Research Article

The definition of exosome and extracellular vesicles (EV)

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Membrane trafficking by exosomes has been the subject of intense investigation due to their diagnostic and therapeutic potential. However, although exosomes have been referred to by most authors as extracellular vesicles of endosomal origin, there are no extracellular vesicle-specific markers that can distinguish exosomes from other vesicle types. Moreover, the material isolated from different fluids such as cell culture conditioned media or plasma, generally contains a mixture of different vesicle subtypes. In this short article, we describe how the definition of the terms extracellular vesicles and exosomes varies in the literature, therefore prompting the need for a standardization of the terminology used to describe these membrane tracking particles. Such harmonization will ultimately help define the potential importance of specific vesicles as markers for various disease states.

Keywords: Extracellular vesicles, exosomes, definition

Introduction

Membrane trafficking is the process by which proteins and other macromolecules are moved throughout a cell or released into extracellular space. Membrane trafficking employs extracellular vesicles (EVs) produced by the cell membrane which then act as a transport intermediary [1]. As depicted in figure 1, EVs maybe formed by different mechanisms such as endocytosis of clathrine-coated vesicles or direct shedding of the plasma membrane. However, although exosomes have been referred to by most authors as EVs of endosomal origin, there are no EV-specific markers that can distinguish exosomes from other vesicle types. Moreover, the material isolated from different fluids such as cell culture conditioned media or plasma, generally contains a mixture of different vesicle subtypes [2]. In this short article,we describe how the definition of the terms EVs and exosomes varies in the literature, therefore prompting the need for a standardization of the terminology used to describe these membrane tracking particles. Such harmonization will ultimately help define the potential importance of specific vesicles as markers for various disease states.

Discussion / analysis of recent literature

The term ' exosome' was originally used to define the small membrane vesicles (50-90nm diameter) released by reticulocytes during their final stage of maturation into red blood cells[3][4][5][6] and during the process of transferrin internalization. However, exosomes have been subsequently described as the vesicles which develop into endosomes[7], particles with a specific physiological role and even the vesicles found in the sediment after centrifugation at 100,000xg of fluids such as cell culture conditioned media, blood plasma, urine, milk or saliva (table 1).

In recent years, the diagnostic and therapeutic potential of the exosomes has become the subject of intense research yet the lack of standardisation in terminology precludes a clear and accurate description of the relevant 'exosomes' used as medical tools.

We selected a few publications on exosomes published between January 1983 and December 2017 (table 1). Indeed the term exosome was described for the first time in Johnstone et al., 1987 [8]. Here it is shown that vesicles are released during the in vitro culture of sheep reticulocytes which can be harvested by centrifugation at 100,000xg for 90 min. These vesicles contain a number of activities, characteristic of the reticulocyte plasma membrane, which are know to diminish or disappear upon reticulocyte maturation. The activities include acetylcholinesterase, cytochalasin B binding (glucose transporter), nucleoside binding (i.e. nucleoside transporter), Na+-independent amino acid transport and the transferrin receptor. Cultures of whole blood , mature red cells, or white cells did not yield comparable levels of these activities, supporting the conclusion that the activities arise from the reticulocytes. It was suggested that vesicle externalization may be a mechanism for shedding of specific membrane functions which are known to diminish dring maturation of reticulocytes to erythrocytes. Previously, in Harding et al. [9]was described that after endocytosis, ransferrin was released, apparently intact, into the extracellular space. At 37 degrees C colloidal gold-transferrin (AuTf) clustered in coated pits and then appeared inside various intracellular membrane-bounded

compartments. Larger multivesicular endosomes became heavily labeled after longer (20-35 min) incubations. Multivesicular endosomes apparently fused with the plasma membrane and released their contents by exocitosis. These data suggested that transferrin was internalized via coated pits and vesicles and demonstrated that transferrin and its receptor were recycled back to the plasma membrane after endocytosis. Recycling appears to occur from multivesicular endosomes (MVE), but transferrin may also recycle directly from small vesicles or tubules to the cell surface.

