Opinion

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The role of extracellular vesicles in the progression of neurodegenerative disease and cancer

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Extracellular vesicles (EVs) are released from many cell types, including normal and pathological cells, and range from 30 to 1000 nm in size. Once thought to be a mechanism for discarding unwanted cellular material, EVs are now thought to play a role in intercellular communication. Evidence is accruing that EVs are capable of carrying mRNAs, miRNAs, noncoding RNAs, and proteins, including those associated with neurodegenerative diseases and cancer, which may be exchanged between cells. For this reason, neurodegenerative diseases and cancers may share a common mechanism of disease spread via EVs. Understanding the role EVs play in disease initiation and progression will aid in the discovery of new clinically relevant biomarkers and the development of better targeted molecular and biological therapies.

Intercellular communication

Within the central nervous system (CNS), communication between cells has been widely explored. Intercellular communication is required for the nervous system to function, including synaptic plasticity, trophic support, ion regulation, and electrical activity [1]. Much of this communication has been studied at the synapse, where neurotransmitter release from synaptic vesicles and receptor-mediated signaling cascades play a major role in neural communication. Synaptic signaling occurs over a very short distance, requiring the postsynaptic cell to have receptors for the neurotransmitter released into the synapse from the presynaptic cell. In neurons, chemical signals trigger electrical signals that travel quickly throughout the CNS, where circuits are in place for communication to occur across brain structures [2]. In the absence of electrical signaling, chemical communication can occur locally using autocrine or paracrine signaling, or over long distances as in the case of the endocrine system [3]. However, long-distance signaling places free-floating proteins and nucleic acids at risk for degradation.

Intercellular transport of macromolecules is known to utilize well-characterized exocytic and endocytic release and uptake machinery, including multivesicular bodies (see Glossary). The intercellular transfer of viruses (e.g., pseudorabies), lectins, toxins (e.g., tetanus, cholera), and macromolecules in the CNS has been studied as a means for understanding mechanisms of axonal transport as well as recently being exploited for uncovering the CNS connectome [2–4]. Certain diseases are capable of taking advantage of neuronal circuitry by infecting connected brain structures (e.g., the basal ganglia in Parkinson's disease), leading to multisystem spread, atrophy of associated cellular networks, and loss of tissue integrity and function [2]. There is mounting evidence that extracellular

Glossary

Adeno-associated virus (AAV): a small virus which infects mammalian cells used as a viral vector to drive gene expression in infected cells in the laboratory and for gene therapy.
Autophagy: a process by which a cell digests parts of its own contents for turnover or removal via degradation by the lysosome. The role of autophagy is to support cellular maintenance and protect cells from undergoing apoptosis by removing damaged or excess organelles.
Blood–brain barrier (BBB): the BBB separates circulating blood from the CNS and is composed of endothelial cells, astrocytic end feet, and pericytes. The BBB restricts large molecules from entering the CNS, making it difficult to design drugs that are small enough to cross the BBB and have their actions on the brain.
Connectome: is a comprehensive map of the brain which diagrams cellular connections. The connectome is used to understand how cells and structures are organized and related to each other and contribute to diverse nervous system functions and behaviors.
Glial cells: are unique to the nervous system and are distinct from neurons. Microglia are found within the CNS and are involved in the immune response. Macroglia include oligodendrocytes and astrocytes in the CNS, and Schwann cells in the peripheral nervous system. Astrocytes are known to play a role in growth factor and ion exchange, whereas oligodendrocytes and Schwann cells are responsible for myelination of nerve axons.
Lysosome: an organelle responsible for the degradation of macromolecules using a low pH.
MicroRNA (miRNA): noncoding RNAs involved in post-transcriptional regulation of gene expression.
Multivesicular body/endosome: endosomes are membrane-bound compartments within the cell that are involved in the sorting and recycling of molecules. The early endosome can mature into a late endosome (also known as a multivesicular body, MVB) in which molecules are sorted into vesicles. Contents within the MVB will either be degraded or recycled back to the plasma membrane.
Neoplasia: abnormal cellular growth or division.
Neurodegenerative disease: a disease that causes cell death within the nervous system.
Prion disorder: occurs with the conversion of normal protein into a disease-causing isoform which mimics viral and bacterial pathogens. Accumulation of mutant prion protein may cause neurodegeneration and can be lethal.
Proteinopathy: a cellular pathology caused by a misregulated protein.
Virulence factor: molecule expressed and secreted by a pathogen that facilitates the ability of the pathogen to spread disease.

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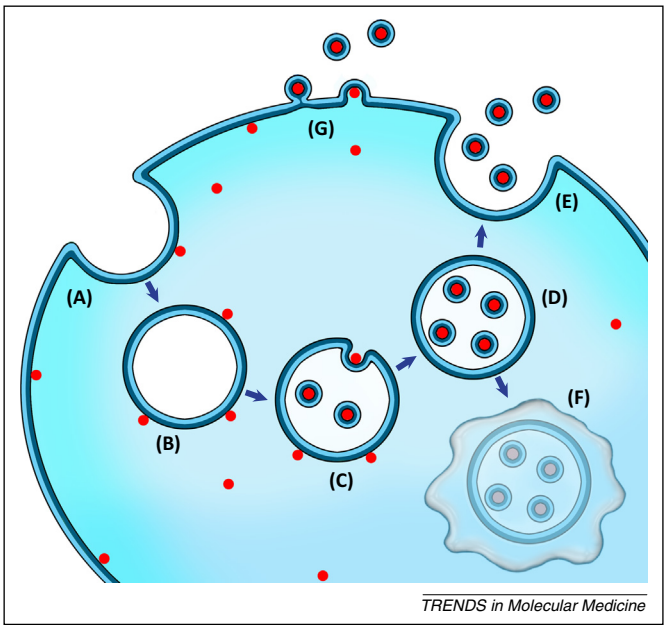

