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Exosomes function in cell-cell communication during brain circuit development

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Abstract

Exosomes are small extracellular vesicles that mediate intercellular signaling in the brain without requiring direct contact between cells. Although exosomes have been shown to play a role in neurological diseases and in response to nerve trauma, a role for exosome-mediated signaling in brain development and function has not yet been demonstrated. Here we review data building a case for exosome function in the brain.

Introduction

Development and maintenance of neuronal circuits requires a complex series of events involving coordinated communication between multiple cell types over multiple length scales of space and time. The mechanisms known to underlie cell-cell communication during brain development include gap junctions, cell adhesion, and release of bioactive molecules such as neurotransmitters and growth factors. The possibility that exosomes, a type of extracellular vesicles (EVs), function as a novel form of cell-cell communication to establish and maintain brain circuits is beginning to be explored. Exosomes are released from cells and interact with other recipient cells to mediate physiological changes [1–3]. They can transfer bioactive lipids, proteins, non-coding RNAs, microRNAs, and mRNAs between cells without requiring direct contact between donor and recipient cells. Virtually all cell types in the brain release exosomes, including neural stem cells, neurons, astrocytes, microglia, oligodendrocytes, Schwann cells and endothelial cells [4–10]. Here we review evidence for exosome-mediated intercellular signaling in nervous system development and highlight critical open questions that would clarify their role(s).

Biogenesis and Release

Exosomes range in size from 40–110 nm and are usually identified by differential centrifugation, density, size, and biochemical markers [3,11]. Three biogenic pathways for exosomes have been identified (Figure 1). Exosomes are typically generated in endosomal compartments called multivesicular endosomes (MVE) or multivesicular bodies (MVBs) by

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invagination of the MVB membrane, engulfing cytoplasmic components of the cell. Exosome biogenesis in MVBs can be either dependent or independent of the endosomal sorting complex responsible for transport (ESCRT)-machinery. MVBs fuse with the plasma membrane, whereupon exosomes are released into the extracellular milieu. In a third biogenic pathway, thus far seen only in non-neuronal cells, EVs are generated by direct budding from the plasma membrane [12]. It was recently suggested that exosomes be defined by their biogenic pathway through MVBs and that EVs formed by direct budding from the plasma membrane be called 'ectosomes' [13]. Although this distinction may ultimately help identify distinct classes of EVs, practically speaking, the methods typically used to collect and enrich exosomes isolate EVs by density and biochemical markers, which do not distinguish EVs by biogenic pathway [3]. Although each of the three biogenic pathways may correlate with specific exosome cargo and unique signaling functions [11–14] diagnostic markers for EVs generated by different biogenic pathways are required to resolve these ambiguities.

Cellular and molecular characterization of the biogenic pathways has shed light on potential functions of exosomes generated by each pathway, and importantly, offered the means to manipulate exosome biogenesis. For instance, interfering with expression or function of ESCRT proteins decreases biogenesis of intraluminal vesicles and release of exosomes. Heparan sulfate proteoglycans have long been recognized as essential for many aspects of brain development and, with respect to exosome signaling, are known to facilitate both secretion and signal transduction of the WNT family of morphogens [15,16]. In non-neuronal cells, ALIX, a protein that interacts with several ESCRT components, was also shown to interact with the transmembrane heparan sulphate proteoglycan, syndecan, through its cytoplasmic adaptor, syntenin, to regulate the biogenesis of exosomes in MVBs [17]. Furthermore, heparanase increases exosome release [18]. Syndecans are coreceptors for growth factors and cell adhesion molecules like integrins and play a role in diverse functions in the brain including synaptic maturation, neuronal migration and axon pathfinding [19–21]. It is possible that deficits in brain development previously ascribed to individual proteins, such as syndecan, syntenin, growth factors and cell adhesion molecules, may be linked through a common pathway of exosome-mediated intercellular signaling. In addition, ESCRT components have been implicated in a host of neurodegenerative diseases like dementia, ALS, and Huntington Disease [22]. The ESCRT III component CHMP2B is involved in frontotemporal dementia and mutations in CHMP2B impair dendritic spine maturation in cultured hippocampal neurons [23]. Many of these neurodegenerative diseases attributed to malfunctioning ESCRT proteins may in fact result from deficits in exosome/microvesicle signaling. Exosome biogenesis by the ESCRT-independent pathway requires the sphingomyelin ceramide and can be decreased by blocking sphingomyelinase [4]. Exosomes generated by this pathway are reportedly released from oligodendrocytic and neuronal cell lines [4,24] and from motor neurons in *Drosophila* larvae [25]. Importantly, studies in the *Drosophila* neuromuscular junction (NMJ) indicate that exosomes generated through both the ESCRT-dependent and independent pathways are co-released at the NMJ and are both required for development of the NMJ in vivo [25–27].