One decade later, G. Raposo et al., 1996[10] found by immnunoelectron microscopy a similar process happened in B lymphoblastic cells. They observed that the limiting membrane of the late endocytic compartments MIICs (major histo-compatibility complex [MHC] class II-enriched compartment) could fuse directly with the plasma membrane, resulting in release from the cells of internal MHC class II-containing vesicles. These secreted vesicles, previously named exosomes (**how can you tell were the same type of EVs and mechanism as described by Jonhstone et al. [4], in a totally different system and cells?**[11][12]) , were isolated from the cell culture media by differential centrifugation for 60 min. at 100,000xg. Furthermore, on 2006, it was discovered that certain cells, including Jurkat T cells, possess discrete domains of plasma membrane which were enriched for exosomal and endosomal proteins, retained the endosomal property of outword vesicle budding and served as sites of immediate exosome biogenesis[13] (**then, 'exosomes' like described by Raposo et al. 1996[7] also bud directly from plasma membrane as the microvesicles (MVs)???**)

During the period from 2007 to 2017, what we named the 'boom period' of 'exosome' research, most authors stated that exosomes were vesicles of endosomal original what is curious since there is currently no way of determining the origin of the different vesicles as well as **endosomal proteins seem to present including in MVs (??)**[13]. Moreover, not all authors provide a definition or even mention the possibility that 'exosomes' may originate from endosome-like plasma membrane patches as described in Booth et al., 2006[13]. Moreover, the isolation of 'exosomes' via ultracentrifugation (UC) using similar protocols is described by virtually all authors (table 1), suggesting that they are potentially describing vesicles of different origin since the material isolated by UC generally contains a mixture of EVs, being impossible to ascertain the origin of each different type of EV. Since EVs play a functional role in many biological and pathological processes as demonstrated by some authors (table 1), it is important that they are accurately classified as **presently are used different unreliable endosomal markers which are present in different types of EVs**[14]

Conclusions

Given the potentially broad range of functions attributed to vesicles, it is important that researchers clearly define the origin of these vesicles. One potential solution to the characterisation of these particles when obtained by UC in terms of their physiological or pathophysiological roles maybe through flow cytometry (FC). Over the last two decades, FC has been regarded as one of the most commonly used technique for EV analysis, with the ability to determine the cellular orign of single EVs. Although having limitations in detecting small EVs, namely 'exosomes', this method enables the analysis of thosands of EVs in one sample, with simultaneous determination of multiple markers [15]

Competing interests

No competing interests were disclosed

Caption

Articles	Theoretical definition of exosomes				The working definition of exosomes				Function
	Description	diameter in nm	Origin	Markers	Isolation method	diameter in nm	Source	Markers	
Johnstone et al, 1987 Harding et et al, 1983	N.D.	N.D.	PM	Transferrin receptor	N.D.	N.D.	Reticulocytes	Transferrin receptor	Ferritin transport
Raposo et al., 1996	Small vesicles contained within the lumen of the MIICs	N.D.	N.D.	N.D.	2 · 30 min at 10,000 g, 60 min at 70,000 g, and 60 min at 100,000 g	N.D.	B cells	MHC class II molecules	N/A
Clayton et al, 2007	nanometer-sized vesicles, secreted by normal and neoplastic cells	30–100 nm in diameter	MVB	CD9, CD81, LAMP-1, glyceraldehyde-3-phosphate dehydrogenase, hsp70, hsp90, β1-integrin, and ICAM-1	ULTRACENTRIFUGATION	30–100 nm in diameter	cultured cancer cells	CD9, CD81, LAMP-1, glyceraldehyde-3-phosphate dehydrogenase, hsp70, hsp90, β1-integrin, and ICAM-1	impair lymphocyte responses to interleukin-2
Clayton et al, 2008	Originating within late endosomal compartments, as multivesicular bodies, or perhaps from endosome-like plasma membrane patches	N.D.	L.E.M.V or PM	N.D.	30% sucrose/D2O, and 100,000 × g for 3 h/ 100,000 × g , 2 h	N.D.	Tumor cell lines	MHC class I TSG101	down-modulate NKG2D expression
Skog et al, 2008	inward budding of endosomal membranes or shed directly by outward budding of the plasma membrane	30-100	ND	ND	110,000 × g for 70 min.	--------	Glioblastoma	-------	Tumor growth
Nilsson et al., 2009	Two types of microvesicles	30–100	N.D.	N.D.	16 000 g for 20 min at 10°C, followed by	N.D.	prostate secretions	N.D.	N/A

