The definition of exosome and extracellular vesicles (EV)

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Abstract

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]

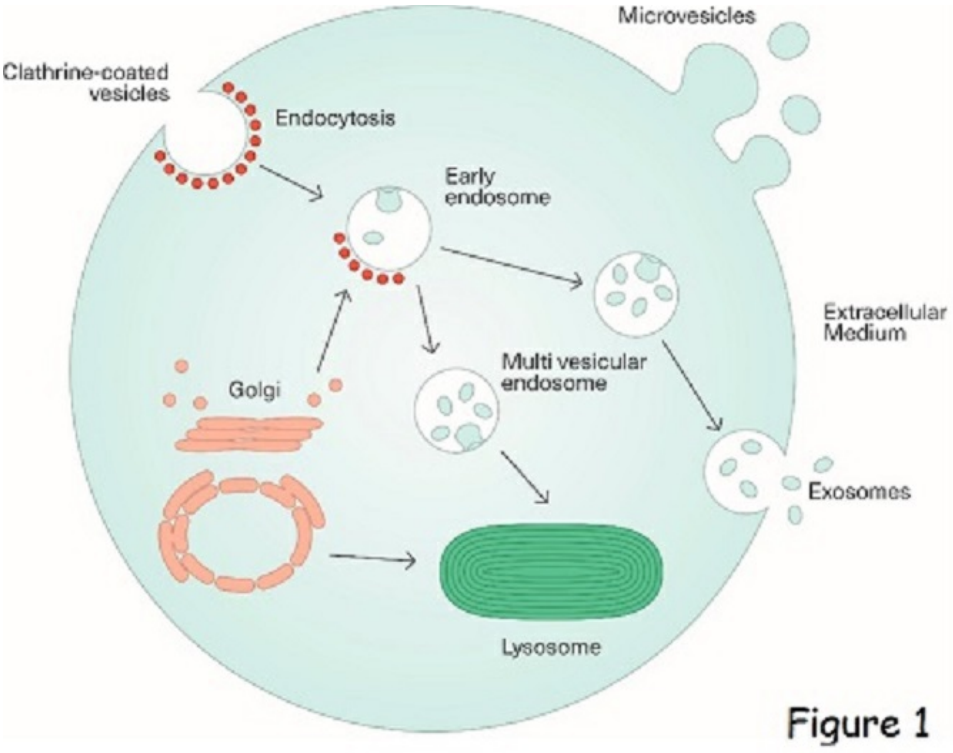
Competing interests

No competing interests were disclosed

Articles	Theoretical definition of exosomes				The working definition of exo	
	Description	diameter in nm	Origin	Markers	Isolation method	dia in r
Johnstone et al, 1987 Harding et et al, 1983	N.D.	N.D.	PM	Transferrin receptor	N.D.	N.D
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
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-nm dia

Caption

Table 1



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

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