar proton pumps (V_1V_o-ATPase) from MVBs, which prevents acidification of the MVB lumen and allows MVB–PM fusion and exosome release. Accordingly, knockout of ATG5 or ATG16L1 significantly reduces exosome release and attenuates the exosomal enrichment of lipidated LC3B. Moreover, treatment with lysosomal or V-ATPase inhibitors rescues exosome release in ATG5-knockout cells, which further supports the role of luminal pH in controlling whether MVBs undergo lysosomal degradation or plasma membrane fusion. Importantly, ATG7 knockout did not affect exosome release, suggesting that the formation of autophagosomes or LC3B lipidation was not required. This study thus provides a mechanism where autophagy-related proteins directly regulate the fate of MVBs and subsequent exosome biogenesis. While the biological function of LC3B in exosomes remains unclear, its localization on the lumen side of ILVs as shown in the Guo et al. study suggests a LAP-like lipidation event either at the MVB membrane or at membrane invaginations that subsequently become ILVs. The eventual release of intact LC3B-positive exosomes points to non-degradative functions of the LAP-like mechanism (Guo et al., 2017).

The ATG12–ATG3 complex that catalyzes LC3B conjugation has also been found to regulate exosome biogenesis through its interaction with ALG-2-interacting protein X (ALIX, also known as PDCD6IP), an ESCRT-associated protein crucial to exosome biogenesis (Murrow et al., 2015). Here, loss of ATG12–ATG3 altered MVB morphology, impeded late endosome trafficking and reduced exosome biogenesis. ALIX knockdown also reduced basal

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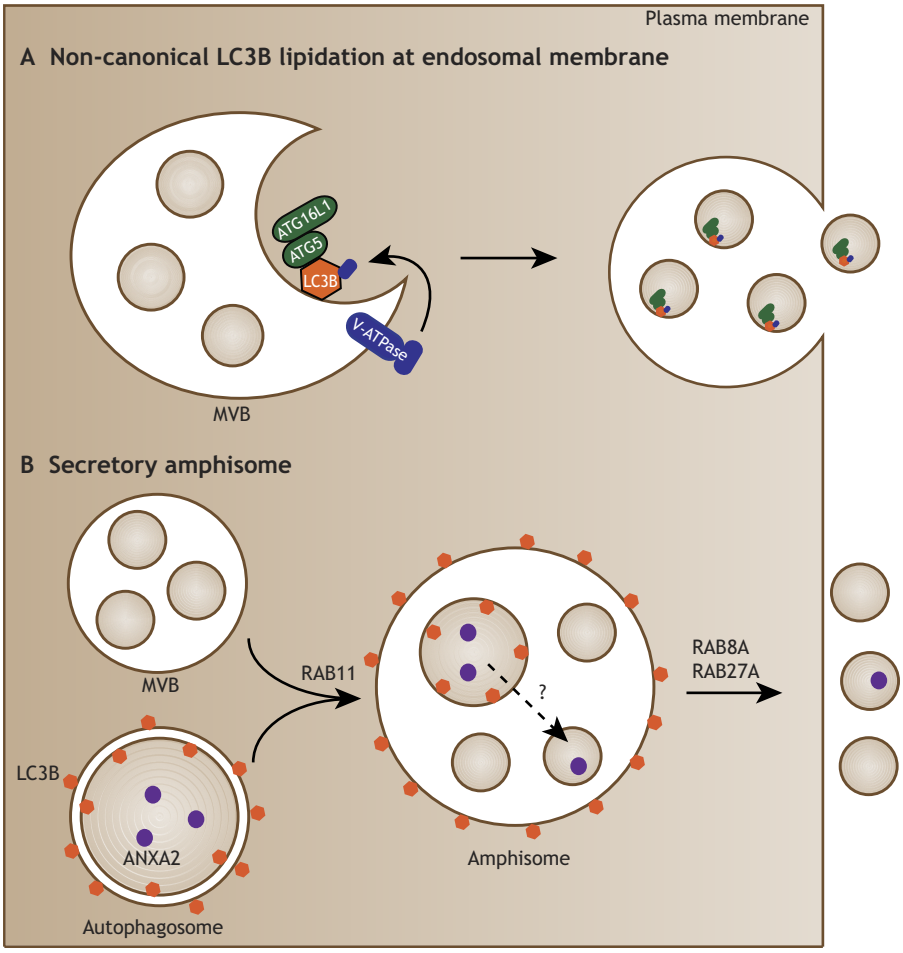

