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Subpopulations of extracellular vesicles and their therapeutic potential

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ABSTRACT

Extracellular vesicles (EVs), such as exosomes and microvesicles, have over the last 10–15 years been recognized to convey key messages in the molecular communication between cells. Indeed, EVs have the capacity to shuttle proteins, lipids, and nucleotides such as RNA between cells, leading to an array of functional changes in the recipient cells. Importantly, the EV secretome changes significantly in diseased cells and under conditions of cellular stress. More recently, it has become evident that the EV secretome is exceptionally diverse, with many different types of EVs being released by a single cell type, and these EVs can be described in terms of differences in density, molecular cargos, and morphology. This review will discuss the diversity of EVs, will introduce some suggestions for how to categorize them, and will propose how EVs and their subpopulations might be used for very different therapeutic purposes.

1. Introduction

Since the 1940s, when the first indication of the existence of extracellular particles and vesicles was observed in platelet-free serum (Chargaff and West, 1946), a massive number of studies on extracellular vesicles (EVs) have been conducted. Seventy years of research and over 6000 publications illustrate a substantial diversity in isolated vesicles based on differences in isolation protocols, cell origins, and biological functions, all leading up to an overwhelming and sometimes confusing and conflicting nomenclature. Over these years, several types of EVs have been identified and assigned various names based on *size*, including nanovesicles, microvesicles, virus-like particles, exosome-like vesicles, and microparticles; based on *biogenesis*, including exosomes, membrane particles, outer membrane vesicles, and shedding membrane vesicles; or based on *cell origin* or *function*, including platelet-dust, oncosomes, matrix-vesicles, ectosomes, dexosomes, texosomes, epididymosomes, cardiosomes, prostasomes, rhinosomes, apoptotic bodies, and tolerosomes. It is obvious that there are overlaps between the different subgroups; for example, tolerosomes are vesicles that can induce tolerance in the immune system (Karlsson et al., 2001), and they can be considered nanovesicles based on size or as exosomes if they are formed in the multivesicular body (MVB) pathway. However, it is also clear that within these subpopulations there are even further subpopulations based on vesicle size, density, RNA, protein, and DNA cargo as well as morphology. In this article, we will review how EVs might be subdivided into a vast array of subpopulations, and furthermore we discuss their potential as therapeutics.

2. Current state of the art

Currently, EVs are classified into three broad classes based on their biogenesis as exosomes, microvesicles, and apoptotic bodies. The population with the largest vesicle size, apoptotic bodies, consists of vesicles that are heterogeneous in size, with a diameter range from 200 nm to 5 μm, and they are shed from the plasma membrane of dying cells undergoing programmed cell death. Microvesicles, or ectosomes as they are sometimes referred to, are considered to be shed from the plasma membrane of viable cells and are 100–800 nm in size. Exosomes are 30–150 nm in size and are released into the extracellular space when MVBs fuse with the plasma membrane and release their intraluminal vesicles (Gyorgy et al., 2011; Mathivanan et al., 2010; Thery et al., 2009; van der Pol et al., 2012). However, it has been shown that vesicles that are larger than 100 nm are present within the endosomal pathway (Ronquist and Brody, 1985) and that vesicles smaller than 100 nm

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can bud off directly from the plasma membrane (Booth et al., 2006), which highlights some of the limitations of this decades-old classification. Therefore, totally different approaches to understanding the diversity of extracellular vesicles will be required for future classifications.

Particles of different size have different sedimentation properties, and therefore EVs can be isolated by differential centrifugation (Cvjetkovic et al., 2014; Jeppesen et al., 2014). Apoptotic bodies are considered to be pelleted at 2000–10,000 × *g*, microvesicles at 10,000–20,000 × *g*, and exosomes at >100,000 × *g* (Crescitelli et al., 2013). Additionally, EVs can be further purified and separated based on their density. Thus, density gradients and cushions using sucrose or iodixanol have been widely used during EV isolation. Depending on the type of EV-producing cell and type of EV subpopulation isolated, various densities has been reported (Table 1). However, the vesicles isolated in these preparations can be very heterogeneous, probably because of co-isolation of different subpopulations of EVs during the different purification steps. Therefore, these protocols will, at least to some degree, isolate mixed populations of EVs, and it has been suggested that the vesicles isolated in this way should more properly be called 2 K, 10 K, and 100 K vesicles, or possibly large, middle-sized, and small EVs, or high and low density EVs, because the biogenesis of each vesicle preparation is uncertain and might be independent of their size and density (Kowal et al., 2016).

