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REVIEW

Role of exosomes in the protection of cellular homeostasis

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ABSTRACT

Due to their ability to shuttle proteins, lipids and genetic material between distant cells, exosomes promote extensive phenotypic changes in recipient cells, modulating immune responses, cellular migration, cancer metastasis or the spreading of neurotoxic protein aggregates in neurodegenerative diseases. Besides intercellular communication, exosome biogenesis and secretion permit the rapid release of a selective repertoire of compounds, conferring cells with an additional mechanism to fight alterations in protein, lipid or RNA homeostasis during stress or pathological conditions. Here, we review the dual role of the different quality control mechanisms arising from the endolysosomal system and the diverse situations that control the decision between degradation or secretion. The crosstalk between exosome secretion and the different cellular degradation mechanisms confers an additional layer of protection to maintain cellular integrity and homeostasis in a number of physiological and pathological conditions.

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Introduction

Throughout their entire lifetime, cells are exposed to different harmful conditions, including thermal, physical, chemical, and oxidative stresses, altogether contributing to molecular damage. Cells have evolved various and interconnected organelle-specific quality control mechanisms to recognize and respond to this damage at different subcellular locations of the cell, including the cytosol, the endoplasmic reticulum (ER), the nucleus, mitochondria and the plasma membrane.[1-3] A complex network of chaperones recognizes unfolded proteins and supports their refolding. However, when refolding fails, chaperones assist in protein degradation via the ubiquitin-proteasome (UPS) or autophagy-lysosomal pathways.[4] Notably, under some circumstances or when load of proteins destined for degradation saturates the capacity of the proteolytic systems, cells can defend themselves against proteotoxicity through the release of toxic protein products to the extracellular media associated or not to lipid vesicles or involving other types of unconventional secretion.[5-9]

Exosomes are small lipid vesicles that are secreted to the extracellular environment upon the fusion of endosomal compartments with the plasma membrane.[10] Once in the extracellular milieu, exosomes can be taken up by nearby cells or, after reaching the blood stream, they can be taken up by distant cells, modulating the activity and fate of receptor cells.[11] Hence, exosomes are currently being recognized as important mediators for cell-to-cell communication in many physiological[12] and pathological situations, including immune response, cancer progression and metastasis, neuronal communication, cardiovascular diseases and progression of neurodegenerative diseases.[13-20]

During the last years, a lot of effort has been placed trying to elucidate the role of exosomes as vehicles for cell-to-cell communication. However, less attention has been placed on the impact that the biogenesis and secretion of exosomes has in producing cells. Exosomes may participate in the control of cellular homeostasis by promoting the release of intracellular harmful components, including proteins, lipids or nucleic acids. In this review, we emphasize the role of exosomes as a quality control mechanism that maintains intracellular homeostasis by promoting the selective release of intracellular harmful components. Clearance of damaged or toxic material, including proteins, lipids and even nucleic acids through exosomes, might alleviate intracellular stress and contribute to the preservation of cellular homeostasis.[21]

Here, we outline the evidence that selective incorporation and release of cellular compounds in exosomes is a quality-control strategy to alleviate intracellular stress and preserve cellular homeostasis. We highlight our current understanding on the physiological functions of

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exosomes and the endolysosomal system, and how they interact to preserve cellular homeostasis.