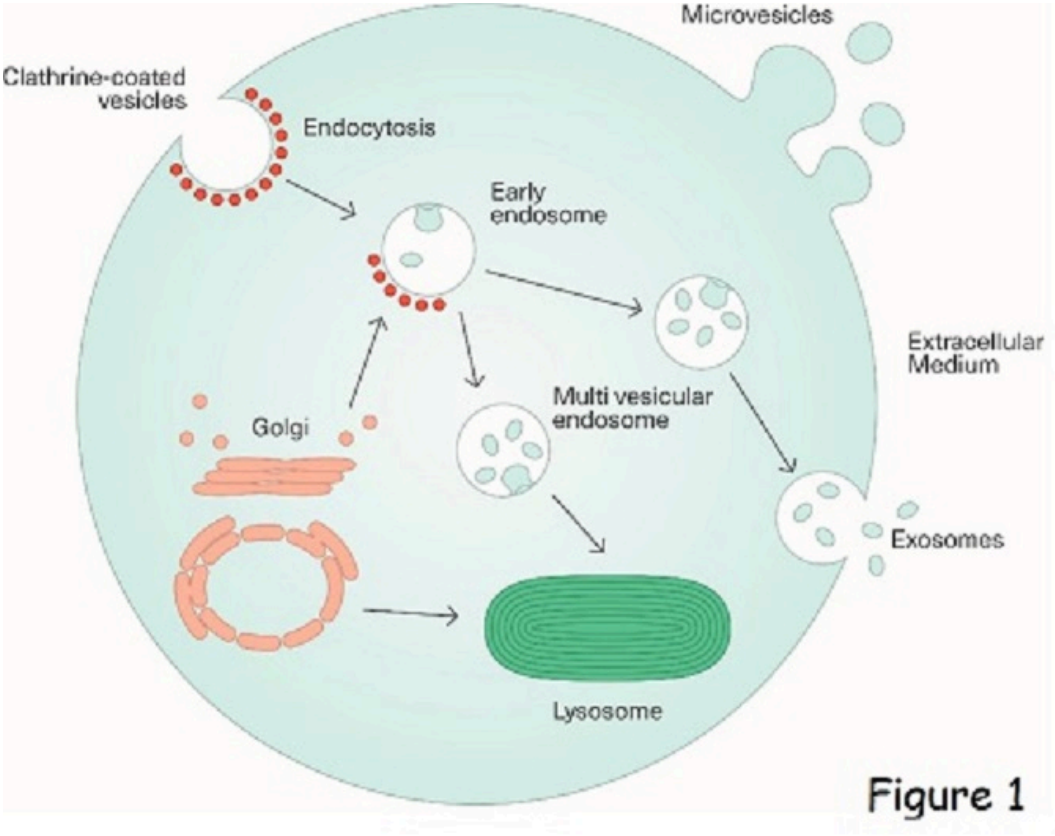
	are present in prostate secretions: (1) prostasomes (150–500 nm), and (2) exosomes, (30–100 nm) with a cup-shaped morphology				filtration through a 0.45 μm filter device/ 100 000 g for 90 min at 10°C				
Miranda et al, 2010	Exosomes are classically formed from the inward invagination and pinching-off of the late endosomal membrane	40–100	L.E.M.V	N.D.	2x 118,000 g for 70 min at 4 °C/ Pre-processed urine was added to the filtration concentrator and 4000 g 4 min	N.D.	urine	N.D.	N/A
Welton et al., 2010	originate from multivesicular bodies of the endocytic origin	N.D.	L.E.M.V		10,000 × g for 30 min/ 30% sucrose, D2O cushion / 100,000 × g for 2 h.	N.D.	HT1376 cells (bladder cancer)	TSG101	N/A
								MHC classI	
								CD9	
								CD81	
								LAMP1	
				N.D.				HSP90 α/β	
Dragovic et al, 2011	originate from multivesicular bodies of the endocytic origin	30-100	L.E.M.V	ND	150,000 g for 1 hour at 4°C	-------	Placenta	---------	N/A
Peinado et al., 2012	Exosomes are small membrane vesicles (30–100 nm) derived from the luminal membranes of multivesicular bodies	30–100	L.E.M.V	N.D.	20,000 × g for 20 min/ 2x 100,000 × g for 70 min	N.D.	Plasma from melanoma patients	Hsp90	Tumor microenvironment /tumor progression
							melanoma cells	Hsp70	
								Hsc70	
Bobrie et al, 2012	Exosomes are small vesicles (50–100 nm in diameter)	50–100	L.E.M.V	N.D.	10,000xg 30 min/ 2x 100,000 × g for 70 min	N.D.	mammary carcinoma cells	Alix	tumor microenvironment /tumor progression
								Hsc70	