Fig. 2. Emerging interplay between autophagy and exosomes. Existing data suggest that there are multiple possible interactions between the autophagy machinery and exosome biogenesis. (A) Subsets of the autophagy machinery may contribute to exosome biogenesis. As an example, shown here is the ATG5–ATG16 complex, which localizes to MVBs and mediates non-canonical lipidation of LC3B. The ATG5–ATG16 complex also facilitates the dissociation of V-ATPase, preventing the acidification of MVBs and their subsequent lysosomal degradation. MVBs then fuse with the plasma membrane (right) to release exosomes. (B) Amphisomes can fuse with the PM and secrete their contents. Shown here is the autophagy-dependent secretion of annexin A2 (ANXA2), where an amphisome intermediate is required for the release of cytosolic ANXA2 in exosomes.

autophagy flux, demonstrating a reciprocal regulation between autophagy and exosome biogenesis. Importantly, starvation-induced autophagy remained intact despite loss of ALIX or disruption of the ATG12–ATG3 complex, implying that different regulatory machineries control basal and stress-induced autophagy, as well as the interactions of these pathways with endocytic compartments (Murrow et al., 2015). As elaborated below, context dependency is a recurring theme in exosome–autophagy crosstalk, with both processes responsive to various forms of cellular stress.

A past study has also implicated ATG9, the only transmembrane ATG, as being involved in the formation of ILVs in *Drosophila*. Under basal conditions, loss of ATG9 impaired autophagy flux and reduced the number of ILVs in amphisomes and autolysosomes (Bader et al., 2015). However, whether the ILVs, in this case, were released as exosomes remains unknown.

Although not designated as ATGs, the class III PI3K complex is required for autophagy and endocytosis by phosphorylating phosphatidylinositide to produce PI(3)P that regulates membrane trafficking. VPS34 (also known as PIK3C3), Beclin1 and p150 (also known as PIK3R4) constitute the core mammalian PI3K complex that is shared between the endocytosis and autophagy processes, and association with different regulatory proteins determines the function of the complex. For example, association with ATG14L mediates autophagosome expansion, whereas the association with UVRAG facilitates endosome maturation (Kihara et al., 2001). Association of the PI3K complex with Run domain Beclin-1-interacting and cysteine-rich domain-containing protein (Rubicon), an effector of RAB7, has been shown to suppress autophagy and endocytosis (Sun et al., 2010), and to also be required for LC3-associated phagocytosis (Martinez et al., 2015). The destabilization of the PI3K complex that occurs upon suppressing Beclin1, either via siRNA-mediated knockdown or Spautin-1 treatment, reduces both exosome release and autophagy flux in chronic myeloid leukemia (CML) cells (Liu et al., 2016). Given its role in endocytosis and autophagy, the impact of various components of the PI3K complex in exosome biogenesis is worthy of further investigation. While the utilization of the same protein complexes in both autophagy and exosome biogenesis is not entirely surprising, autophagy might also play a more direct role in the making of exosomes, which will be discussed below.

The amphisome – a degradative compartment and novel secretory organelle

Historically, amphisomes have been defined as degradative compartments in the cell. Nascent autophagosomes fuse with MVBs to produce hybrid organelles termed amphisomes, which can subsequently fuse with lysosomes for content degradation (Gordon et al., 1992; Liou et al., 1997) (Fig. 1). Antagonistic interactions between autophagy and exosome release in the form of amphisome degradation have been well documented. In the erythroleukemic cell line K562, starvation or rapamycin treatment induces autophagy, increases autophagosome–MVB fusion and decreases exosome release (Fader et al., 2008), perhaps as cells attempt to recycle MVBs for energy instead. Failure to release exosomes can also lead to the redirection of MVBs to autophagic degradation. For example, in mammalian cell line and mouse models, conjugation of the ubiquitin-like protein ISG15 (known as ISGylation) promotes

