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Review article

Extracellular vesicles: Novel promising delivery systems for therapy of brain diseases

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ABSTRACT

Extracellular vesicles (EVs) are cell-derived membrane vesicles virtually secreted by all cells, including brain cells. EVs are a major term that includes apoptotic bodies, microvesicles and exosomes. The release of EVs has been recognized as an important modulator in cross-talking between neurons, astrocytes, microglia and oligodendrocytes, not only in central nervous system (CNS) physiology but also in neurodegenerative and neuroinflammatory disease states as well as in brain tumors, such as glioma. EVs are able to cross the blood brain barrier (BBB), spread to body fluids and reach distant tissues. This prominent spreading ability has suggested that EVs can be exploited into several different clinical applications ranging from biomarkers to therapeutic carriers.

Exosomes, the well-studied group of EVs, have been emerging as a promising tool for therapeutic delivery strategies due to their intrinsic features, such as the stability, biocompatibility and stealth capacity when circulating in bloodstream, the ability to overcome natural barriers and inherent targeting properties. Over the last years, it became apparent that EVs can be loaded with specific cargoes directly in isolated EVs or by modulation of producer cells. In addition, the engineering of its membrane for targeting purposes is expected to allow generating carriers with unprecedented abilities for delivery in specific organs or tissues. Nevertheless, some challenges remain regarding the loading and targeting of EVs for which more research is necessary, and will be discussed in this review. Recently-emerged promising derivations are also discussed, such as exosome associated with adeno-associated virus (AAV) vectors (vexosomes), enveloped protein nanocages (EPNs) and exosome-mimetic nanovesicles.

This article provides an updated review of this fast-progressing field of EVs and their role in brain diseases, particularly focusing in their therapeutic applications.

1. Introduction

Extracellular vesicles (EVs) are membrane-contained vesicles originated from the endocytic pathway or from plasma membrane, that are released into the extracellular space by virtually all cells, playing an important role in intercellular communication during physiological and pathological processes [1,2].

There are currently three types of EVs, classified based on their intracellular origin and size (see Fig. 1). Exosomes are the smallest vesicles (30–150 nm), originated from the inward budding of multivesicular bodies (MVB). They can be degraded upon fusion with the lysosome or can release intraluminal vesicles (ILVs) into the extracellular space upon fusion with the plasma membrane, secreting exosomes [3,4]. Slightly larger than exosomes, microvesicles or ectosomes (50 nm–1 μm) result from the outward budding of the plasma membrane and are released in physiological conditions or in response to specific stimuli, such as changes in ATP levels [3]. The third type of EVs are apoptotic bodies (50 nm-5 μm) which are produced by cells undergoing apoptosis [5]. Finally, in an oncogenic context, a specific type of EVs derived from large cellular protrusions of cancer cells that can range from 1 to 10 μm has also been considered and designated as oncosomes [6–8].

Due to their different cellular ancestries, EVs contain specific proteins, lipids and genetic material, including messenger RNAs (mRNAs), microRNAs (miRNAs) and other small non-coding RNAs, and genomic DNA (gDNA) from their progenitor cells [9–11].

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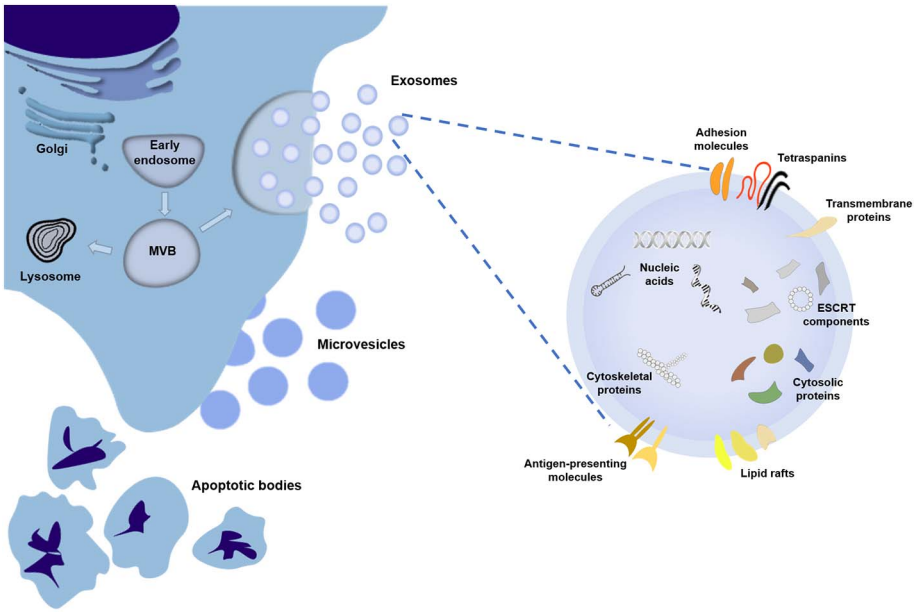

Fig. 1. Representation of EVs biogenesis and composition. Extracellular vesicles (EVs) comprise exosomes (30–150 nm), microvesicles (50 nm–1 μm) and apoptotic bodies (50 nm–5 μm). Exosomes originate from the inward budding of endosomal multivesicular bodies (MVB). MVB can be degraded upon fusion with the lysosome or can release intraluminal vesicles (ILVs) into the extracellular space upon fusion with the plasma membrane, being designated as exosomes. Microvesicles are larger than exosomes and result from the outward budding of the plasma membrane, while apoptotic bodies, the largest ones, are produced by fragmented cells undergoing apoptosis. Exosomes, in particular, are enriched in lipid rafts (e.g. cholesterol and flotillin-1), nucleic acids (DNA and RNA species) and they can also harbor a plethora of proteins (e.g. adhesion molecules, tetraspanins, cytosolic proteins and endosomal sorting complexes required for transport (ESCRT) components) [3,4,9,11].