	formed intracellularly in endocytic multivesicular compartments							CD63 Tsg101	
Van der Pol, 2012	Plasma membrane endosomes	50-100	ND	ND	ULTRACENTRIFUGATION	------	-----	----	N/A
Borges et al., 2013	These 30- to 100-nm vesicles are formed by inward invagination in endosomal compartments known as multivesicular bodies	30–100	L.E.M.V	N.D.	filtration on 0.1-μm pore filters/ 100,000×g for 60 minutes	N.D.	Kidney Mouse proximal tubular cells NIH-3T3 mouse embryonic fibroblasts kidney interstitial fibroblasts (TFBs)	CD63	tissue regenerative functions and fibrosis
Raposo and Stoorvegel, 2013	Endosomal origin	40-100	L.E.M.V		differential ultracentrifugation	-------	---------	----------	N/A
Akers et al., 2013	inward invagination in endosomal compartments known as multivesicular bodies	30–100	L.E.M.V	CD9 CD63	------	-------	-------	---------	N/A
Kahlert et al, 2014	Exosomes are small vesicles (50-150 nm) of endocytic origin that are released by many different cell types	50-150	L.E.M.V	N.D.	150,000 × g at 4 °C overnight	N.D.	Serum pancreatic cancer patients	TSG101 CD63	N/A
Melo et al, 2014	Exosomes are nano-vesicles of 50–140 nm in size that contain proteins, mRNA, and	50–140	N.D.	N.D.	filtration of the supernatant with 0.2 μm filters and ultracentrifugation for 2 hr	N.D.	Serum pancreatic cancer patients	Flotillin	microRNA biogenesis / tumorigenesis.

	microRNAs (miRNAs) protected by a lipid bilayer						Pancreatic cancer cells	TSG101	
								CD9	
Thakur et al., 2014	Exosomes, small membrane vesicles (30-100 nm) of endocytic origin secreted by most cell types	30-100	L.E.M.V	N.D.	20,000 × g for 20 min/ 2x 100,000 × g for 70 min	N.D.	cancer cells	Tsg101	N/A
								Hsc70	
								CD9	
Melo et al, 2015	Exosomes are secreted membrane enclosed vesicles of a size range of 50 to 150 nm diameter. Formed during the inward budding of late endosomes, they develop into intracellular multivesicular endosomes	50-150	L.E.M.V	N.D.	2x 100,000g for 2 hrs at 4°C or 150,000g overnight at 4°C/ 150,000g at 4°C for 2 hrs	N.D.	Serum pancreatic cancer patients	CD81, Flottilin1	N/A
							Pancreatic cancer cells		
Hoshino et al. 2015	No definition	N.D.	N.D.	N.D.	10 min -12,000g for 20 min. - at 100,000g for 70 min. pelleted again by ultracentrifugation	N.D.	cancer cells	Alix	Organotropyc metastasis
							Plasma cancer patients		
Costa-Silva et al., 2015	Exosomes, membrane vesicles of endocytic origin ranging in size from 30 to 150nm	30-150	L.E.M.V	N.D.	12,000g for 20 min. - at 100,000g for 70 min. pelleted again by ultracentrifugation	N.D.	Serum pancreatic cancer patients	No markers	Pre-metastatic niche
Kowal et al, 2016	Cells release heterogeneous vesicles of different sizes and	N.D.	L.E.M.V	N.D.	40 min at 10,000 × g / 90 min at 100,000 × g /recentrifuged at the same speed	N.D.	Dendritic cells	CD63, CD9, and CD81 co-enriched	N/A

	intracellular origins, including small EVs formed inside endosomal compartments (i.e., exosomes)								

Table 1



Mechanisms of formation of different types of EVs (Adapted from Guenat et al., 2017)[16]

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