Journal of Cell Communication and Signaling (2019) 13:443–450
https://doi.org/10.1007/s12079-019-00524-8

REVIEW

Exosomes and autophagy: rekindling the vesicular waste hypothesis

Johann Mar Gudbergsson [1] · Kasper Bendix Johnsen [2]

Received: 4 April 2019 / Accepted: 31 May 2019 / Published online: 6 June 2019

Abstract

Exosomes were first described as waste carriers implicated in reticulocyte maturation but has during the past decade been associated with many other cellular functions. The biogenesis of exosomes has been extensively studied and several protein machineries have been identified to dictate their production and release. The newly discovered branches of the autophagy system implicate secretion of waste in endosomal-derived vesicles as is thought for exosome release. Many of the proteins that have been identified as responsible for the formation and release of these vesicles are the same as those identified in exosome biogenesis. In this Perspective, we discuss the possibility of exosomes being a part of the autophagy machinery and the consequences this could have on interpretation of exosome functions.

Keywords Exosomes · Extracellular vesicles · Autophagy · Secretory autophagy · EV · Vesicles

Introduction

Conventional protein secretion typically requires a signal peptide to guide proteins through the endoplasmic reticulum (ER) and Golgi apparatus. Secretion of proteins from cells without involvement from the ER-Golgi network can also occur, a process defined as unconventional secretion. Several types of unconventional secretion have been described including secretion of vesicles derived from the autophagosome or endosome (Rabouille 2017). Vesicular secretion of cellular content has gained great attention, and the understanding of the many functions and mechanisms governing these processes is continuously growing. Different pathways of vesicle release from cells have been identified during normo- and pathophysiological processes, including the endosomal-derived exosomes, plasma membrane-derived microvesicles (or ectosomes), lysosomal vesicle secretion, and secretory autophagy (Yáñez-Mó et al. 2015; Ponpuak et al. 2015; Galluzzi et al. 2017). Extracellular vesicles (EVs) is an umbrella term, which generally covers exosomes, microvesicles, and apoptotic bodies, whereas lysosomal vesicle secretion and secretory autophagy vesicles are typically not associated with this particular field of research. Although these vesicles definitely are EVs in principle terms, we will in this paper not include them when addressing EVs but rather mention them separately to avoid confusion.

The field of EV research has seen a surge in publications as indicated by 8.000 out of the 12.000 publications within this field have been published during the past 5 years only. The functions of EVs have been suggested to span widely, being included in various cell-cell communication pathways within many different organisms, liquid biopsy and biomarker discovery, and as drug delivery vehicles (Johnsen et al. 2014; Yáñez-Mó et al. 2015). Exosomes are generated in cells by inward budding of early endosomal membranes creating intraluminal vesicles (ILVs), hereby generating multivesicular bodies (MVB)/late endosomes inside the cell. For exosomes to be released to the external environment, the MVB fuses with the cell membrane via Rab-GTPases and SNAREs. EVs, including exosomes, can be isolated from a wide variety of body fluids such as blood plasma/serum, cerebrospinal fluid, synovial fluid, urine, and cell culture medium (Hessvik and Llorente 2018). Numerous isolation methodologies exist with the most widely used being differential centrifugation with ultracentrifugation, density gradient ultracentrifugation, size-exclusion chromatography and polymer-based precipitation (Böing et al. 2014; Gudbergsson et al. 2015; Johnsen et al. 2018).

Autophagy governs cellular homeostasis and ensures cell survival by adapting cellular metabolism to stressful environments.

✉ Johann Mar Gudbergsson
jmg@hst.aau.dk

[1] Department of Health Science and Technology, Laboratory of Immunology and Cancer Biology, Aalborg University, Fredrik Bajers Vej 3B, 9220 Aalborg, Denmark

[2] Department of Health Technology, Center for Nanomedicine and Theranostics, Technical University of Denmark, Kongens Lyngby, Denmark

Several types of autophagy exist including the canonical macroautophagy, chaperone-mediated autophagy, and the less studied microautophagy (for review see (Galluzzi et al. 2017)). Two subsets of microautophagy have recently been described, namely the endosomal (micro)autophagy (EA) and secretory autophagy (SA). Typically, compounds destined for macroautophagy are sorted into intermediate structures called omegasomes that mature into autophagosomes, and autophagosomes then deliver the content to lysosomes for degradation. In EA, proteins are sorted into endosomal vesicles (ILVs) by inward budding into endosomal membranes via Hsc70 (HSPA8) to generate MVBs (Sahu et al. 2011; Uytterhoeven et al. 2015; Mejlvang et al. 2018). MVBs can then fuse with lysosomes to degrade the content. EA can be induced by starvation and has therefore been called starvation-induced autophagy. SA encompasses the fusion of an autophagosome with a MVB/late endosome forming the amphisome. This initial fusion initiates a process resulting in the release of autophagic content to the extracellular milieu by fusion of the amphisome with the plasma membrane (Ponpuak et al. 2015).