protein aggregation and degradation, along with a decrease in the number of MVBs and reduced exosome release (Villarroya-Beltri et al., 2016). ISGylation of TSG101, an ESCRT-1 accessory protein, is sufficient to impair exosome biogenesis. Prevention of endosome–lysosome fusion through the use of bafilomycin A1, a dominant-negative mutant form of RAB7 or inhibition of autophagy all rescue exosome release, which suggests that autophagy is involved in the lysosomal degradation of MVBs that contain ISGylation-induced aggregates (Villarroya-Beltri et al., 2016). Another recent report has demonstrated autophagic clearance of aberrant endocytic vacuoles caused by CD63 knockout, where inhibition of autophagy partially rescued exosome biogenesis in the CD63-null cells (Hurwitz et al., 2018). These studies illustrate the prevalence of autophagic degradation of MVBs in diverse contexts. However, as discussed below, additional evidence indicates that MVB and autophagosme fusion may perform additional functions in some contexts.

Recently, non-degradative functions of amphisomes have been revealed. In mouse intestinal goblet cells, LC3B was found to colocalize with the endosomal markers EEA1, RAB7 and RAB11 on amphisome-like organelles, which are vital to the production of reactive oxygen species (ROS) that regulate the secretion of mucin granules (Patel et al., 2013). Another report further demonstrated the possibility of amphisomes serving secretory functions in lung epithelial cells. Here, interferon-γ (IFN-γ)-induced autophagy-dependent exosome secretion of annexin A2 (ANXA2), which likely took place through amphisomes (Chen et al., 2017). IFN-γ treatment caused the colocalization of LC3B, CD63 and ANXA2 on amphisomes. This colocalization and subsequent exosome release were dependent upon ATG5, RAB11 and RAB27A, suggesting that the formation of autophagosomes, MVBs and the fusion of amphisomes with the plasma membrane were vital to the process (Chen et al., 2017) (Fig. 2B). However, care must be taken to differentiate the autophagy-dependent unconventional secretion from exosomal secretion. For example, while functional MVBs are required for optimal autophagy-dependent secretion of IL-1β (Zhang et al., 2015), autophagosome–lysosome fusion is dispensable (Kimura et al., 2017), suggesting that LC3B-positive IL-1β carrier vesicles fuse directly with the plasma membrane. The reliance on MVB functionality could be due to the extensive crosstalk between autophagy and endocytosis (Tooze et al., 2014). Curiously, IFN-γ-induced exosomal secretion of ANXA2 requires RAB8A (Chen et al., 2017), a known mediator of autophagy-dependent IL-1β secretion (Dupont et al., 2011). These observations suggest a potential overlap between autophagy-mediated unconventional secretion and exosome release, but further studies are required to delineate the possible connections between these processes.

Exosome–autophagy crosstalk is exploited by viruses

Studies of viral infections provide a fascinating perspective on interactions between autophagy and exosome production. Viruses are known to hijack the exosomal pathway to evade the host immune system and increase infectivity (Gould et al., 2003). Increasing evidence suggests that viruses may also take advantage of the autophagy–exosome crosstalk to facilitate their replication and release. The hepatitis C virus (HCV) offers a unique model to delineate the links between autophagy and exosome biogenesis. HCV infection has been shown to lead to the upregulation of autophagy, as well as the release of virus-containing exosomes (Bukong et al., 2014; Liu et al., 2014). Knockdown of Beclin1 or ATG7 decreases the level of extracellular exosome-associated HCVs (Shrivastava et al., 2016), suggesting that the core autophagy machinery plays a role in the packaging of HCV particles into exosomes. Indeed, increased autophagosome–lysosome fusion reduced the release of HCV particles, suggesting that a portion of HCV particles or its replication machinery could reside within autophagosomes (Ren et al., 2016). Curiously, HCV infection differentially regulates autophagy at different time points. In the early stages of HCV infection, upregulation of Rubicon, a negative regulator of autophagosome–lysosome fusion, suppresses autophagy flux, indicating that HCV viruses may exploit the build-up of autophagosomes for replication (Wang et al., 2015). Later on during the infection, UVRAG expression is induced (Wang et al., 2015). Given the role of UVRAG in enhancing endosomal transport and endosome maturation (Liang et al., 2008), its delayed induction in HCV infection may reflect altered endosomal trafficking, which facilitates virus escape via exosomes. Delineating the egress route of HCV particles may thus provide crucial insights into the molecular links between autophagy and endocytic pathways in the context of infections.

