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Extracellular vesicles shuffling intercellular messages: for good or for bad

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The release of extracellular vesicles (EVs) is a highly conserved process exploited by diverse organisms as a mode of intercellular communication. Vesicles of sizes ranging from 30 to 1000 nm, or even larger, are generated by blebbing of the plasma membrane (microvesicles) or formed in multivesicular endosomes (MVEs) to be secreted by exocytosis as exosomes. Exosomes, microvesicles and other EVs contain membrane and cytosolic components that include proteins, lipids and RNAs, a composition that differs related to their site of biogenesis. Several mechanisms are involved in vesicle formation at the plasma membrane or in endosomes, which is reflected in their heterogeneity, size and composition. EVs have significant promise for therapeutics and diagnostics and for understanding physiological and pathological processes all of which have boosted research to find modulators of their composition, secretion and targeting.

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Introduction

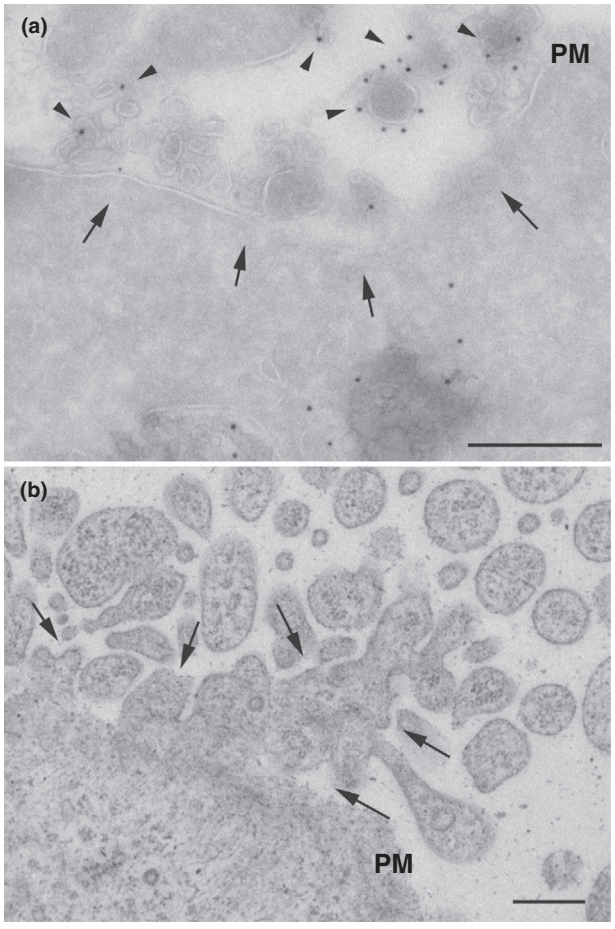
EVs (exosomes, prostasomes, microvesicles, ectosomes and oncosomes among others) are targeted to recipient cells where they exchange proteins, lipids and nucleic acids involved in essential cellular processes from immune regulation to neuronal communication, including tissue physiology and regeneration. Pathophysiological processes from tumor progression to pathogen transmission appear to be influenced by EVs. EVs carry a common set of components involved in their biogenesis, structure and putatively, their interaction with target cells. Unique subsets of proteins are present in EVs released by different cell types [1,2]. Discovered in the early 1980s, the term exosome was used to demarcate small (30–100 nm) vesicles of endosomal origin secreted by reticulocytes [3,4]. Exosome secretion was further described in immune cells, where small vesicles stimulate immune responses leading to tumor eradication [5,6]. A clear understanding of the different types of EVs and their respective functions has been difficult to achieve as cells can release not only exosomes but vesicles generated by budding of the plasma membrane, commonly called Microvesicles (MVs) [1] (Figure 1). The isolation methods are not yet optimal to recover highly enriched samples of each vesicle subtype [7]. Efforts are currently underway to standardize purification and characterization protocols for the different types of vesicles [8]. As an example, an improved characterization of different EV subtypes has been proposed based on lipid-protein ratios and lipid properties [9]. It is also essential to develop an acceptable nomenclature in the field [10]. In this review we will summarize recent advances in understanding the biogenesis, secretion and functions of EVs with special emphasis on exosomes.