Exosomes were originally described by *Johnstone* et al. in 1987 as possible secreted containers of waste during reticulocyte maturation (Johnstone et al. 1987) but has since then been described to be much more than that (Yáñez-Mó et al. 2015). Still, several mechanisms in EV biogenesis seem to overlap with different autophagy processes although only very few studies investigate both aspects at the same time (see below). This stimulates the following question: Could some or most of the vesicles secreted from the cell have the function of getting rid of waste material as a part of maintaining cellular homeostasis through autophagy, as was originally hypothesized in the early work on exosomes? The new branch of secretory autophagy certainly suggests an interconnection between EA/SA and exosomes since they share characteristics with respect to both formation and secretion. Many specific functions have been attributed to EVs in a wide variety of diseases, insinuating that we know what they are and what their purpose is. This implies that we 1) know all the different types of EVs and where they originate from, and 2) know how to isolate and quantify the specific subsets of EVs. At this point, the latter is not possible (Sódar et al. 2016; Simonsen 2017; Johnsen et al. 2018). In this Perspective, we wish to highlight the similarities between subsets of the autophagy system and exosome biogenesis to discuss the possibility of exosomes being an integrated part of the autophagy system.

Interconnection between exosome and autophagy pathways

To understand any possible interconnection between the exosome- and autophagy-related secretory pathways, one can study the protein machinery employed to facilitate the two processes. Many proteins involved in different autophagy processes have also been discovered in EV experiments (Table 1) (Baixauli et al. 2014; Xu et al. 2018). Leading experts in autophagy research recently published a review in the EMBO Journal defining autophagy processes based on key proteins involved (Galluzzi et al. 2017). We compared this list of key proteins against the EV protein database Vesiclepedia (Table 1) (Kalra et al. 2012) and found that almost all proteins had been identified in EV experiments to varying degrees. Four of the proteins related to autophagy were in the Top 100 of most widely identified EV proteins, namely HSPA8 (3/100), HSP90AA1 (8/100), VCP (24/100), and Rab7A (81/100). This could indicate an interconnection between the two pathways, and in the coming sections we will discuss this possible connection in more detail.

Exosomes

Mechanisms regarding exosome formation and release have been studied widely during the past decade where several protein machineries have been identified (see reviews (Kowal et al. 2014; Hessvik and Llorente 2018)). Exosomes are formed by inward budding into the early endosome resulting in maturation into late endosome/MVB. Several proteins are key in the process of inward budding and cargo sorting, such as the ESCRT proteins Hrs, CHMP4, TSG101, STAM1, VPS4, other proteins such as the Syndecan-syntenin-ALIX complex, nSMase2, PLD2 and CD9, alongside different post-translational modifications of proteins (Fig. 1) (Colombo et al. 2013; Ghossoub et al. 2014; Kowal et al. 2014; Villarroya-Beltri et al. 2014; Hessvik and Llorente 2017). After formation, the MVB can either fuse with the lysosome to degrade its content or fuse with the plasma membrane to release the ILVs as exosomes. The release of exosomes to the extracellular milieu is driven by proteins of the Rab-GTPase family, including RAB2B, 5A, 7, 9A, 11, 27 and 35. SNARE family proteins VAMP7 and YKT6 have also been implicated in the release (Fader et al. 2009; Ostrowski et al. 2010; Gross et al. 2012; Kowal et al. 2014; Hessvik and Llorente 2018).

Endosomal autophagy

Starvation of cells, a key inducer of autophagy, induces enlargement of MVB structures and a co-localization of Rab11 and LC3 in these structures, an indication of autophagy-related processes associated to the MVB (Fig. 1) (Fader et al. 2008). The sorting of autophagy-related cargo into the MVBs is dependent on Hsc70 (HSPA8), VPS4 and TSG101 and independent on LAMP-2A, hereby excluding a role of the lysosome (Sahu et al. 2011). Hsc70 was responsible for sorting specific proteins with the pentapeptide sequence KFERQ into MVBs (Sahu et al. 2011), and for MVB-

Table 1 List of key autophagy proteins from Galluzzi et al. 2017 and the number of times these proteins have been identified in EV experiments according to Vesiclepedia