Coordination of autophagy and exosome release

The crosstalk between autophagy and exosome biogenesis is largely context dependent. Autophagy and exosome release offer some functional redundancy in eliminating unwanted proteins whereby each route may compensate for a deficiency in the other. Defective MVBs and their contents may be subject to autophagic degradation, and inhibition of autophagy may rescue exosome release from MVBs that would otherwise be degraded (Villarroya-Beltri et al., 2016). Alternatively, exosome release and autophagy may act in concert to counter cellular stress (Kumar et al., 2014). These interactions are best illustrated in the context of diseases, which will be discussed below.

Autophagy–exosome crosstalk in neurodegeneration

Studies focusing on amyloid transmission have unveiled many interesting links between autophagy, endocytosis and exosome biogenesis (Borland and Vilhardt, 2017). Neuronal cells frequently utilize autophagic degradation and exosome secretion to eliminate protein aggregates to reduce proteotoxicity (Fig. 3). α-Synuclein (SNCA) has been well studied because of its relevance in Parkinson's disease, where cell-to-cell transmission of SNCA from diseased to healthy neurons is believed to propagate neurodegeneration (Gitler et al., 2009). The ATPase ion pump ATP13A2 has been found to regulate both autophagic degradation of SNCA and its exosomal release (Bento et al., 2016). Depletion of ATP13A2 suppresses autophagy in multiple neuronal cell lines through downregulation of SYT11, which then impairs lysosomal function and hence SNCA degradation. Conversely, overexpression of ATP13A2 in neurons alleviates the detrimental effect of high levels of SNCA, presumably by inducing its autophagic degradation (Bento et al., 2016). ATP13A2 has also been found to closely associate with autophagosomes and MVBs; here, elevated levels of ATP13A2 enhances the externalization of SNCA through exosomes, which is proposed to be accomplished through ATP13A2-mediated modulation of intraluminal zinc ion levels in MVBs (Kong et al., 2014).

When defects in autophagy or lysosomal function prevent the efficient degradation of intracellular protein aggregates, exosome release may be enhanced to alleviate the proteotoxic stress. For instance, overexpression of tubulin polymerization-promoting protein (p25α, also known as TPPP) inhibits autophagosome maturation and promotes autophagy-dependent secretion of SNCA instead (Ejlerskov et al., 2013). The secretory membrane carrier