It is interesting to note that tetraspanins, a family of proteins that organizes microdomains in the plasma membrane and regulates exosome biogenesis, cargo and signaling, are also important in brain function and have been specifically associated with X-linked mental retardation [28–30]. The tetraspanin, CD81, plays a role in neurite outgrowth [31], and CD81-null mice have a significant increase in brain size, that is attributed to increased astrocytes and microglia [32]. This series of papers provides another mechanistic link between exosome biology and brain development.

Once released, exosomes fuse with the plasma membrane of recipient cells, releasing their contents into the cytoplasm, transferring lipid and protein to the plasma membrane, or signaling directly by interacting with receptors on the cell surface. Exosomes may also be internalized by macropinocytosis or endocytosis. Exosome fusion or endocytosis may be mediated by receptor-mediated mechanisms. These diverse mechanisms for transfer of materials can initiate fundamentally different signaling cascades in recipient cells.

Function: Intercellular Signaling

Although exosomes and other EVs were once considered as a means to dispose of unwanted cellular debris, key studies demonstrated that exosomes released from cancer cells deliver proteins and nucleic acids that affect several aspects of tumor biology [1,14]. Within the nervous system, as in cancer, exosomes play both beneficial and pathological roles [2,14]. Neurons and glia release exosomes in vitro and in vivo [2,14,33–35]. Trophic support from oligodendrocytes and astrocytes to neurons is thought to be conveyed by exosomes [34]. For instance, Schwann cells transfer materials to damaged axons via exosomes [33] and the intercellular crosstalk between axons and oligodendrocytes during myelination appears to be mediated by exosomes [36]. By contrast, exosomes released from oligodendrocytes are reported to inhibit myelination [10], highlighting the importance of identifying specific exosome cargo to elucidate exosome-mediated intercellular communication. Two recent studies have implicated exosome-mediated signaling in the stimulation of neurite growth by astrocytes and multipotent mesenchymal cells [5,6]. In addition, microvesicles released from microglia can reportedly increase neuronal synaptic activity in vitro and in vivo [37,38], suggesting that this form of intercellular signaling could play a role in neuronal plasticity.

The best evidence so far for an effect of exosome-mediated signaling on brain development in vivo is from *Drosophila*, where the Wnt binding protein, Evenness Interrupted/Wntless/Sprinter (Evi), packages Wnt into exosome-like vesicles and is required for development and maintenance of the NMJ. WNTs are hydrophobic secreted morphogens that have diverse roles in nervous system development and function including development, maturation and plasticity of synapses [39]. WNTs are transported to target cells by various mechanisms including exosomes [16,26,40]. In the first demonstration of in vivo exosome-mediated transfer of WNT, Budnik and colleagues [26] showed that secretion of exosome-like vesicles, identified by the transmembrane protein, Evi, is required for intercellular transport of wingless (Wg), the *Drosophila* homolog of WNT1, at the NMJ. The release of Evi-containing vesicles depends on Rab11 and Syntaxin 1A [25]. Providing some of the most compelling evidence for the role of exosome-mediated signaling at synapses, Budnik's lab further showed that Rab11-dependent release of exosomes from presynaptic boutons transported the transmembrane protein, Synaptotagmin 4 (Syt4) from the motor neuron to the muscle fiber, where it is required for assembly of the NMJ [27]. Syt4 is particularly interesting cargo since it could provide a mechanism for calcium-dependent release of a retrograde signal from the muscle to the presynaptic terminal, thereby ensuring activity-dependent matching of pre- and postsynaptic components during synaptogenesis. It would be fascinating to determine whether a comparable mechanism operates for synapse assembly in the CNS.