Gene	# of times identified in EV experiments	Gene	# of times identified in EV experiments
ACBD3	39	NBRF2	0
ACBD5	21	OPTN	34
AMBRA1	1	PARK2	1
ATG2A,B	10	PEX2	1
ATG3	46	PEX3	3
ATG4A,B,C,D	33	PEX5	2
ATG5	1	PEX13	1
ATG7	37	PEX14	16
ATG9A, B	21	PHB2	76
ATG10	2	PIK3C3 (VPS34)	5
ATG12	1	PIK3R4 (VPS15)	34
ATG13	2	PINK1	2
ATG14	1	PLEKHM1	2
ATG16L1	3	PSMD4 (RPN10)	45
ATG101	0	RAB7A, B*	216
BCL2	30	RAB11A	115
BCL2L13	22	RB1CC1	6
BECN1	10	RNF166	1
BNIP3 (NIP3)	18	RUBCN (RUBICON)	2
BNIP3L (NIX)	1	SMURF1	5
CALCOCO2	4	SNX4	29
EI24 (EPG4)	22	SNX18	51
EPG5	28	SQSTM1 (p62)	73
ENDOG	2	STX17	2
FAM134B	1	TAX1BP1	51
FANCC	1	TBK1	27
FUNDC1	14	TECPR1	8
GFAP	36	TFEB	24
HSPA8 (HSC70)*	292	TGM2	93
HSP90AA1*	270	TOLLIP	125
INPP5E	3	TRIM5	1
LAMP1	145	ULK1 (ATG1), ULK2	8
LAMP2	115	UVRAG	23
LGALS3	105	VCP*	214
LGALS8	36	WDFY3 (ALFY)	24
MAP1LC3A,B,B2 (LC3)	35	WIPI1,2,3,4	2
GABARAP, GABARAPL1,L2	65	VMP1	20
MTOR	73	WAC	3
NBR1	4	ZFYVE1	2

*Top 100 proteins most frequently identified in EV experiments (Vesiclepedia – microvesicles.org)

mediated protein turnover at neuronal synapses (Uytterhoeven et al. 2015). Cellular starvation also induce a rapid EA response within 30–60 min (Mejlvang et al. 2018). A study by *Mejlvang* et al. showed the presence of p62 in ILVs in MVBs, and by blocking MVB dynamics with the cholesterol transporter inhibitor U18666A, the EA response was abrogated. To

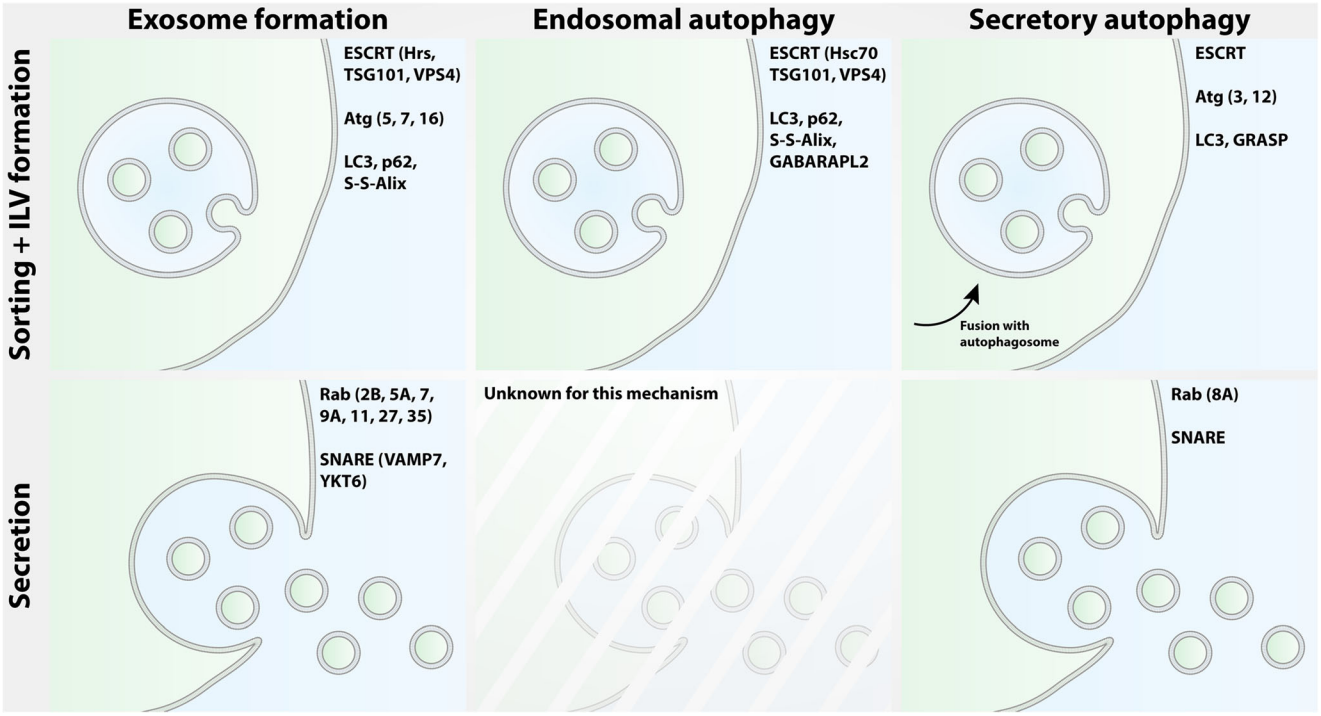

Fig. 1 Schematic representation of proteins involved in the biogenesis and secretion of exosomes, endosomal autophagy and secretory autophagy