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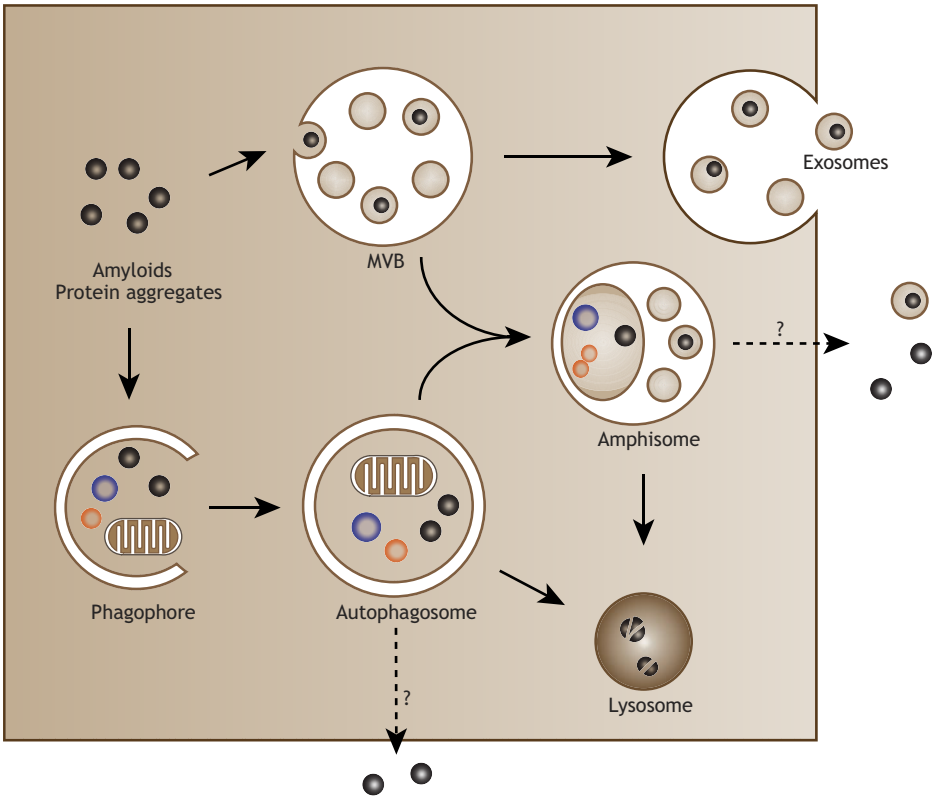

Fig. 3. Exosomes and autophagy in neurodegeneration. Abnormal protein accumulation and aggregation is a hallmark of multiple neurodegenerative disorders. Exosome release and autophagic degradation are two coordinated pathways through which amyloids and protein aggregates can be eliminated. Cytosolic protein aggregates may be sequestered into autophagosomes or MVBs and degraded through lysosomes. Secretory autophagy may also play a role in externalization of protein aggregates through mechanisms yet to be delineated. Alternatively, and especially during lysosomal or autophagic dysfunction, MVBs may release ILVs containing protein aggregates or amyloids as exosomes, decreasing the proteotoxic stress in the releasing cells, yet propagating protein aggregates to neighboring cells.

protein 5 (SCAMP5) also promotes re-direction of cargos from autophagic degradation to exosome secretion (Yang et al., 2017). Indeed, upregulation of SCAMP5 in response to protein stress prevents autophagosome–lysosome fusion. Overexpression of SCAMP5 also facilitates the clearance of SNCA and huntingtin, the causal agent of Huntington's disease, by redirecting them into exosomes (Yang et al., 2017). In SNCA-overexpressing cell lines and animal synucleinopathy models, lysosomal inhibition with bafilomycin A1 increases the secretion of SNCA in the exosome fraction (Poehler et al., 2014; Alvarez-Erviti et al., 2011). Recently, lysosomal inhibition was also reported to increase the number of amphisomes in the neuronal cell line H4, and to increase the levels of SNCA and autophagy-related proteins in exosome-like EVs (Minakaki et al., 2017), further supporting a model where cells utilize exosome secretion to remove protein aggregates during lysosomal or autophagic dysfunction. Ironically, this behavior might be responsible for the propagation of the disease phenotype when neighboring neurons take up these exosomes (Poehler et al., 2014; Minakaki et al., 2017).

Autophagy–exosome crosstalk in cancer

The significance of exosome signaling in cancer, and the roles of autophagy in multiple stages of tumorigenesis have been individually reviewed (Galluzzi et al., 2015; Azmi et al., 2013) (also see Box 1 for a brief overview), but the importance of the interplay between exosome and autophagy in cancer is only beginning to be recognized (Fig. 4).