The endolysosomal pathway in the control of cellular homeostasis

The endolysosomal system is a highly dynamic compartment in which different membrane compartments modulate intracellular protein and lipid trafficking through regulated processes of internalization, sorting, recycling, degradation or secretion. Endocytosis allows the internalization of adhesion receptors, growth-factor receptor–ligand complexes, nutrient transporters, lipids, extracellular material and pathogens. Vesicles formed from the plasma membrane fuse and deliver their membrane and protein content to Rab5- and EEA1-positive early endosomes, which later undergo conversion from Rab5- to Rab7-positive endosomes.[22] During this conversion, a significant amount of the internalized content is recycled back to the plasma membrane through Rab11-positive recycling endosomes, while the remaining material is sequestered in intraluminal vesicles (ILVs) in late endosomes, also known as multivesicular bodies (MVBs).[23] Ubiquitinated membrane proteins are recruited to endosomes by the ESCRT machinery. ESCRT-0, ESCRT-I and ESCRT-II directly bind to ubiquitinated membrane proteins, while ESCRT-III and the ATPase Vps4 drive membrane-remodeling reactions that result in ILV invagination and scission. ILV biogenesis can also occur through ESCRT-independent mechanisms.[24] Tetraspanin proteins, such as CD63 and CD81, are proposed to act as regulators of ILVs formation.[25-28] The accumulation of ceramide in the endosomal membrane is also relevant for the formation of ILV.[29] The effect of ceramide on ILV formation has been proposed to be mediated by the local production of its downstream metabolite sphingosine-1-phosphate (S1P).[30] Recently, it has been described that not only membrane proteins but also cytoplasmic proteins can be selectively packaged into ILVs through a process called endosomal microautophagy (MA). MA depends on the ESCRT machinery and the electrostatic binding of Hsc70 to endosomal acidic phospholipids. Hsc70 interacts and introduce into ILVs cytoplasmic proteins containing KFERQ-motifs.[31]

Once ILV are formed, MVBs can degrade their cargo by fusing with lysosomes or, alternatively, MVBs can secrete their ILVs by fusing with the plasma membrane, eliminating the incorporated components through secretion to the extracellular environment. The molecular mechanisms and cellular situations that regulate the fate of MVBs are not completely understood. MVB are decorated with tethering complexes (CORVET, HOPS) and SNAREs (e.g. Stx7, Stx8, VTI1b and Vamp7) that facilitate the fusion with lysosomes or with the plasma membrane.[32] Hence, 2 quality-control mechanisms converge at the endolysosomal compartment for dealing with damaged, unwanted or toxic intracellular components. It can mediate the degradation and recycling of damaged or toxic intracellular components through lysosomal degradation, or allow the sequestration and release of these compounds in exosomes.

Role of exosomes in the preservation of intracellular homeostasis

Exosomes were first described in the 80s as a cellular mechanism by which reticulocytes get rid of the transferrin receptor (TfR) during their maturation into erythrocytes. TfR molecules were found in small vesicles inside endosomal compartments, which were released to the extracellular medium upon exocytosis of these endosomes, supporting the role of exosomes as vehicles to eliminate unwanted cellular compounds.[33,34] The protein composition of exosomes supports the function of exosomes as a cellular mechanism to get rid of obsolete, and toxic material. Misfolded and prion proteins are released in exosomes[35] and have been involved in the propagation of neurodegenerative diseases, such as Huntington disease, Alzheimer disease, and Parkinson disease. Several self-aggregating neurotoxic proteins including amyloid β, APP C-terminal fragments, Tau, α-synuclein, SOD1 and the prion protein (PrP) can be released from cells in exosomes.[36-41] These findings delineate a protein quality control pathway that, unlike degradation-based mechanisms, promotes protein homeostasis by exporting misfolded proteins through exosomal route.

The extracellular release of RNAs or microRNAs in exosomes may be a rapid way to regulate gene expression during cellular activation or transformation. For example, during an induced muscle atrophy process, myotubes secrete miR-23 and miR-182 to the extracellular environment through exosomes. miR-182 represses FoxO expression and its extracellular release in exosomes allows FoxO expression and the transcriptional program required for the acquisition of atrophy phenotype.[42,43] Similarly, upon activation, lymphocytes down-modulate the intracellular levels of miR-150 , a key repressor of lymphocyte differentiation and function, by releasing it in exosomes.[44] let-7 miRNAs generally play a tumor-suppressive role targeting oncogenes such as RAS or HMGA2. It has been proposed that cancer cells release let-7 miRNAs via exosomes into the extracellular environment to maintain their oncogenesis.[45] Inhibition of exosome secretion by silencing Rab27 proteins leads to impaired microRNA release and increased miRNA activity in the parental cell.[46]