investigate whether this response was lysosomal-dependent, knockdown of SNARE STX17 was performed to inhibit lysosomal fusion, but no effect on EA was seen, indicating a strict endosomal response (Mejlvang et al. 2018). The existence of a rapid autophagy response to cellular stress was further demonstrated by Wang et al. (2019). By applying mechanical stress to tumor cells for up to 60 min., increases in LC3II and p62 was seen in the EV fraction. These results clearly point towards an intersection of EA processes with exosome formation and secretion since both pathways are characterized by MVB formation. Whether the ILVs generated in EA are secreted in the same manner as exosomes has not, to our knowledge, been reported. However, since the ESCRT machinery is instrumental in both biogenesis pathways (Raiborg and Stenmark 2009; Lefebvre et al. 2018), the potential of EA ILVs being secreted as exosomes is not farfetched. Besides the involvement of ESCRT proteins, EA also relies on core autophagy receptors such as p62/SQSTM1, LC3 and GABARAPL2 (Mejlvang et al. 2018). According to the EV database Vesiclepedia (microvesicles.org) these proteins have been identified in numerous EV experiments and in published EV studies (Kalra et al. 2012; Miao et al. 2015; Hessvik et al. 2016).

Secretory autophagy

SA has only been scarcely studied in mammalian cells but drawing parallels to yeast homologs has aided in understanding the process in mammals (Ponpuak et al. 2015). Several proteins are involved in the regulation and biogenesis of SA compartments such as GRASPs, LC3, Rab8a, ESCRTs, SNAREs along with several Atg proteins (Fig. 1) (Dupont et al. 2011; Patel et al. 2013; Ponpuak et al. 2015). SA biogenesis has been related to omegasomes (equivalent to yeast CUPS) that are rich in PI3P, which ultimately end up maturing into the autophagosome (Axe et al. 2008; Ponpuak et al. 2015). *Griffiths* et al. showed that autophagosomes could fuse with MVBs to form the amphisome and release vesicles to the external environment (Griffiths et al. 2012). Since both SA and EA are characterized by endosomal influence, there could be an overlap between these two newly described pathways. In line with this, SA has also been shown to be induced by starvation (Pallet et al. 2013; Ponpuak et al. 2015). The first protein to be described as secreted by SA was IL-1β, which is also considered an EV-associated cytokine (Dupont et al. 2011; Yáñez-Mó et al. 2015). The overlap between EA, SA and exosome proteins could indicate that at least parts of these pathways intersect. Similarities between autophagy-related pathways and exosomes has previously been mentioned in other studies and reviews (Record et al. 2014; Baixauli et al. 2014; Ponpuak et al. 2015; Hessvik et al. 2016; Hessvik and Llorente 2018; Xu et al. 2018; Pleet et al. 2018; Levine and Kroemer 2019). These studies describe crosstalk between the two pathways but do not directly describe exosomes as being a part of the autophagy system. In the coming sections, we discuss the possibility of exosomes being an integrated part of the autophagy machinery.

Tampering with canonical autophagy-lysosome pathways affects vesicle secretion

Several studies investigating secretion of EVs or SA have shown that by inhibiting or stimulating canonical autophagy pathways, exosome biogenesis and release was altered. The autophagy proteins Atg12-Atg3 were shown to form a complex with Alix, and by knocking either of these proteins down, ILV formation in MVBs and exosome secretion was reduced (Murrow et al. 2015). In support of this, knockdown of Atg5 and Atg16L1 was also shown to reduce exosome secretion, and Atg5 and LC3 were demonstrated to be responsible for de-acidification of MVBs, another stimulating factor in the release of exosomes (Guo et al. 2017). Inhibition of autophagy with chloroquine or wortmannin resulted in an increased secretion of the EV-associated proteins CD63, TSG101 and Alix (Hurwitz et al. 2017). *Hessvik* et al. showed that inhibition (using apilimod) and knockdown of the phosphoinositide kinase, PIKfyve, whose substrate PI(3)P is heavily involved in canonical autophagy, increased exosome secretion (Hessvik et al. 2016). Interestingly, the EVs became more enriched with autophagy-related proteins such as NBR1, p62, LC3 and WIPI2, and it was observed using immuno-electron microscopy that p62 and CD63 were localized to the same exosomes (Hessvik et al. 2016). A similar mechanism was observed with the autophagy-inhibitor Bafilomycin A1, where exosome release increased and enrichment of LC3 and SQSTM1 in exosomes was seen as a result (Minakaki et al. 2018).

Two sides of the same coin?