In contrast to the beneficial effects of glia-derived exosomes mentioned above, some exosomes released from microglia and astrocytes induce inflammation that damages brain tissue [14]. In neurodegenerative diseases, exosome may transfer toxic proteins between cells in the brain, including synuclein in Parkinson's Disease and mutant SOD1 in ALS [2,14]. Conditions that result in packaging and release of damaging or beneficial exosome cargo in specific cell types are not understood. For instance, it is not clear if in disease conditions, toxic proteins generated in cells are packaged into exosomes 'passively', simply

reflecting the amount of protein in the donor cell, or if toxic cargo are actively targeted to exosomes. It is also possible that in disease recipient cells are more sensitive to bioactive molecules transferred by exosomes. In this case, the cellular pathology would rest more in the response to exosome signaling by recipient cells. So far, relatively little is known about the cellular mechanisms underlying toxicity in exosome signaling in the nervous system.

Dynamic regulation of exosome cargo and signaling

Exosomes deliver a variety of noteworthy cargo such as nucleic acids, (mRNA, noncoding RNAs, microRNA, viral RNA and mitochondrial DNA), proteins and lipids. Extensive proteomic and nucleic acid profiling of exosomes from a variety of cell types and body fluids indicates that exosome cargo vary according to the source or cell type of origin, as well as the physiological state or the health of animal/person/cells from which exosomes were collected [35,41–43], as described in the online resources Exocarta and Vesiclepedia [13]. These data suggest that packaging of exosome cargo in neurons and glia can change in response to different external stimuli, developmental stage or functional state of the neural circuit in which the cells participate. It is essential to determine the identity and relative quantities of cargo in neural exosomes to determine the functions for exosomes in intercellular communication during CNS development. In depth basic science investigation of exosome cargo dynamics and signaling is required to clarify the function of these fascinating organelles.

Activity-dependent exosome signaling

Exosome release from neurons, astrocytes and neural cell lines is triggered by depolarization-induced increased intracellular calcium [8,44,45], and SNARE complex-mediated membrane fusion[46], suggesting that more active neurons within a circuit could release more exosomes than less active or immature neurons. This observation also leads to the interesting possibility that activity-dependent regulation of exosome release provides a mechanism to control temporal features of exosome signaling and suggests that exosome-mediated communication could encode a ‘historical perspective’ reflecting prior activity in the cells/circuits. Furthermore, exosome biogenesis, cargo and secretion may be differentially distributed in apical and basal compartments of neurons, as in other polarized cells [47]. For instance, alphaB-crystallin, a protein involved in neurodegenerative disorders, is released in exosomes from the apical compartment of adult human retinal pigment epithelial cells [48]. Consequently activity-dependent regulation of exosome release from neurons and glia adds interesting possibilities for spatial and temporal control of which cells contribute exosomes to the extracellular signaling milieu.

Exosome Signaling within Neural Circuits

Exosome-mediated intercellular signaling has been implicated in neurodegenerative diseases, including ALS, Parkinson’s disease, prionic disease propagation, multiple sclerosis and Alzheimer’s Disease[7,45,49–55]. Similarly, recent studies have reported transmission of tau protein and α -synuclein (both found in the exosomal fraction) between synaptically connected neurons in vivo[56–58]. Based on the concept that diseases arise when cellular mechanisms in healthy cells go awry, it seems likely that exosome-mediated signaling serves a constructive function in the development and maintenance of neural circuits. One plausible hypothesis is that restricted exchange material via exosomes may occur between synaptically-connected neurons, as suggested by exosome-mediated intercellular signaling at the *Drosophila* NMJ [26,27]. Key challenges to explore intercellular communication pathways are the development of strategies to identify and manipulate exosome release and uptake in the intact brain. In this regard valuable attempts have been made to characterize exosomes recovered from the intact tissue[49], and to manipulate exosome release in vitro

[4,25,59,60,61,62]]. A challenge will be to develop tools that specifically target EV release without cytotoxic effects attributed to general interference with vesicular trafficking. Improvements in isolation/detection of exosomes by biochemical or histological strategies combined with the manipulation of their signaling in vivo would offer valuable information about the role of exosome-mediated signaling in brain development and function.

Manipulation of exosome cargo

Considerable progress has been made in developing exosomes as a tool to deliver bioactive reagents. One successful strategy has been to load exosomes with specific cargo, which is then delivered to and active in recipient cells[1,63,64]. This targeted strategy has yielded exciting results in several contexts, for instance decreasing proliferation in cancer cell lines[1,64] and decreasing atherosclerotic lesions in mice [63]. An important advance was targeting exosomes to CNS neurons for use in intact animals by expressing a fusion protein of a portion of the rabies virus glycoprotein on the exosome surface of dendritically-derived (ie non-immunogenic) exosomes[65]. Exosomes were then loaded with siRNA against BACE1 by electroporation and injected intravenously into APP-overexpressing mice as a model for Alzheimers Disease. These studies demonstrate that exosomes can be loaded with specific cargo, introduced into animals and ameliorate CNS disease models.