Interestingly, exosomal miRNA secretion is a mechanism whereby cells rapidly dispose miRNAs in excess of their targets to adjust miRNA:mRNA ratio. Physiological (cell-activation dependent) or artificial overexpression of miRNA target sequences promotes a relative miRNA enrichment in P-bodies and depletion from MVBs and exosomes. Conversely, artificial overexpression of a miRNA enriches it in MVBs and exosomes.[47]

Exosomes participate in the regulation of intracellular RNA homeostasis by promoting the release of misfolded or degraded RNA products. yRNAs are involved in the degradation of structured and misfolded RNAs. Recent analysis of the RNA content of exosomes by deep-sequencing techniques has shown a remarkable enrichment of yRNA fragments and mRNA degradation products in exosomes.[48] Interestingly, exosomes may be also involved in the release of toxic RNAs since expanded trinucleotide repeat RNAs, such as the CAG repeats that underlie RNA toxicity in Huntington disease, are released from the cell in exosomes.[49] Other evidences support the relation between RNA degradation and its export to extracellular vesicles. In this regard, proteins involved in RNA processing are abundant in exosomes, and secreted RNAs have almost twice shorter half-life times than intracellular mRNAs.[50,51] Altogether, all these reports support that cells maintain intracellular RNA homeostasis through the release of distinct RNA species in extracellular vesicles.

Although less known, exosomes are involved in the control of lipid homeostasis. Exosomes have been shown to alleviate cholesterol accumulation in Niemann-Pick type C disease, a lysosomal storage disease in which cells accumulate unesterified cholesterol and sphingolipids within the endosomal and lysosomal compartment.[52] Antipsychotics drugs carry serious side effects such as the disruption of lipid homeostasis. Antipsychotics induces intracellular accumulation of LDL and impair intracellular cholesterol trafficking. When curcumin is administrated to cells under the effects of antipsychotics drugs, LDL is released into exosomes improving lipid homeostasis.[53]

Genomic and mitochondrial DNA have been found in exosomes. Evidence shows that genomic DNA is secreted in exosomes by cancer cells. One of the fragments of double stranded genomic DNA (dsDNA) secreted correspond to mutations of the suppressor gene p53.[54] Other works also showed that exosomes released from tumor cells lines contain high levels of ssDNA. Mitochondrial DNA (mtDNA) has been found in glioblastoma and astrocyte exosomes.[55] Telomeric repeat-containing RNA (TERRA) is release into exosomes by cells that present telomere dysfunction.[56,57] Although several works have reported the presence of DNA in exosomes, the function of exosomes in the regulation of DNA homeostasis is far from been elucidated.

Degradation *vs* secretion to preserve intracellular homeostasis

Whereas extracellular components and plasma membrane receptors are transported to the degradation/secretion pathway by the endosomal/exosomal pathway, intracellular components are transported to the lysosome by the process of autophagy, a 'self-eating' catabolic pathway that is used by cells to capture their own cytoplasmic components destined for degradation and recycling.[58] Autophagy can handle degradation not only of cytosolic macromolecules, but also of much larger structures such as excess or dysfunctional organelles, protein aggregates and intracellular pathogens.

Autophagy begins when double-membraned structures called phagophores engulf portions of cytosol that can include aggregates, lipids, carbohydrates, damaged organelles or invading pathogens. The phagophore expands and grows into a double-membrane compartment, known as the autophagosome.[59] Autophagosomes must undergo a series of maturation steps in part by fusing with endocytic vesicles, including early and late endosomes and MVBs.[60,61] The resulting hybrid organelles, called amphisomes, are more acidic and fuse with lysosomes to form degradative autolysosomes. Proper maturation of the autophagosome requires an intact endocytic trafficking pathway, components of the endosomal sorting complex required for transport (ESCRT) pathway,[62-64] and components involved in endocytic vesicle fusion.[65,66] Autophagosomes traffic along microtubules toward the microtubule-organizing center, where lysosomes are concentrated, enabling fusion and degradation of the contents by lysosomal hydrolases (Fig. 1).