The cellular state or "health" is reflected in the content of EVs, where both protein and RNA signatures changed in response to starvation (de Jong et al. 2012). Mechanisms for sorting cargo into MVBs and exosomes include ubiquitination, SUMOylation and phosphorylation of proteins (Villarroya-Beltri et al. 2013; Moreno-Gonzalo et al. 2018). The same post-translational modifications are implicated in sorting of cargo to autophagosomes (Mizushima and Komatsu 2011; Grumati and Dikic 2018). In neurodegenerative diseases such as Parkinson's and Alzheimer's, exosomes were identified as extracellular carriers of the aggregate-prone proteins, such as α-synuclein and ß-amyloid, respectively (Alvarez-Erviti et al. 2011; Yuyama and Igarashi 2017; Sardar Sinha et al. 2018). From an autophagy perspective, this vesicle (exosome) secretion could possibly be dictated by the autophagy machinery. This was evidenced by a reduction of extracellular ß-amyloid plaques in Atg7 knockout/amyloid precursor protein (APP) knock-in mice compared to APP mice with functional Atg7 (Nilsson et al. 2013, 2015). α-synuclein secretion was shown to be increased four-fold by inhibiting autophagosome fusion with lysosomes in neuron-like cells expressing the aggregation-prone α-synuclein$_{A30P}$, suggesting a secretion-based compensation for the lack of α-synuclein degradation (Ejlerskov et al. 2013). Similarly, another study showed that EV-mediated α-synuclein secretion could be increased up to five-fold by inhibiting conventional macroautophagy with bafilomycin A1 (Minakaki et al. 2018).

Another similar process that seems to overlap between these cellular machineries is the system that viruses use for propagation. It has been proposed that different viruses hi-jack the MVB-exosome pathway for budding (Nolte-'t Hoen et al. 2016; Sadeghipour and Mathias 2017; Hurwitz et al. 2017; Pleet et al. 2018), and knockdown of autophagy proteins Beclin1 and Atg7 was shown to inhibit exosome-mediated release of HCV particles to the extracellular space (Shrivastava et al. 2016). *Miao* et al. showed that cells were capable of non-lytic expulsion of bacteria via delivery of the bacteria from autophagosomes to MVBs, which were then ultimately secreted into urine in exosomes (Miao et al. 2015). They also demonstrated that the encapsulated bacteria were positive for both the exosome marker CD63 and the autophagy marker LC3, and showed that knockdown of TSG101 or Alix markedly decreased the bacterial expulsion (Miao et al. 2015). Exosome secretion could also relieve cellular stress by carrying harmful DNA in the excosome, including chromosomal fragments, to prevent the generation of too many reactive oxygen species (Takahashi et al. 2017). This could indicate that exosome secretion could be an autophagy-related response to discard compounds that are harmful to the cell, and to compensate if canonical degradative autophagy pathways are defect or overloaded. In support of this, several of the top 100 EV proteins on Vesiclepedia are of cytoskeletal (actinins, actins, myosin, cofilin) or nuclear (histones) origin, which could indicate that the EVs are implicated in cell homeostasis via autophagy-driven pathways (Kalra et al. 2012; Luo et al. 2016).

Navigating the vesicular fog: Is the waste the message?

EVs can be isolated from various fluids to be analysed for their functional characteristics or have their composition determined for use in biomarker discovery (Johnsen et al. 2018). However, these isolation methodologies has severe limitations with respect to obtaining a pure EV sample, especially for blood plasma and serum (Simonsen 2017). We recently showed through a systematic review of blood EV concentrations that the EVs constitute a minority of particles in blood, severely outnumbered by lipid nanoparticles (approximately 1:1,000,000) (Johnsen et al. 2018). This represents a large technical hindrance of isolating a pure EV sample, but what about the EV heterogeneity itself? (Kowal et al. 2016). To

avoid contamination of EVs from serum, isolation of EVs from cell cultures is frequently done by adding EV-depleted serum to the culture medium, reducing the serum concentration or using serum-free medium for the period of EV production (Gudbergsson et al. 2015). In turn, these conditions induce cellular starvation to various degrees that induces a rapid autophagy response, which could result in more vesicle release from the cells through EA and SA (Mejlvang et al. 2018). The immediate autophagy response to starvation is in part to rapidly rid the cell of anabolic proteins through endosomal and secretory autophagy, which might be secreted in vesicles (Ponpuak et al. 2015; Mejlvang et al. 2018). This could severely bias conclusions on EV function, for example when assessing effects of EVs on cancer cell growth or metastasis since the EV composition would favour these characteristics due to the starvation during conditioning of the culture medium. Various chemotherapeutics also enhance EV secretion and alter the EV proteomic profile in different ways to present a pro-metastatic, proliferative, or apoptotic phenotype, including the potential of modulating the cell cycle and matrix degradation (Kavanagh et al. 2017; Bandari et al. 2018; Keklikoglou et al. 2018). To cells, chemotherapy is an existential threat and thus they elicit a response to this threat, which includes autophagy. The autophagy response depends on the type of chemotherapy used and can stimulate pro-survival or pro-death responses (Sui et al. 2013). The fact that chemotherapeutics induce autophagy could indicate that the enhancement in EV secretion and alteration of their content is due to EA and SA, but research is needed to elucidate this. In support of this, the isolation protocols to obtain SA vesicles has been shown to be similar to that of general EV isolation protocols, utilizing differential centrifugation followed by ultracentrifugation (Griffiths et al. 2012; Ejlerskov et al. 2013; Hessvik et al. 2016).