Cancer cells frequently face the onslaught of chemotherapy, radiation and the host immune system, where cellular stress responses are crucial to their survival. Although stressors such as hypoxia have been found to increase exosome secretion (King et al., 2012), as well as autophagy flux (He et al., 2012) in breast cancer cells, the extent to which these two pathways are coordinated in cancer remains mostly unexplored. ER stress is known to upregulate autophagy in multiple types of normal and cancerous cells (Verfaillie et al., 2010). Recently, ER stress was shown to increase MVB formation and exosome release in HeLa cells, whereas loss of IRE1a and PERK (also known as ERN1 and EIF2AK3, respectively) two arms of unfolded protein response (UPR) signaling, abolished the increase in exosome production (Kanemoto et al., 2016). In breast and prostate cancer cell lines, rotenone-induced mitochondrial damage leads to increased levels of endosomal tetraspanins (CD9, CD63 and CD81) and ATG7 in the cytoplasm, which coincides with an upregulation of autophagy and increased exosome release in what appears to be a collective stress response (Kumar et al., 2014). A study of regulatory proteins in vesicular trafficking pathways has identified that GAIP-interacting protein C-terminus (GIPC) simultaneously regulates autophagy and exosome production in pancreatic cancer cells. Knockdown of GIPC leads to decreased mTOR activity and increased autophagy flux as well as increased exosome production (Bhattacharya et al., 2014). Taken together, these studies suggest that under certain conditions, autophagy and exosome biogenesis may work in concert to counter cellular stressors (Fig. 4A).

Recently, the FYVE-type zinc finger-containing phosphoinositide kinase (PIKfyve) was found to regulate the redirection of proteins from autophagic degradation to exosomal release in prostate cancer cells (Hessvik et al., 2016). Inhibition of PIKfyve with apilimod or its downregulation via siRNA reduced autophagy flux, but increased the release of EVs that bear the typical exosomal markers TSG101 and ALIX, as well as also a subset of ATGs (Hessvik et al., 2016). This possible redirection of degradative cargos to exosomal release suggests the existence of a mechanism that is utilized by cancer cells to maintain homeostasis. Therefore, the notion of exosome release and autophagy induction as inter-related stress adaptation mechanisms for cancer deserves further attention.

The importance of the crosstalk between cancer cells and the surrounding normal cells is widely recognized. While exosomes are accepted as crucial messengers in cell–cell communication, the non-

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Box 1. Autophagy and exosomes in cancer

Autophagy in cancer

Cell-autonomous autophagy is recognized as having a dual role in cancer, typically, performing a tumor-suppressing role in normal cells and acting as tumor-promoting in transformed cells. Autophagy contributes to cellular homeostasis in normal tissue by removing potentially damaging proteins and organelles and therefore acts to prevent tumorigenesis (Galluzzi et al., 2015). In established tumors, however, autophagy supports tumor progression, for example, by maintaining mitochondrial integrity in cancer cells with oncogenic Ras mutations (Guo et al., 2011; Amaravadi et al., 2016). At the genomic level, the core autophagy machinery is often intact in many types of cancer, which supports its indispensability for tumorigenesis (Lebovitz et al., 2015). Hence, autophagy inhibition is under investigation as a potential anti-cancer therapy strategy. *In vitro* and animal studies have demonstrated the growth-suppressing and cell-killing effect of autophagy inhibition in cancer cells (Sun et al., 2011; He et al., 2015; Chittaranjan et al., 2014). However, a thorough understanding of the context-dependent roles of autophagy in cancer is needed for more precise and efficient autophagy modulation for therapeutic benefits (Levy et al., 2017). In comparison, cell non-autonomous roles of autophagy in cancer are less explored, although autophagy-dependent secretion has been implicated in some aspects of tumorigenesis (Keulers et al., 2016).