Figure 1. Extracellular vesicle (EV) biogenesis and transfer. Depiction of cargo loading into EVs such as nucleic acids and intracellular proteins where endocytosis of the plasma membrane **(A)** results in the uptake of proteins, nucleic acids, and membrane-associated molecules (red dots), and formation of the early endosome **(B)**. Upon transformation of the early endosome into the late endosome **(C)**, exosomes are formed by inward budding of the late endosome/multivesicular body (MVB) with the content, including mRNA, miRNA, DNA, and protein, in a similar orientation as at the plasma membrane **(D)**. Fusion of the MVB with the plasma membrane allows for the release of exosomes into the extracellular space **(E)**. Alternatively, the MVB may fuse with the lysosome for degradation **(F)**. Other EVs, such as ectosomes, can be formed directly at the plasma membrane **(G)**. Released EVs, along with their content, may be taken up by an adjacent or a distant cell.

vesicles (EVs) are involved in intercellular communication in many systems throughout the body, including the CNS. EVs are released by many cell types and have been found to carry cargoes including DNA, mRNA, miRNA, lipids, and/or proteins (Figure 1) [3,5,6]. EVs from brain-derived cells have been shown to carry molecules associated with neurodegenerative diseases [7–10]. Cancer cells have been shown to release EVs carrying oncoproteins and RNAs that support their viability [5,11–13]. We propose that neurodegenerative diseases and cancer share a common route of transport of disease-related proteins and nucleic acids that could mirror normal neurocytological transport mechanisms. In this way, we hypothesize that affected cells can take up and release a myriad of molecular and biological agents that have the potential to affect, as well as potentially infect, large numbers of other cells in distant circuitries and spread degenerative as well as neoplastic disease throughout the brain or the body.

Extracellular vesicles

Ranging from 30 to 1000 nm in size, EVs include distinct subtypes such as exosomes, ectosomes, microvesicles, oncosomes, and shedding bodies [3,14]. Exosomes arise from inward budding of the late endosome and are thus bound by a lipid bilayer similar in composition to the cell membrane from which the vesicle derived (Figure 1). Held within the cell by multivesicular bodies (MVBs), exosomes face degradation if the MVB fuses with a lysosome [15]. Alternatively, exosomes can be released at the plasma membrane when association of an MVB at the cell membrane occurs at lipid rafts. Other types of EVs, such as ectosomes, can be shed directly from the plasma membrane (Figure 1G) [16]. Endocytosis of an EV by another cell is thought to occur through clathrin- and receptor-mediated processes [3]. Exosomes are derived from the late endosome, and thus can be identified using markers for components of the late endosome, including for example, CD63, ALG-2-interacting protein (Alix), and Tumor susceptibility gene 101 (Tsg101), in combination with their size (30–100 nm), morphology (saucer shape upon fixation for transmission electron microscopy), and density (1.15–1.19 g/ml in sucrose) [3]. Apart from these identification methods, exosomes are difficult to distinguish from other subtypes experimentally, and much of the literature regarding this topic utilizes different terms and definitions to describe this population of vesicles. We will therefore refer to the whole population of secreted and released vesicles as EVs throughout this article.

By enclosing their cargo within a lipid bilayer, EVs provide a way for cells to communicate long distances as the cargo is protected from extracellular degradative proteases and RNases. The generation and release of EVs can be an extremely rapid cellular process that reflects dynamic cellular state and specific aspects of disease [17]. EVs have been found in many biological fluids including plasma, cerebral spinal fluid (CSF), and urine [3], making them ideal candidates for biomarker studies.

Although EVs have been studied for many years, especially in the field of immunology, there has been a recent surge of attention devoted to EVs in other fields [16]. Recent reports show that EVs are secreted from cells in the brain [18–22] and, in addition to a functional role in the CNS under normal conditions, may contribute to pathogenesis and disease progression. Given the recent attention to brain-derived EVs, it is important to investigate their normal function and how they might contribute to disease progression.

Normal EV communication in the brain

EVs are produced by several cell types in the brain, including neurons, oligodendrocytes, astrocytes, and microglia [18–22]. Neuronal interactions with glial cells are mediated by EVs through the transfer of proteins, mRNAs, and miRNAs, where vesicle release into the extracellular space is taken up by recipient cells [18,23–25]. Locally, these cargoes have so far been shown to regulate neurite growth and synapse formation [20,23,26]. In addition, because EVs shed from primary neurons contain the adaptor protein Ndfip1 (neural precursor cell expressed developmentally downregulated protein 4 family-interacting protein 1), they are hypothesized to play a role in protein removal and trafficking [27].

Normal cells release EVs constitutively, but increase their release in response to stress, such as hypoxia, DNA damage, exposure to a bacterium or virus, and cellular senescence [3,28]. One putative functional role for this stress response is to alert the immune system via activation of heat shock proteins (Hsp), as in the case of exosome-mediated Hsp70 release from astrocytes [29]. Additionally, ATP release stimulates EV shedding from microglia via