The countless different proteins detected in EV samples and the limited knowledge on EV and secretory vesicle biology in general adds complexity to the secretome equation. Where are the vesicles originating from, and why are they produced by the cell? Is it an autophagy-lysosomal process of maintaining homeostasis and a response to stressful environments, or is the cell sending a specific message to its surroundings independent of a metabolic response, or both? In case of both, how do we distinguish between these messages? Could the waste itself be the message, providing a real-time status of cellular health to the surrounding resident cells and immune cells? If we assume that all or some vesicle release from cells is related to the autophagy and lysosome machinery, does it make EVs less relevant in a functional or biomarker context? When interpreting the function of EVs, knowing *how* and *why* they are produced is essential, and if there are several different subsets of EVs tied to different cellular pathways, being able to distinguish them is crucial when estimating a certain function. For biomarker discovery, EVs could still represent an asset since it reflects the cellular health status, however, there are still serious technical limitations with co-precipitation of other particles from blood and thus concluding that the biomarker is "enriched in EVs" could be wrong. Considering this, the EV and SA fields still suffer from being the new players on the field. To further dissect the relationship between these two fields, more fundamental research on the basic biology and technology for analysis is needed.

Concluding remarks

The discovery of EVs has opened a new and exciting avenue of research and adds a new layer of complexity to cell biology and intercellular communication. The parallel emergence of EV research and the secretory branch of autophagy might have led to scientific progress within the fields without them crossing paths. Here, we argue that the understanding of EV biology needs to include cellular autophagy in the equation since many proteins involved in the regulation of both machineries seem to be shared. To fully determine EV function and their use in biomarker discovery, more fundamental research into the underlying mechanisms is crucial. As EVs were first described as waste carriers, perhaps that notion is not completely farfetched. We encourage researchers working with EVs or the secretory branch of autophagy to further dissect the underlying mechanisms behind these pathways as we believe this could help understanding the connection between regulation of individual and collective cellular health and intercellular communication.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Alvarez-Erviti L, Seow Y, Schapira AH et al (2011) Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. Neurobiol Dis 42:360–367. https://doi.org/10.1016/j.nbd.2011.01.029

Axe EL, Walker SA, Manifava M et al (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 182:685–701. https://doi.org/10.1083/jcb.200803137

Baixauli F, López-Otín C, Mittelbrunn M (2014) Exosomes and Autophagy: Coordinated Mechanisms for the Maintenance of Cellular Fitness. Front Immunol 5:403. https://doi.org/10.3389/fimmu.2014.00403

Bandari SK, Purushothaman A, Ramani VC et al (2018) Chemotherapy induces secretion of exosomes loaded with heparanase that degrades extracellular matrix and impacts tumor and host cell behavior. Matrix Biol 65:104–118. https://doi.org/10.1016/j.matbio.2017.09.001

Böing AN, van der Pol E, Grootemaat AE, et al (2014) Single-step isolation of extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles 3. https://doi.org/10.3402/jev.v3.23430

Colombo M, Moita C, van Niel G et al (2013) Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci 126:5553–5565. https://doi.org/10.1242/jcs.128868

de Jong OG, Verhaar MC, Chen Y, et al (2012) Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. J Extracell Vesicles 1. https://doi.org/10.3402/jev.v1i0.18396

Dupont N, Jiang S, Pilli M et al (2011) Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1β. EMBO J 30:4701–4711. https://doi.org/10.1038/emboj.2011.398

Ejlerskov P, Rasmussen I, Nielsen TT et al (2013) Tubulin polymerization-promoting protein (TPPP/p25α) promotes unconventional secretion of α-synuclein through exophagy by impairing autophagosome-lysosome fusion. J Biol Chem 288:17313–17335. https://doi.org/10.1074/jbc.M112.401174

Fader CM, Sánchez D, Furlán M, Colombo MI (2008) Induction of autophagy promotes fusion of multivesicular bodies with Autophagic vacuoles in K562 cells. Traffic 9:230–250. https://doi.org/10.1111/j.1600-0854.2007.00677.x

Fader CM, Sánchez DG, Mestre MB, Colombo MI (2009) TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. Biochim Biophys Acta, Mol Cell Res 1793:1901–1916. https://doi.org/10.1016/J.BBAMCR.2009.09.011

Galluzzi L, Baehrecke EH, Ballabio A et al (2017) Molecular definitions of autophagy and related processes. EMBO J 36:1811–1836. https://doi.org/10.15252/embj.201796697

Ghossoub R, Lembo F, Rubio A et al (2014) Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. Nat Commun 5:3477. https://doi.org/10.1038/ncomms4477

Griffiths RE, Kupzig S, Cogan N et al (2012) Maturing reticulocytes internalize plasma membrane in glycophorin A-containing vesicles that fuse with autophagosomes before exocytosis. Blood 119:6296–6306. https://doi.org/10.1182/blood-2011-09-376475

Gross JC, Chaudhary V, Bartscherer K, Boutros M (2012) Active Wnt proteins are secreted on exosomes. Nat Cell Biol 14:1036–1045. https://doi.org/10.1038/ncb2574