Concluding Remarks

While recent studies have provided important information on the composition and function of exosomes, several fundamental aspects of exosome signaling are yet to be established. The most simplistic model is that exosomes are a vehicle for intercellular transfer of cargo. A more speculative model is that exosome signaling in the nervous system is dynamically regulated between particular donor and recipient cells. Future studies focusing on the functional role as well spatial and temporal regulation of exosome signaling in the developing and mature nervous system will provide essential information about exosomes as a novel intercellular signaling mechanism in health and disease.

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Highlights

Exosomes are a mode of intercellular communication within the nervous system.

Although more widely studied in the context of neurological disease, exosomes may serve a beneficial function during brain circuit development.

Exosome signaling function may be dynamically regulated by modulating exosome cargo loading and release from donor cells and by modulating receptivity and signaling in recipient cells.

Elucidation of the function of exosomes in brain development and disease requires the generation of tools and reagents to identify and manipulate exosome signaling in the intact brain.

Figure 1. Exosome Biogenesis

A) Exosomes may have unique cargo and signaling capacity depending on the cellular compartment of their biogenesis and release. Cartoon of a neuron, dendrites, cell body and axons, boxed, from which exosomes with distinct signaling capacity could be released. B) Three possible mechanisms have been proposed for biogenesis of exosomes. The first 2 mechanisms fit the general consensus that exosome biogenesis and secretion involve multivesicular bodies (MVBs) or multivesicular endosomes (MVEs). These involve ESCRT-dependent (1) and ESCRT-independent (2) vesicle formation at MVB. In the third mechanism, observed so far only in non-neuronal cells, ESCRT-dependent vesicle formation by direct budding from the plasma membrane generates a heterogeneous population of extracellular vesicles (EVs) ranging in size from 40–1000nm. EVs in the size range of 40–110 nm label with some of the molecular markers of exosomes [12]. While EVs generated by each of these biogenetic pathways share some molecular markers, they are likely to carry different cargo and serve different functions [13,14].

Figure 2. Intercellular signaling mediated by exosomes in the nervous system

A.) Diagram of intercellular signaling mediated by exosomes between different cell types of the central and peripheral nervous systems. (a) Astrocytes stimulate neuronal arbor growth by releasing synapsin-containing exosomes [6]. (b) Mesenchymal progenitor cells seem to have similar effects by releasing and transferring microRNAs to neuronal cells[5]. (c) Microglia-derived microvesicles reportedly increase neuronal synaptic activity possibly as a homeostatic mechanism to maintain neuronal connectivity after synapse refinement or pruning[38]. In the crosstalk between neurons and microglia, exosomes derived from neurons can be collected by microglia as a mechanism for removal of toxic molecules [4]. (d) In addition, exosomes seem to play an important role in the bidirectional communication between neurons and myelinating cells (oligodendrocytes and Schwann cells) [7,36]. (e) Autocrine inhibitory exosomes derived from oligodendrocytes could play an important developmental role regulating its own expansion and the growth of the myelin sheath [10]. (f) Interneuronal signaling through exosomes has been reported under pathological conditions and may be responsible for transfer of proteins between synaptically connected cells [50,54,56]. (h) Evidence that exosomes play an important role in synaptogenesis comes from recent studies at the neuromuscular junction (NMJ), where exosomes transfer signaling molecules transsynaptically which coordinate synaptic growth, maturation [25–27]. B) Model hypothesizing the role for exosomes in synaptogenesis. Evi/Wg containing exosomes are released from neurons at *Drosophila* NMJ regulate synaptic growth and maturation [26]. Synaptotagmin 4 (Syt4) was shown to be delivered to the postsynaptic muscle via exosomes

and is important for synaptogenesis through retrograde signaling from the muscle to the presynaptic motor neuron [27]. Exosomes carrying Syt4 within the vesicle lumen could fuse with the postsynaptic membrane to create a guidepost for calcium-dependent fusion of vesicles releasing a retrograde signal. SVs = synaptic vesicles; lightning bolt depicts activity.