Short review

Exosomes: From biogenesis and secretion to biological function

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Abstract

Exosomes are small microvesicles that are released from late endosomal compartments of cultured cells. Recent work has shown that exosome-like vesicles are also found in many body fluids such as blood, urine, ascites and amniotic fluid. Although the biological function of exosomes is far from being fully understood, exosomes may have general importance in cell biology and immunology. The present review aims to address some of the facets of exosome research with particular emphasis on the immunologist’s perspective: (i) exosomes as a novel platform for the ectodomain shedding of membrane proteins by ADAMs and (ii) recent findings on the role of exosomes in tumor biology and immune regulation.

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1. Introduction

Exosomes are small microvesicles released from cells, and have been the subject of intensive research in recent years. Originally described as a mechanism for the removal of cell surface molecules in reticulocytes, the pioneering work of several labs showed the importance of exosomes for general cell biology and in particular for the immune system. The reader is also encouraged to refer to other excellent reviews on exosomes biology that have recently been published [1,2].

1.1. The endosomal pathway, exosome biogenesis and secretion

Eukaryotic cells stay in contact with the environment by receiving signals such as cytokines or chemokines, the uptake of nutrients and the secretion of proteins into the extracellular space. For uptake and secretion, each cell has a complex network of membranes inside the cell. Using these compartments, cells not only take up macromolecules from the exterior environment (endocytosis) but also release newly-synthesized proteins or carbohydrates (exocytosis).

1.1.1. Exocytosis

There are different mechanisms by which cells release proteins into the extracellular space. The most common process is the release of large biomolecules through the plasma membrane by a process called exocytosis. In multicellular organisms, exocytosis has a regulatory or signaling function. According to the mechanism of release, exocytosis can be divided into two different modes: (1) constitutive (non-calcium-triggered) or (2) regulated (calcium-triggered) exocytosis. Constitutive exocytosis occurs in all cells and functions to either secrete extracellular matrix components or to incorporate newly-synthesized proteins into the plasma membrane after fusion with transport vesicles. Regulated exocytosis is particularly important in neurological signaling, where synaptic vesicles fuse with the membrane at the synaptic cleft and induce nerve impulses [3,4].

Fusion of multivesicular bodies (MVBs) with the plasma membrane and the subsequent release of their cargo represents another mechanism of exocytosis. Since the development of these membrane vesicles has an endocytic origin, this mechanism is a secretion process of the endosomal system. Other components of the endosomal system include endocytic vesicles, early endosomes, late endosomes and lysosomes. Endocytic vesicles arise through clathrin- or non-clathrin-mediated endocytosis at the plasma membrane and are transported to early endosomes. Late endosomes develop from early endosomes by acidification, changes in their protein content and their tendency to fuse with vesicles or more generally with other membranes.
These different vesicles can be distinguished by their physical shape and cellular location. In particular, early endosomes display a tubular appearance and are located at the outer margin of the cell, whereas late endosomes are spherical in shape and are located close to the nucleus. The key step in the formation of MVBs from late endosomes is reversed budding. During this process, the limiting membrane of late endosomes buds into their lumen, which results in a continuous enrichment of internal luminal vesicles.

1.1.2. Fates of vesicles within MVBs: degradation versus exosome secretion

The fusion of a MVB with the lysosome results in the degradation of the vesicle-associated proteins and lipids. This process allows the cell to remove transmembrane proteins as well as excessive membranes. The degradation of transmembrane proteins plays an important role in the down-regulation of activated cell surface receptors. For example, many activated growth factor receptors become internalized by ligand-induced endocytosis and are sorted into the luminal vesicles of the MVB to then undergo further degradation in the lysosome. Normal internalization without degradation is in some cases insufficient in diminishing signaling by the receptors. As depicted in Fig. 1A, even following internalization into early endosomes, the C-terminal phosphorylated docking sites and kinase domain of the activated epidermal growth factor receptor (EGFR/ErbB1) retain access to the cytosol where further signal transduction can occur. However, sorting into MVBs isolates the activated receptors from the cytosol and thereby from binding partners. Interestingly, mice carrying a mutation resulting in defective sorting of the EGFR into MVBs have a higher risk of developing tumors.

In dendritic cells (Fig. 1B), MVBs play a critical role as a storage compartment for MHC class II molecules and their associated invariant chain. In this case, the MVB compartment is called the MHC class II compartment (MII). Following internalization of antigen by dendritic cells, the invariant chain is removed and the MHC complex becomes loaded with the antigen peptide. These internal vesicles of the MII then fuse with the plasma membrane resulting in the presentation of antigen-loaded MHC class II molecules on the cell surface.