Exosomes in cancer

Exosomes have been recognized as a significant cell–cell communication pathway in cancer. Many recent reviews have discussed the roles of exosomes in the tumor microenvironment (Minciacchi et al., 2015; Azmi et al., 2013; Kharaziha et al., 2012; Tkach and Théry, 2016) and anti-tumor immune responses (Greening et al., 2015; Bobrie and Théry, 2013; Filipazzi et al., 2012). Overall, tumor-derived exosomes are known to alter the microenvironment of a pro-cancer phenotype, facilitate immunosurveillance evasion and promote local invasion and distant metastases. In a metastatic breast cancer model, expression of different sets of surface integrins on tumor-derived exosomes has been shown to determine their target cells and thereby influence the organotropism of subsequent metastases (Hoshino et al., 2015). Exosomes from metastatic melanoma cells can alter the phenotype of bone marrow progenitor cells, leading to an increase in the size of the primary tumor, as well as in the size and number of metastases (Peinado et al., 2012). Owing to their presence in bodily fluids, including blood and urine, circulating exosomes are under investigation for their potential to serve as biomarkers for cancer progression or treatment response. Serum exosomes have been shown to carry DNA with a mutation profile that is almost identical to that of the primary tumor (Kahlert et al., 2014). Thus, the detection of biomarkers in exosomes may reveal small, hidden tumors at early stages of the disease (Melo et al., 2015). It may be possible in the future to survey the change in exosome composition as the disease progresses or responds to treatment, and so obtain crucial insight into the change in disease status without the need for invasive sampling. Furthermore, engineered exosomes are promising carriers for drugs and nucleic acids. Tumors that actively scavenge nutrients through macropinocytosis, such as Ras-transformed pancreatic cancer cells (Commisso et al., 2013), may be especially responsive to such an exosome-mediated drug delivery.

cell-autonomous roles of autophagy are only beginning to emerge, such as its participation in interactions between tumor cells, stromal cells and immune cells (Maes et al., 2013). Given that autophagy can influence exosome release, it would be interesting to determine whether non-cell autonomous roles of autophagy are accomplished, in part, via exosomal signaling.

A recent study has found that breast cancer cells released exosomes that alter autophagy flux in recipient breast epithelial cells (Dutta et al., 2014). Through mechanisms yet to be determined, human breast epithelial cells produce increased levels of ROS upon exosome uptake, which plays a role in the upregulation of autophagy flux. Subsequently, the breast epithelial cells secrete pro-tumor growth factors as the result of the uptake of exosomes that were derived from the cancer cells (Dutta et al., 2014) (Fig. 4B). Secretory autophagy in stromal cells has been reported to mediate the release of nutrients or growth factors that promote cancer cell growth (Sousa et al., 2016; Chiavarina et al., 2011), so it would be interesting to investigate whether exosome-mediated signaling is able to regulate autophagic secretion.

Acquired resistance to chemotherapies and targeted therapies is one of the major obstacles in combating cancers and remains a field of active investigation. Understanding how cancer cells withstand chemotherapy and develop resistance is crucial to successful cancer control. Upregulation of autophagy and exosome release have been documented following drug treatments (Ertmer et al., 2007; Sun et al., 2011), suggesting that they constitute a part of the cancer cell stress response or survival mechanism against chemotherapy. In support of this possibility, increases in the levels of autophagy flux and exosome production in various types of chemotherapy-resistant cancers have been reported (Yang et al., 2011; Yu et al., 2015). For example, increased release of exosomes has been observed in platinum-resistant ovarian cancer cell lines, as well as in serum from patients with cisplatin-resistant tumors (Yin et al., 2012), while increased autophagy flux has also been found in platinum-resistant ovarian cancer cells (Pasto et al., 2016). Although it is unknown whether these changes are part of the resistance mechanism or merely a consequence of the shifting cellular phenotype, these observations provide the basis to investigate the therapeutic value of inhibiting autophagy and disrupting exosome release to counter chemotherapy resistance.

The downstream signaling effects of exosomes released from chemotherapy-resistant cancer cells are also essential to consider. It has been proposed that exosomes might propagate drug-resistant phenotypes through the transfer of miRNA or multidrug-resistant transporter (MDR) proteins (Azmi et al., 2013; Torreggiani et al., 2016; Bach et al., 2017). In another context, exosomes from gefitinib-treated EGFR-mutant PC-9 cells have been shown to increase autophagy flux in recipient cancer cells that were subsequently less responsive to cisplatin treatment (Li et al., 2016) (Fig. 4C). These studies illustrate the potential capacity of the crosstalk between tumor-derived exosomes and autophagy to influence tumor behavior and its interactions with the microenvironment.