A significant number of proteins found in EVs is involved in their biogenesis process, such as tetraspanins (CD9, CD63, CD81) and Rab proteins (Rab11, Rab27a, Rab27b) (see Fig. 1) [12]. Moreover, proteins of the endosomal sorting complex required for transport (ESCRT) pathway, which is thought to be the main driver of exosomal biogenesis, may also be present [13]. This fact suggests that protein loading of EVs is controlled through a wide range of regulated pathways, depending on cellular conditions and types of EVs involved, indicating a role for a sorting mechanism [14].

In addition, some nucleic acids, such as miRNAs, which typically bind to proteins, namely argonaute 2 (AGO2), heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) or synaptotagmin-binding cytoplasmic RNA-interacting protein (SYNCRIP), form complexes that can be selectively sorted into EVs [15–17]. This discriminating sorting mechanism seems to be able to package specific mRNAs and miRNAs resulting in considerable differences between the RNA profile of EVs and their progenitor cells [18–20]. After cell release, the lipid membrane protects EVs content from RNAse digestion and facilitates internalization by recipient cells [21]. Although EVs lipid membrane is similar in composition to their progenitor cells, the main difference is an apparent enrichment in phosphatidylserine, which determines EVs ability to be internalized by cells [22].

The regulatory properties of EVs are generally determined by their progenitor cell type, and once released they can be internalized by recipient cells and change their function [23].

EVs uptake also depends on the physiologic state of the recipient cells and on the EVs surface ligands, such as heparin sulfate proteoglycans (HSPGs) [24,25] or cell surface receptors, such as scavenger receptor type B-1 (SR-B1) [26]. Recently, Hoshino and collaborators have found that exosomal integrins present a tropism to specific organs during metastization [27]. After uptake, the content can be degraded by lysosomes or it can perform its natural function, such as silencing of mRNA, as is the case for miRNAs [28,29].

This mechanism of carrying specific information, protected by a natural lipid bilayer, confers stability in the bloodstream and keeps the payload safe from the immune system (stealth capacity). Beyond that, EVs lipid membranes can help bypassing natural barriers, such as the blood-brain barrier (BBB) [30,31]. All these characteristics make this system a promising therapeutic tool to address brain diseases, which will be prospected in this review.

2. Roles of extracellular vesicles in brain physiology and disease

In addition to direct, paracrine, endocrine and synaptic cell-cell interactions, EVs also contribute to intercellular communication in the brain through their basal release and uptake by surrounding cells, or release into the cerebrospinal fluid (CSF) and blood [1,32].

To get into target cells, EVs demand the fusion with cell membrane, which may occur either directly with the plasma membrane or with the endosomal membrane upon endocytic uptake. In fact, endocytosis seems to constitute the major EVs mechanism of entry (reviewed in [23]). EVs may enter into cells by a wide range of endocytic pathways, including clathrin-dependent endocytosis [33] or clathrin-independent endocytosis that covers micropinocytosis [34], phagocytosis [35], caveolin-mediated [36] and lipid raft-mediated uptake [37]. The uptake mechanism may be influenced by proteins and glycoproteins present on the surface of both the vesicles and the target cells [25,38].

Virtually all brain cells secrete EVs, including neurons [39,40], astrocytes [41], microglia [42,43] and oligodendrocytes [44,45]. There is evidence that synaptic glutamatergic activity regulates secretion of EVs in neurons, through *N*-methyl-D-aspartic acid (NMDA) receptors upon calcium entrance [39]. Indeed, Chivet and colleagues have shown that exosomes released upon activation of glutamatergic synapses bind selectively to other neurons instead of being internalized by glial cells [46].

A similar mechanism of regulation has been reported to promote the release of EVs carrying myelin-associated and other proteins from oligodendrocytes to neurons, establishing communication between these two neural cell types and contributing to myelination and neuronal integrity [44,47].

Simultaneously, there is evidence that EVs carrying antigens from oligodendrocytes can be taken up by microglia through a macropinocytotic mechanism [34]. Altogether, these results emphasize the role of EVs in interneural communication within the CNS.

In brain diseases, there is evidence that EVs are able to play a dual role: on one side, cells use EVs to remove toxic proteins and aggregates out of their cytoplasm; on the other side, these EVs can interact with healthy cells delivering their toxic cargoes and spreading pathology. Accordingly, it has been reported that neuron-derived EVs in Alzheimer's disease (AD) stimulate aggregation and degradation of amyloid-beta (Aβ) peptides and tau proteins [48,49]. In an oncogenic context, EVs carrying tumor antigens are also able to reach blood