MVBs can also fuse with the plasma membrane leading to the release of the internal vesicles into the extracellular space (Fig. 1C). The released vesicles are then called exosomes. Many cell types release exosomes via this mechanism including hematopoietic cells, reticulocytes, B- and T-lymphocytes, dendritic cells, mast cells, platelets, intestinal epithelial cells, astrocytes, neurons and tumor cells. Depending on their origin, exosomes have previously been named dexosomes.

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Fig. 1. Different fates and functions of internalized vesicles. (A) Lysosomal degradation: some cell surface receptors such as the EGFR are internalized following ligand binding and activation. Degradation of the receptor following trafficking to lysosomes functions to down-regulate receptor signaling. (B) MHC class II storage compartment: antigens taken up into vesicles are degraded into shorter peptides which bind to MHC class II molecules in the MHC class II storage compartment (MCII). After delivery of the loaded MHC complexes to the cell surface, they can be recognized by CD4+ T cells. (C) Release of exosomes: multivesicular bodies can fuse with the plasma membrane and release internal vesicles (exosomes) into the extracellular environment.
(dendritic cell-derived exosomes) or exosomes (T-cell exosomes or tumor exosomes).

1.1.3. Sorting into the MVB and exosomes

Only very little is know about the sorting signals which are responsible for the sorting of proteins into vesicles within MVBs, which can be subsequently released as exosomes. As mentioned earlier, binding of a ligand to cell surface receptors results in receptor activation and initiation of signal transduction pathways. The fate of different activated receptors can be highly variable. Some receptors pass multiple cycles of uptake and recycling to the plasma membrane, whilst others, which are destined for degradation, are directly transported to lysosomes. In the case of most cellular transport mechanisms, such as nuclear translocation, proteins intended for a specific compartment display a characteristic amino acid sequence, which acts as a sorting signal [22]. For the recruitment of proteins into MVBs, there is currently no common sorting signal known for all cells. However, as discussed below, in certain cases some signals have been identified, which are necessary for internalization and trafficking of proteins to MVBs and exosomes.

For the EGF receptor, a point-mutation has been identified which does not affect receptor trafficking to the outer membrane of the MVBs, but prevents inward budding which leads to the formation of luminal vesicles containing the EGFR [23]. In Saccharomyces cerevisiae, ubiquitinylation of the G-protein coupled receptor (GPCR) Ste2 leads to trafficking into MVBs and subsequent degradation in lysosomes [24,25]. The highly conserved ubiquitin polypeptide is processed by a cascade of enzymes and becomes ligated to lysine residues of substrate proteins. The ligation of a single ubiquitin moiety (mono-ubiquitinylation) acts as a signal for endocytosis and the delivery into MVBs. However, the attachment of multiple ubiquitin chains (poly-ubiquitinylation) directs proteins for degradation in the proteasome. Mutation of lysine residues in Ste2 blocks downregulation via ubiquitin-triggered internalization. The formation of MVBs relies on ubiquitin-binding proteins [26,27]. The endosomal sorting complex required for transport (ESCRT) recognizes the ubiquitinylated cargo via Vps-27. Vps-27 then recruits another ESCRT complex and Tsg-101, which activate AP3/Alix. This complex drives the cargo into the budding vesicles. Although mono-ubiquitinylation triggers the uptake into MVBs, not all proteins in exosomes are ubiquitinated. It appears that there is also a passive mechanism involved in protein sorting to MVBs. The responsible signals for this processes are in some cases the presence of tetraspan enriched [28] or cholesterol enriched (=lipid rafts) membrane microdomains [29].

1.2. Exosomal structure and integral constituents

Exosomes are classically defined as membranous vesicles with a diameter of 30–100 nm. Many groups have performed proteomic analysis of vesicles derived from cell lines or body fluids such as urine, blood and ascites. Such analyses have shown that all mammalian exosomes share some common characteristics such as structure (bilipidic layer), size, density and overall protein composition. Some proteins are located on the surface or in the lumen of nearly all exosomes (exosomal markers). Notably, these include cytoplasmic proteins such as tubulin, actin, actin-binding proteins, annexins and Rab proteins as well as molecules responsible for signal transduction (protein kinases, heterotrimeric G-proteins) [30–32]. Most exosomes also contain MHC class I molecules [33,34] and heat-shock proteins such as Hsp70 and Hsp90 [30,35]. The protein family most commonly associated with exosomes is the tetraspinins including CD9, CD63, CD81 and CD82 [36–38]. Conversely, other exosomal proteins directly represent the proteome of the source cells. For example, analysis of urinary vesicles showed a link between exosomes containing aquaporin-2 and their origin from the urogenital tract [39]. Meanwhile, urinary vesicles have been examined for their potential use in the detection of malignancy-associated proteins and from these analysis it was concluded that exosomes could present novel biomarkers for renal diseases [39]. As exosomes are also found in serum and ascites fluids of tumor patients, it is possible that exosome analysis may eventually become important for diagnosis and biomarker analysis. In some tumors, certain overexpressed markers can also be detected in exosomes [40]. Consistent with their endosomal origin, there are typically no proteins of the nucleus, mitochondria, or endoplasmic reticulum detectable in exosomes [41]. In contrast, all exosomal proteins are typically found in the cell cytosol or at the plasma membrane.