Features and composition of exosomes

Electron Microscopy (EM) is the only method to visualize exosomes, as their size (30–100 nm) is under the limit of optical resolution and it remains to be demonstrated that individual exosomes can be detected by super resolution microscopy. Exosomes from cell culture media or body fluids analyzed by 'whole mount' electron microscopy appear as cup shaped vesicles [6]. This feature, often used to define vesicles as exosomes, is rather an artefact of fixation, contrasting and embedding procedures. Exosomes visualized by cryo microscopy in a close-to-native state appear as round vesicles with a well-defined bilayer [1,11]. However, such morphological features do not attest to the endosomal origin of exosomes as similar small sized vesicles may also originate from plasma membrane budding (Figure 1). A clue that small vesicles isolated from the cell culture media by differential ultracentrifugation at 100 000 g are likely to correspond to exosomes are the observations in situ, in cells, by electron microscopy of fusion profiles of MVEs with the plasma membrane [6] (see also Figure 1). Immunocytochemistry, performed on isolated vesicles is often ideal to confirm enrichment of components also present in ILVs of the MVEs. However, it sets a 'high bar' for confirmation as the process is highly dynamic and generally only few events are captured after fixation for EM procedures.

The composition of exosomes from different cells is gathered in the ExoCarta database although some entries

Figure 1



Visualisation of exosomes and MVs by electron microscopy. **(a)** Melanocytes were processed for ultrathin cryosectionning and immunogold labelled for the melanosomal enzyme TYRP1 (Protein A-Gold 10). Note in the extracellular space the membrane vesicles (exosomes) with a size ranging from 50 to 100 nm close to the deformed plasma reminiscent of an exocytic fusion event (arrows). Some vesicles are labelled (arrowheads). It cannot be excluded that the larger vesicles do not correspond to MVs that result from budding from the plasma membrane. **(b)** Breast cancer cells were fixed and processed for conventional electron microscopy. Vesicles of very different sizes are present in the extracellular space and some (MVs) bud directly from the plasma membrane (arrows). The smaller vesicles, not in continuity with the plasma membrane may correspond to endosome derived exosomes secreted in the same area. PM: plasma membrane. Bars: 500 nm.

Table 1

Examples of common proteins that are present in vesicles with exosomal features released by different cells. The protein content was determined by mass spectrometry analysis of exosomes from dendritic cells, melanocytes, Schwann cells, neurons, intestinal epithelial cells

Classifications	Proteins
Antigen presentation	MHC class I, MHC class II
Adhesion molecules	Tetraspanins: CD63, CD81, CD9, CD37, CD53, CD82 Integrins: α3, α4, αM, αL, β1, β2 MFG-E8
Membrane trafficking	Annexins: I, II, IV, V, VI, VII, XI Syndecan-1 Rab 2, Rab 5c, Rab 10, Rab 7 Arf 3, Arf 6, Arf 5 Clathrin
ESCRT proteins Heat-shock proteins	Alix, Tsg101 Hsc70, Hsp90
Cytoskeletal proteins	Actin, Cofilin 1 Moesin Tubulin: α1, α2, α6, β5, β3
Enzymes	Pyruvate kinase Alpha enolase GAPDH
Signal transduction	14-3-3 ξ, γ, ε Gβ1, Gi2α Syntenin-1
Lipid raft	Flotillin-1, Flotillin-2
Others	Lactadherin Elongation factor 1α Lamp2

include EVs using different modes of isolation and characterization. Other two databases, Vesiclepedia [12] and EVpedia [13], include published compositional data (proteins, lipids and RNAs) of both exosomes and MVs but always with caveat of suboptimal isolation methods. Table 1 shows exosomal proteins compiled from mass spectrometry data, obtained by us and main collaborators, after analysis of vesicles with exosomal features released by different cell types. A major limiting factor in the characterization of extracellular vesicles has been the absence of trustworthy methods for quantifying vesicle release. A reliable method to analyze small amounts of isolated exosomes is an optimized FACS procedure based on the labelling of isolated vesicles with fluorescent probes [14,15]. As a complement, the NanoSight LM10 based on light scattering automatically tracks and sizes nanoparticles on an individual basis although it is difficult to discriminate between small aggregates and vesicles [16].

Exosomes and microvesicles carry genetic materials

The identification of mRNA and miRNA in exosomes and the ability of the transferred exosomal mRNA to be translated in target cells is a major breakthrough in exosome science [17]. Further studies confirmed the presence of RNAs and short DNA sequences associated with EVs (obtained by ultracentrifugation not preceded by differential centrifugation steps) [18,19]. The approach used to characterize EV/exosome-associated miRNA among the various studies often make it unclear if the