3. Emerging subpopulations of extracellular vesicles

3.1. Subpopulations of extracellular vesicles based on morphology

For a long time, it was believed that all EVs were spherical in shape and having a very particular and quite limited size distribution. When examined with negative stain by transmission electron microscopy (TEM), they were also described as cup-shaped, with no information on their interior structure. More recently, EVs have also been examined by Cryo-TEM, a technique that uses no fixatives and thus allows the studied vesicles to retain their natural state. It was revealed that the cup-shaped morphology was an artifact from the fixation steps included in the negative stain technique (Gould and Raposo, 2013) and that the morphology of EVs was much more diverse than previously believed.

In a recent publication, we categorized EVs that had been released by a human mast cell line and isolated by floating at approximately at 1.11–1.12 g/cm^3. Most of the isolated vesicles (>75%) had a single membrane and were smaller than 100 nm in diameter. However, within this isolate we could in total define nine different categories of vesicles as illustrated in Fig. 1, including one or more vesicles within another vesicle, double membrane vesicles,

Table 1
List of publications studying subpopulations of EVs based on density.

EV source	Isolation protocol	Subpopulations of EVs	Analyses	Reference
Human seminal fluid from vasectomized men	3000 × *g* 10 min; 2 × 10,000 × *g* 20 min; supernatant top-loaded on density cushion (0.7M/2.0M sucrose) 100,000 × *g* 75 min; SEC column (Sephacryl S-1 000), pooled fractions were loaded onto a sucrose gradient (2.0–0.4M), bottom-loaded, 16 h, 190,000 × *g*	Low density (~1.09–1.16 g/cm^3) High density (~1.23–1.26 g/cm^3)	EM, LC-MS/MS, WB	(Aalberts et al., 2012)
Human seminal fluid from vasectomized men	3000 × *g* 10 min; 2 × 10,000 × *g* 20 min; supernatant was loaded onto a SEC column (Sephacryl S-1 000), fractions were pooled and loaded onto a sucrose gradient (2.0–0.4M), bottom-loaded, 16 h, 190,000 × *g*	Low density (~1.13–1.17 g/cm^3) High density (~1.24–1.26 g/cm^3)	Cryo-TEM, lipid analysis, WB	(Brouwers et al., 2013)
Mouse melanoma cell line (B16F10)	2000 × *g* 10 min; 10,000 × *g* 30min; 110,000 × *g* 70 min; 110 K pellet was loaded onto a sucrose gradient (2.5–0.4M), bottom-loaded, 16 h, 200,000 × *g*	Low density (~1.12–1.19 g/cm^3) High density (~1.26–1.29 g/cm^3)	EM, nanoLC-MS/MS, RNA size distribution (Bioanalyzer), gene expression analysis in recipient cells, WB	(Willms et al., 2016)
Human monocyte-derived dendritic cells (from blood samples of healthy donors)	300 × *g* 10 min; 2000 × *g* 20 min; 10,000 × *g* 40 min; 100,000 × *g* 90 min; 10 K and 100 K pellets were loaded onto an iodixanol gradient (10%, 20%, 30%), bottom-loaded, 1 h, 350,000 × *g*	F3 10 K pellet (~1.115 g/cm^3) F5 10 K pellet (~1.145 g/cm^3) F3 100 K pellet (~1.115 g/cm^3) F5 100 K pellet (~1.145 g/cm^3)	EM, WB, LC-MS/MS	(Kowal et al., 2016)
Human mast cell line (HMC-1)	300 × *g*, 10 min; 16,500 × *g* 20 min; 120,000 × *g* 70 min; 120 K pellet was loaded onto a sucrose gradient (2.5–0.4M), bottom-loaded, 16 h, 175,000 × *g*	Low density (~1.09–1.21 g/cm^3) High density (~1.24–1.31 g/cm^3)	EM, RNA size distribution, miRNA and mRNA microarray, next generation sequencing, LC-MS/MS, WB, Flow cytometry	(Lässer et al., 2017)
Bone marrow-derived mesenchymal stem cells	1500 × *g*, 20 min; supernatant concentrated with ultrafiltration (3 kDa); iodixanol gradient (5%, 10%, 20%, 40%), top-loaded, 18 h, 100,000 × *g*	Low density (~1.01–1.06 g/cm^3) Medium density (~1.08–1.15 g/cm^3) High density (~1.17–1.27 g/cm^3)	EM, miRNA (qRT) PCR, protein array, proliferation in recipient cells	(Collino et al., 2017)

EM, electron microscopy; WB, western blot; SEC, size exclusion chromatography.

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