1.3. Biological functions of exosomes and other secreted vesicles

In contrast to the fate of proteins trafficked for degradation to the lysosomal system, secreted exosomes are biologically active entities which are important for a variety of pathways. Some examples of roles for exosomes are discussed below.

1.3.1. Morphogen signaling

Exosomes and other cell-derived soluble vesicle compartments can themselves act as biologically active signals. For example, in developmental biology, morphogens play an essential role in tissue patterning. They are released by donor cells and spread through the adjacent tissue at different concentrations. Pattern formation in developing Drosophila tissues occurs in response to the graded distribution of morphogens such as Wingless and Hedgehog [42–44]. Interestingly, Wingless is tightly associated with secreted exosome-like vesicles called argosomes [45,46]. It has been reported that argosomes arise by a mechanism similar to the formation of exosomes from MVBs. The Wingless morphogen gradient is established by multiple transcytosis events. Thereby, the main contingent of argosomes travels through tissues and is found in endosomes, whilst few argosomes become degraded in lysosomes. The argosomes therefore function to spread a morphogen gradient of the Wingless protein.

1.3.2. Exosomes as immunological mediators

Exosomes display a wide variety of immunostimulatory properties. For example, exosomes secreted by Epstein-Barr virus (EBV)-transformed B cells are able to stimulate CD4+ T cells in an antigen-specific manner [15]. Meanwhile,
exosomes produced by mouse dendritic cells pulsed with tumor peptide are able to mediate the rejection of established tumors [13,47]. These antitumor effects were antigen-specific and were associated with the activity of T cells. Direct stimulation of T cells by membrane vesicles from antigen-presenting cells has also been reported [48]. Conversely, it has been suggested that intestinal epithelial cells, T-cell tumors and melanoma cells can secrete exosomes capable of inducing antigen-specific tolerance and FasL-mediated T-cell apoptosis [49,50].

A recent study has shown a role for exosomes in the modulation of T-cell signaling in pregnancy [51]. Exosomes from the serum of pregnant women could suppress the expression of important T-cell signaling components including CD3-ζ and JAK3. This suppression was correlated with exosome-associated FasL and a striking difference was noted between women delivering at term and those delivering pre-term. Marker analysis suggested that the exosomes were derived from the placenta and the release of FasL-containing exosomes may be one mechanism by which the placenta promotes a state of immune privilege.

Exosomes were also shown to play a role in the control of tumor growth [52]. Pretreatment of mice with exosomes derived from murine mammary carcinomas augmented subsequent tumor growth by inhibiting the cytolytic activity of NK cells. On the molecular level, tumor exosomes diminished levels of perforin in NK cells, a molecule that is essential for target cell lysis. It was shown that exosomes are taken up and remain stable in NK cells. Meanwhile, mRNA levels of perforin were not affected by exosomes, suggesting a post-translational regulatory mechanism. One possibility could be that perforin, stored in granula, becomes degraded by exosomes that have entered the NK cell.

As stated above, exosomes from tumor cells or dendritic cells pulsed with tumor-specific peptides can activate the immune system and are a valuable source of material for immunotherapy. Clinical trials are currently being conducted to assess the safety and efficacy of anti-tumor vaccines using exosomes [53]. However, the finding that exosomes mediate positive and negative immune regulatory functions suggests that the application of exosomes in immunotherapy must be carefully examined prior to onset of further clinical applications.

1.3.3. Ectodomain shedding of proteins in the MVB and exosomes

Transmembrane proteins in many cases can undergo cell surface cleavage to generate a soluble form of the molecule (Fig. 2A). This has been demonstrated for a large number of molecules including growth factors and receptors, adhesion molecules and other heterologous proteins [54] and the resulting soluble forms often exhibit alternate roles to the transmembrane form. For example, the transmembrane form of heparin-binding epidermal-like growth factor (HB-EGF) binds via its extracellular ectodomain to diphtheria toxin and the diphtheria toxin receptor-associated protein (DRAP27/CD9) [55]. In addition, the intracellular C-terminus of transmembrane HB-EGF binds the anti-apoptotic protein BAG-1 and this affects cell morphology, adhesion and resistance to apoptosis [56]. Meanwhile, following proteolytic ectodomain shedding, the soluble form of HB-EGF binds the EGFR to mediate a variety of