Grumati P, Dikic I (2018) Ubiquitin signaling and autophagy. J Biol Chem 293:5404–5413. https://doi.org/10.1074/jbc.TM117.000117

Gudbergsson JM, Johnsen KB, Skov MN, Duroux M (2015) Systematic review of factors influencing extracellular vesicle yield from cell cultures. Cytotechnology. https://doi.org/10.1007/s10616-015-9913-6

Guo H, Chitiprolu M, Roncevic L et al (2017) Atg5 disassociates the V 1 V 0 -ATPase to promote exosome production and tumor metastasis independent of canonical macroautophagy. Dev Cell 43:716–730.e7. https://doi.org/10.1016/j.devcel.2017.11.018

Hessvik NP, Llorente A (2017) Current knowledge on exosome biogenesis and release. Cell Mol Life Sci. https://doi.org/10.1007/s00018-017-2595-9

Hessvik NP, Llorente A (2018) Current knowledge on exosome biogenesis and release. Cell Mol Life Sci 75:193–208. https://doi.org/10.1007/s00018-017-2595-9

Hessvik NP, Øverbye A, Brech A et al (2016) PIKfyve inhibition increases exosome release and induces secretory autophagy. Cell Mol Life Sci 73:4717–4737. https://doi.org/10.1007/s00018-016-2309-8

Hurwitz SN, Cheerathodi MR, Nkosi D, et al (2017) Tetraspanin CD63 bridges Autophagic and endosomal processes to regulate Exosomal secretion and intracellular signaling of Epstein-Barr virus LMP1. J Virol 92. https://doi.org/10.1128/JVI.01969-17

Johnsen KB, Gudbergsson JM, Skov MN et al (2014) A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta 1846:75–87. https://doi.org/10.1016/j.bbcan.2014.04.005

Johnsen KB, Gudbergsson JM, Andresen TL, Simonsen JB (2018) What is the blood concentration of extracellular vesicles? Implications for the use of extracellular vesicles as blood-borne biomarkers of cancer. Biochim Biophys Acta Rev Cancer. https://doi.org/10.1016/J.BBCAN.2018.11.006

Johnstone RM, Adam M, Hammond JR et al (1987) Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 262:9412–9420

Kalra H, Simpson RJ, Ji H et al (2012) Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol 10:e1001450. https://doi.org/10.1371/journal.pbio.1001450

Kavanagh EL, Lindsay S, Halasz M et al (2017) Protein and chemotherapy profiling of extracellular vesicles harvested from therapeutic induced senescent triple negative breast cancer cells. Oncogenesis 6:e388. https://doi.org/10.1038/oncsis.2017.82

Keklikoglou I, Cianciaruso C, Güç E et al (2018) Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models. Nat Cell Biol. https://doi.org/10.1038/s41556-018-0256-3

Kowal J, Tkach M, Théry C (2014) Biogenesis and secretion of exosomes. Curr Opin Cell Biol 29:116–125. https://doi.org/10.1016/j.ceb.2014.05.004

Kowal J, Arras G, Colombo M et al (2016) Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1521230113

Lefebvre C, Legouis R, Culetto E (2018) ESCRT and autophagies: endosomal functions and beyond. Semin Cell Dev Biol 74:21–28. https://doi.org/10.1016/j.semcdb.2017.08.014

Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176:11–42. https://doi.org/10.1016/j.cell.2018.09.048

Luo M, Zhao X, Song Y et al (2016) Nuclear autophagy: an evolutionarily conserved mechanism of nuclear degradation in the cytoplasm. Autophagy 12:1973–1983. https://doi.org/10.1080/15548627.2016.1217381

Mejlvang J, Olsvik H, Svenning S et al (2018) Starvation induces rapid degradation of selective autophagy receptors by endosomal microautophagy. J Cell Biol 217:3640–3655. https://doi.org/10.1083/jcb.201711002

Miao Y, Li G, Zhang X et al (2015) A TRP Channel senses lysosome neutralization by pathogens to trigger their expulsion. Cell 161:1306–1319. https://doi.org/10.1016/J.CELL.2015.05.009

Minakaki G, Menges S, Kittel A et al (2018) Autophagy inhibition promotes SNCA/alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophagosome-exosome-like phenotype. Autophagy 14:98–119. https://doi.org/10.1080/15548627.2017.1395992

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147:728–741. https://doi.org/10.1016/j.cell.2011.10.026

Moreno-Gonzalo O, Fernandez-Delgado I, Sanchez-Madrid F (2018) Post-translational add-ons mark the path in exosomal protein sorting. Cell Mol Life Sci 75:1–19. https://doi.org/10.1007/s00018-017-2690-y

Murrow L, Malhotra R, Debnath J (2015) ATG12–ATG3 interacts with Alix to promote basal autophagic flux and late endosome function. Nat Cell Biol 17:300–310. https://doi.org/10.1038/ncb3112