In contrast to degradative autophagy, autophagy has other role in unconventional secretion by a mechanism called secretory autophagy.[67] One of the best studied example of secretory autophagy is the release of the proinflammatory cytokine IL-1β that depends on the autophagy factor Atg5 and the membrane-associated small GTPase Rab8.[68] Rab8a has been involved in the vectorial sorting to plasma membrane of others secretory autophagic cargo proteins like α-synuclein. In this regard, Ejlerskov and colleagues showed evidence that secretion of α-synuclein requires Atg5 and is increased by lysosomal dysfunction.[69] Moreover, silencing of deacetylase HDAC6 increase the levels of acetylated tubulin, impairing the fusion of autophagosomes with lysosome and increasing the secretion of α-synuclein. Interestingly, α -synuclein secretion has been reported to be meditated by exosomes and the secretory autophagy

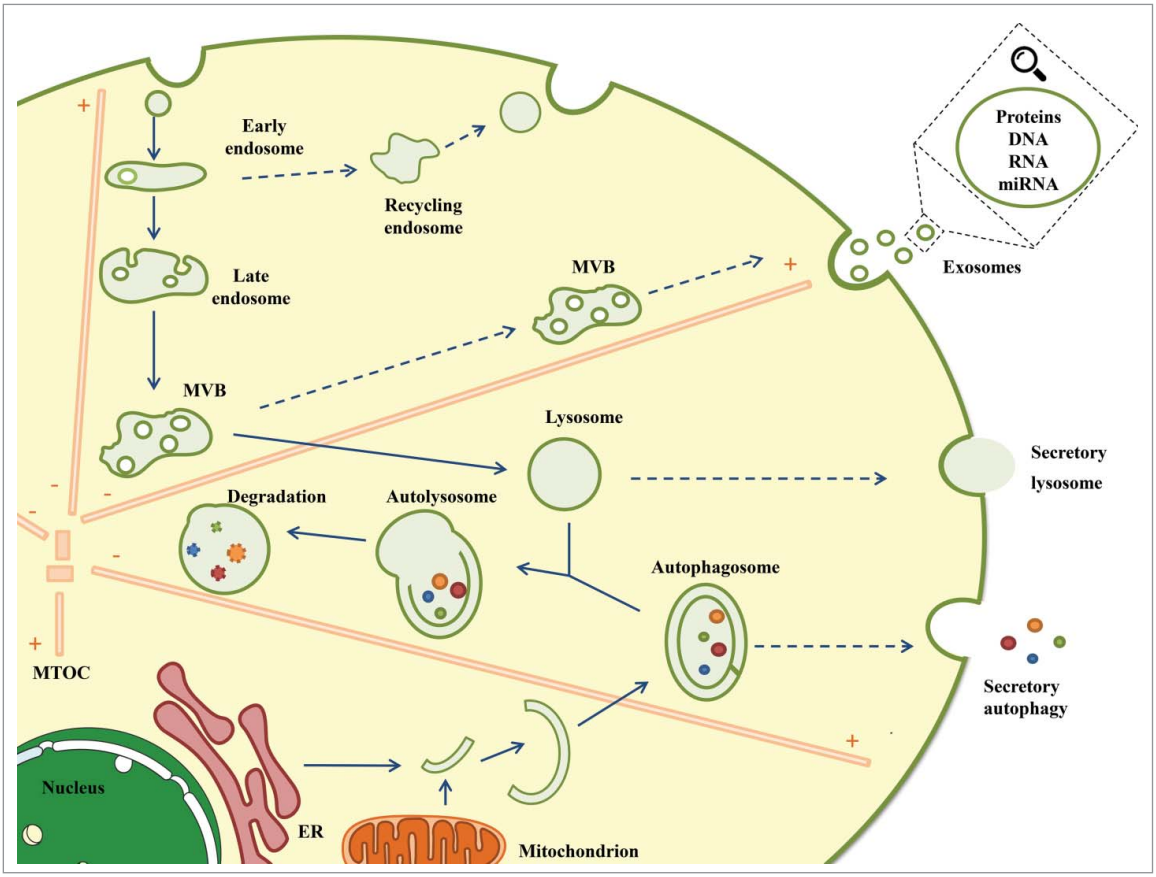

Figure 1. Degradation and secretion converge at the endolysosomal system. To ensure efficient function in the regulation of intracellular homeostasis, the endolysosomal system orchestrates endocytosis, MVB formation, exosome secretion, autophagy induction and lysosomal degradation, coordinating the balance between the degradation and secretion mechanisms. Clearance of damaged or toxic material, including proteins, lipids and nucleic acids through exosomes, secretory autophagy or secretory lysosomes might alleviate intracellular stress and contribute to the preservation of cellular homeostasis. ER: Endoplasmic reticulum, MVB: Multivesicular body; MTOC: Microtubule-organizing center.

pathways, underlining the crosstalk between both pathways to maintain cellular homeostasis. Autophagic cargo, depending on certain circumstances can be destined for autophagic degradation or secretion. Controlling autophagosomal motility along microtubules toward minus-end or plus-end could by an approach to direct autophagosomal organelles toward degradation or secretion.

Lysosomes are the principal degradative organelle of the cell.[70] Lysosomes degrade and recycle unwanted or damaged proteins and organelles to guarantee the continuous renewal of cellular constituents and to prevent the accumulation of toxic components. Besides their catabolic function, lysosomes contribute to different physiological processes including cell signaling, energy metabolism, plasma membrane repair, inflammation, or cell death.[71]