Fig. 2. Exosomes as a novel platform for ADAM-mediated cleavage. (A) Cleavage of cell surface proteins such as L1 and CD44 can occur at the cell surface. A second pathway of cleavage is initiated by the endocytosis of both ADAM proteases and cell surface substrates. During vesicles maturation, additional cargo is derived from the golgi and the trans golgi network (TGN). (B) Proteolysis of substrate transmembrane molecules by ADAMs can also occur in MVBs. The resulting cleaved/soluble forms are secreted by fusion with the plasma membrane.
biological effects including proliferation [57]. Ectodomain shedding events are predominantly mediated by various subclasses of zinc-binding metalloproteases such as the ADAM (a disintegrin and metalloprotease), MMP (matrix metalloprotease) and MT-MMP (membrane-type matrix metalloprotease) families [58]. ADAMs and MT-MMPs are themselves transmembrane proteins and we have demonstrated that the two most widely characterized ectodomain sheddases ADAM10 and ADAM17 (TACE) are also present in secreted exosomes along with substrate transmembrane molecules [59].

The majority of ectodomain shedding reports have suggested that proteolysis of transmembrane molecules occurs at the cell surface. However, in the case of several molecules, proteolytic processing can also occur inside the cell within vesicular compartments in MVBs and within secreted exosomes. For example, we have shown that the L1 adhesion molecule and CD44 undergo proteolytic ectodomain shedding in MVBs by ADAM10 [59–61]. The cleaved/soluble ectodomains of L1 and CD44 can then be directly released from the cell via exocytosis. Concurrently, full-length transmembrane forms of L1 and CD44 are released from the cell within exosomes and can undergo further ADAM10-mediated cleavage to generate soluble forms of each molecule (Fig. 2B). The notion for a role of exosomes in ectodomain shedding and protein secretion is supported by similar findings for the transmembrane proteins CD46 and the tumor necrosis factor receptor 1 (TNFR1). Ovarian adenocarcinoma cell lines release full-length transmembrane CD46 in vesicles [62]. Vesicle-associated CD46 can then become further processed by metalloproteases to generate a soluble form. Meanwhile, TNFR1 is released from vascular endothelial cells within exosomes and also undergoes ectodomain shedding [63]. Most fascinatingly, both CD46 and TNFR1 maintain their biological activity on the exosome surface with regards to ligand binding. These findings suggest a crucial role for exosomes in the biological function and ectodomain shedding of a variety of transmembrane molecules.

Interestingly, exosome secretion and ADAM-mediated ectodomain shedding may be tightly related processes. Stimuli which activate ectodomain shedding such as calcium flux, metalloprotease activators (e.g. 4-aminophenylmercuric acetate) and agents which stimulate cholesterol extraction from the plasma membrane (e.g. methyl-β-cyclodextrin) are also stimulators of exosome release [59]. In addition, inhibitors of ADAM-metalloproteases block exosome formation [63]. This raises the possibility that metalloproteases such as ADAM10 and ADAM17, which are present in endosomes and exosomes, may play a direct role in exosome formation.

2. Conclusions

In the present review we have focused on some recent aspects of exosome biology. Whilst much information on the biosynthesis of exosomes has been demonstrated using in vitro cell lines, many questions regarding the biological role of exosomes in complex cellular systems remain to be addressed. It is clear that exosome-like microvesicles are present in body fluids such as blood, urine, amniotic fluid ascites and pleural effusions under healthy and disease conditions. However, the origin of these exosomes and their destination for stimulation of distal cells remains unclear. It has been demonstrated that exosomes can be taken up by other cells. However, whether this establishes a novel mechanism of cell–cell communication is an intriguing yet unanswered question. The release and subsequent cleavage of transmembrane protein in microvesicles may be a novel mechanism of secretion that is distinct from the classical exocytosis pathway. It is presently unclear how exosomal secretion is regulated; however, recent work has shown that the p53 protein and the p53 regulated protein TSPAP may be involved in this process [64]. Meanwhile, it is worth mentioning that in certain disease conditions, exosomes may play regulatory functions. This is highlighted by the recent finding that β-amyloid peptides, associated with Alzheimer’s disease (AD), are released in association with exosomes and that exosomal proteins were found to accumulate in the plaques of AD patients brains [65]. In addition, prions are released from cells in association with exosomes and trafficking within the body functions as an infectious route for propagation of disease [66, 67]. The release of exosomes from tumor cells may also be a novel mechanism for chemoresistance. For example, enhanced exosomal export of cisplatin was observed in drug-resistant human ovarian carcinoma cells [68], whilst exosome-based secretion of doxorubicin mediates resistance in other cancer cell lines [69]. In conclusion, the studies mentioned in this review highlight multiple roles for secreted exosomes in a range of biological settings including development, immunology and cancer. Whilst functional roles of exosomes are only recently becoming clear, future investigations are likely to indicate the importance of these mediators in biological processes.

References