Nilsson P, Loganathan K, Sekiguchi M et al (2013) Aβ secretion and plaque formation depend on autophagy. Cell Rep 5:61–69. https://doi.org/10.1016/j.celrep.2013.08.042

Nilsson P, Sekiguchi M, Akagi T et al (2015) Autophagy-related protein 7 deficiency in amyloid β (Aβ) precursor protein transgenic mice decreases Aβ in the multivesicular bodies and induces Aβ accumulation in the Golgi. Am J Pathol 185:305–313. https://doi.org/10.1016/J.AJPATH.2014.10.011

Nolte-'t Hoen E, Cremer T, Gallo RC, Margolis LB (2016) Extracellular vesicles and viruses: are they close relatives? Proc Natl Acad Sci U S A 113:9155–9161. https://doi.org/10.1073/pnas.1605146113

Ostrowski M, Carmo NB, Krumeich S et al (2010) Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 12:19–30; sup pp 1-13. https://doi.org/10.1038/ncb2000

Pallet N, Sirois I, Bell C et al (2013) A comprehensive characterization of membrane vesicles released by autophagic human endothelial cells. Proteomics 13:1108–1120. https://doi.org/10.1002/pmic.201200531

Patel KK, Miyoshi H, Beatty WL et al (2013) Autophagy proteins control goblet cell function by potentiating reactive oxygen species production. EMBO J 32:3130–3144. https://doi.org/10.1038/emboj.2013.233

Pleet ML, Branscome H, DeMarino C et al (2018) Autophagy, EVs, and infections: a perfect question for a perfect time. Front Cell Infect Microbiol 8:362. https://doi.org/10.3389/fcimb.2018.00362

Ponpuak M, Mandell MA, Kimura T et al (2015) Secretory autophagy. Curr Opin Cell Biol 35:106–116. https://doi.org/10.1016/j.ceb.2015.04.016

Rabouille C (2017) Pathways of unconventional protein secretion. Trends Cell Biol 27:230–240. https://doi.org/10.1016/j.tcb.2016.11.007

Raiborg C, Stenmark H (2009) The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 458:445–452. https://doi.org/10.1038/nature07961

Record M, Carayon K, Poirot M, Silvente-Poirot S (2014) Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim Biophys Acta Mol Cell Biol Lipids 1841:108–120. https://doi.org/10.1016/j.bbalip.2013.10.004

Sadeghipour S, Mathias RA (2017) Herpesviruses hijack host exosomes for viral pathogenesis. Semin Cell Dev Biol 67:91–100. https://doi.org/10.1016/j.semcdb.2017.03.005

Sahu R, Kaushik S, Clement CC et al (2011) Microautophagy of cytosolic proteins by late endosomes. Dev Cell 20:131–139. https://doi.org/10.1016/j.devcel.2010.12.003

Sardar Sinha M, Ansell-Schultz A, Civitelli L et al (2018) Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathol 136:41–56. https://doi.org/10.1007/s00401-018-1868-1

Shrivastava S, Devhare P, Sujijantarat N et al (2016) Knockdown of autophagy inhibits infectious hepatitis C virus release by the Exosomal pathway. J Virol 90:1387–1396. https://doi.org/10.1128/JVI.02383-15

Simonsen JB (2017) What are we looking at? Extracellular vesicles, lipoproteins, or both? Circ Res 121:920–922

Sódar BW, Kittel Á, Pálóczi K et al (2016) Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. Sci Rep 6:24316. https://doi.org/10.1038/srep24316

Sui X, Chen R, Wang Z et al (2013) Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. Cell Death Dis 4:e838. https://doi.org/10.1038/cddis.2013.350

Takahashi A, Okada R, Nagao K et al (2017) Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. Nat Commun 8:15287. https://doi.org/10.1038/ncomms15287

Uytterhoeven V, Lauwers E, Maes I et al (2015) Hsc70-4 deforms membranes to promote synaptic protein turnover by endosomal microautophagy. Neuron 88:735–748. https://doi.org/10.1016/J.NEURON.2015.10.012

Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F et al (2013) Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 4:2980. https://doi.org/10.1038/ncomms3980

Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, et al (2014) Sorting it out: regulation of exosome loading. Semin Cancer Biol 1–11. https://doi.org/10.1016/j.semcancer.2014.04.009

Wang K, Wei Y, Liu W et al (2019) Mechanical stress-dependent autophagy components release via extracellular Nanovesicles in tumor cells. ACS Nano. https://doi.org/10.1021/acsnano.9b00587

Xu J, Camfield R, Gorski SM (2018) The interplay between exosomes and autophagy – partners in crime. J Cell Sci 131:jcs215210. https://doi.org/10.1242/jcs.215210

Yáñez-Mó M, Siljander PR-M, Andreu Z et al (2015) Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 4:1–60

Yuyama K, Igarashi Y (2017) Exosomes as carriers of Alzheimer's amyloid-ß. Front Neurosci 11:229. https://doi.org/10.3389/fnins.2017.00229