Conclusions and future directions

The interplay between exosome biogenesis and autophagy occurs in multiple different ways. At the molecular level, there are examples of autophagy-related proteins and protein complexes that function in exosome biogenesis. At the organelle level, the exosome and autophagy pathways intersect at amphisomes, the contents of which have multiple fates, including extracellular release or lysosomal degradation. Both exosome biogenesis and autophagy play vital roles in maintaining cellular homeostasis and mitigating cellular stress, with increasing evidence to indicate that these cellular responses are accomplished through a crosstalk between autophagy and exosomes. What has become clear is that the dynamic and context-dependent nature of the interplay between exosome biogenesis and autophagy has important implications not only for normal physiology but also for disease – and thus perhaps also represents therapeutic opportunities if we can better understand its regulation and complexity.

Questions underlying the identity and heterogeneity of various intermediate compartments in exosome–autophagy crosstalk and vesicular trafficking still remain. For instance, it is unclear whether

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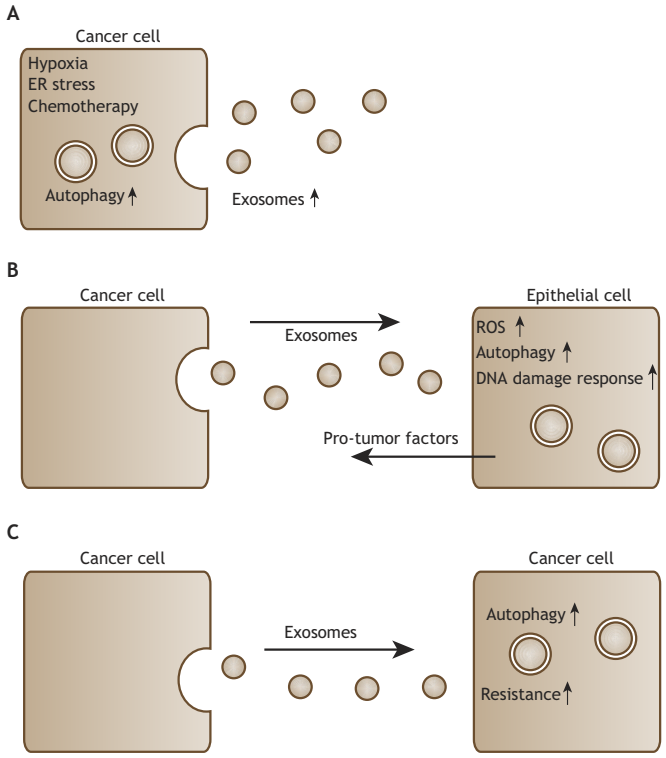

Fig. 4. Means of autophagy–exosome crosstalk in cancer. Autophagy-exosome crosstalk in cancer can be context dependent. (A) In response to cellular stressors, autophagy and exosome release may be concomitantly upregulated in cancer cells. This coordinated activation may constitute an adaptive stress response, the molecular details of which are not yet understood. (B) Cancer cells release exosomes that elevate intracellular ROS levels and induce DNA-damage responses in recipient epithelial cells, as well as upregulate autophagy and the secretion of pro-tumor factors through mechanisms yet to be determined. (C) Treatment of cancer cells with anti-cancer drugs can induce the release of exosomes that up-regulate cytoprotective autophagy in recipient cancer cells and modulate their sensitivity to drug treatment.

separate populations of MVBs exist that are predestined to fuse with either the plasma membrane, lysosome or autophagosome, and, if not, whether there are specific signals that seal the fate of an MVB. Similarly, it is unknown whether there are subpopulations of autophagosomes that preferentially fuse with MVBs or directly with lysosomes, or how secretory and degradative autophagosomes are differentially controlled. Advancements in technology and methodology may lead to a renewed understanding and definition of various subtypes of EVs including exosomes. Novel discoveries continue to add dimensions of complexity into evolving models of exosome–autophagy interactions and vesicular trafficking. Above all, an outstanding question is how organelle identity is established and maintained amidst intersecting pathways and promiscuous machinery in the eukaryotic endomembrane system.

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Competing interests
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