Lysosomes contain a single membrane that isolates and protects the cell from the acidic environment of the lumen by an internal thick glycocalyx. Degradation in the lysosomes is mediated by multiple acid hydrolases, which include members of the proteases, peptidases, sulphatases, glycosidase, lipase and nuclease protein families; this wide repertoire allows degradation of different biological substrates including proteins, nucleic acids and lipids.

It is well known how the flux of cargo through the endolysosomal system and the induction of autophagy/lysosomal degradation are adjusted by mTOR signaling that integrates signaling from nutrients, growth factors, and energy availability.[72] Lysosomes respond to nutrients availability by moving toward the plasma membrane, whereas starvation results in a tighter perinuclear localization.[73] Mitochondrial respiration is absolutely required for lysosomal degradation since in cells that relay on glycolysis as a consequence of mitochondrial dysfunction, there is an impairment of lysosomal degradation and function.[74]

Lysosomes may also act as secretory organelles in multiples cell types.[75] Lysosome release their content to plasma membrane as a mechanism for defense against

parasites and for plasma membrane repair.[76,77] When proteins destined for degradation saturates the capacity of proteolytic system of the cell, as occurs in lysosome storage diseases, cells can defend themselves against proteotoxicity releasing dysfunctional lysosomes.[78,79] Other possibility is that cells compensate for lysosome malfunction by disposal of potentially toxic cargos in exosomes. Hence, it starts to become evident that the functional state of the lysosome affects the release of exosomes.[80-82]

Concluding remarks

To ensure efficient function in the regulation of intracellular homeostasis, the endolysosomal system orchestrates endocytosis, MVB formation, exosome secretion, autophagy induction and lysosomal degradation, and coordinates the balance between degradation and secretion mechanisms (Fig. 1). The decision point between the 2 fates might be regulated by the cellular metabolic state, the nutrient availability or by external signals. Cellular status may affect the positioning of MVBs toward plasma membrane or perinuclear regions by switching microtubule motor proteins. Kinesin proteins may direct MVBs to plus end microtubule releasing MVBs as exosomes and dyneins family could guide MVBs toward lysosomes.[83] Understanding the signaling and the molecular clues that determine the traffic of cargo toward the lysosomes or the plasma membrane will allow us to manipulate the flux between degradation and secretion and could have immense relevance in the control of different pathological situations such as neurodegenerative or inflammatory diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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