Review

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Physiological and pathological roles of exosomes in the nervous system

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Abstract: Exosomes represent a subtype of extracellular nanovesicles that are generated from the luminal budding of limiting endosomal membranes and subsequent exocytosis. They encapsulate or associate with obsolete molecules to eliminate or to transfer their cargos in intercellular communication. The exosomes are also released and transported between neurons and glia in the nervous system, having a broad impact on nerve development, activation and regeneration. Accumulating evidence suggests that the exosomes are attributed to the pathogenesis of several neurodegenerative diseases such as prion disease, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, as well as aging, in which the exosomes lack the capacity for cellular self-repair and spread their enclosed pathological agents among neurons. In this article, we review the current proposed functions of exosomes in physiological and pathological processes in the nervous system.

Keywords: exosome; glial cell; neurodegenerative disease; neuron.

Introduction: the nature of exosomes

In the extracellular space, several types of membrane vesicle are present. These vesicles of cell origin are mainly divided into two groups: shedding vesicles and exosomes. The former represent diverse types of particle, which are generated by directly budding and pinching off from plasma membranes. These include microvesicles (also termed microparticles, ectosomes, or shedding vesicles), irregularly shaped particles of heterogeneous sizes with diameters ranging from 100 nm to 1 μm diameter, and apoptotic blebs (generally referred to as apoptotic bodies), which are 50 nm–4 μm nonuniformly shaped vesicles generated during the late stage of cell death (1). Exosomes are generated via endocytic pathways, not in the plasma membrane. They are formed by inward invagination and fission of a distinct domain of endosomal membranes. The endosomal compartments filled with intraluminal vesicles (ILVs) are termed multivesicular bodies (MVBs), which correspond to intracellular precursor organelles for exosomes. A set of MVBs fuse with the plasma membrane and ILVs are released into the extracellular space. The secreted ILVs are called exosomes. Exosomes are uniformly spherical and the smallest extracellular vesicles (40–100 nm) (2, 3). They were first reported by two groups almost at the same time as transferrin receptor-associated vesicles in reticulocytes (4–6). Since their discovery, exosomes have been reported to be released from a number of cell types *in vitro*, including the cells in the nervous system (3). Moreover, they have been isolated from diverse body fluids, including blood, saliva, breast milk, and cerebrospinal fluids (CSF) (7–10). Early studies on reticulocytes showed that exosomes can transport obsolete contents out of these cells during their maturation to erythrocytes, assuming that the primary function of exosomes might be the disposal of unnecessary cellular components as an alternative degradation pathway. In neuroblastoma cells, a low activity of lysosomes induced by alkalizing agents leads to the enlargement of endosomal compartments, which promotes the release of accumulated proteins and lipids with exosomes (11, 12). Niemann-Pick type C1 disease (NPC1) is an autosomal recessive lysosomal disorder. Loss of function of npc1 reduces lysosomal inactivity, resulting in the accumulation of cholesterol and sphingolipids within lysosomes and endosomes (13). In fibroblasts derived from NPC1 patients, the elevated levels of exosome secretion

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were observed to attenuate the toxic lipid storage (14). The exosome generation has also been associated with the autophagy system. Activation of autophagy by the LC3 overexpression promotes the fusion of MVBs with autophagic vacuoles and subsequently inhibits exosome released from erythroleukemic cells (15–17). These lines of evidence point to a close relationship between exosome secretion and other degradation pathways. On the other hand, exosomes also participate in intercellular transmission and the spread of their cargo. The information exchanges mediated by exosomes have been described as a novel form of intercellular communication in various biological phenomena (18, 19). In this article, we summarize the topics on currently proposed biological events associated with exosomes in the nervous system.

Formation of MVBs is the first step in exosome generation, which proceeds via an endosomal sorting complex required for the transporter (ESCRT) machinery (20, 21). The ESCRT protein complex is composed of four subunits, ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which associate with accessory proteins such as Alix. These subunits are sequentially recruited to the cytosolic surface of the endosomal membrane and drive the inward budding and fission of the membrane to form ILVs. Recent studies have also demonstrated exosome generation in an ESCRT-independent manner. The ESCRT-independent exosome biogenesis includes lipid- and tetraspanin-required pathways. Lipid-dependent formation of ILVs is required for the conversion of sphingomyelin (SM) to ceramide (Cer) (22). The polarhead group of SM, the phosphocholine structure, is degraded by neutral sphingomyelinase (nSMase)-2 at the luminal surface of endosomes, leading to Cer generation. The cone-shaped structure of Cer may physically spontaneously induce a negative curvature in the membrane and promote ILV formation. Alternatively, sphingosine-1 phosphate, a metabolite of Cer, has been reported to induce inward budding through the activation of the G protein-coupled S1P receptor (23). Hydrolyzation of phosphatidylcholine (PC) into phosphatidic acid (PA) by phospholipase D has also been proposed to promote exosome generation (24). Translocation of phospholipase D2 from the cytosol to the endosomal compartment triggers exosome release. The precise mechanism of PA-dependent exosome generation is unclarified; however, similar to the conversion of SM to Cer, the loss of the headgroup of PC might drive ILVs formation through the alternations of the physical properties of endosome membranes (25). Several studies have shown that tetraspanins participate in the generation of MVBs (26–28). In Hela cells, tetraspanin CD63 has been reported to compete with Hrs, a component of ESCRT-III, to generate distinct ILV subpopulations (27). Rab GTPases are essential regulators for vesicle transport including endosome trafficking. Several Rab proteins, Rab11, Rab27, and Rab35, have been identified to be involved in exosome secretion in different culture cell types (29–32).

The compo differs depends on their origin, types and conditions of parental cells. Although exosomes are devoid of proteins associated with the mitochondria, nuclei, endoplasmic reticulum, or Golgi apparatus, they contain membrane and cytoplasmic proteins (2). Exosome biogenesis accounts for the loading of ESCRT proteins such as Alix and Tsg101 into exosomes. The ESCRT complex is also responsible for the binding and sequestration of ubiquitinated proteins into ILVs, resulting in the abundance of ubiquitinated cargos in exosomes (21, 33). Tetraspanins contribute to the sorting of proteins toward exosomes (34). In lymphoblast cultures, proteomic analysis of exosomes and their parental cells revealed selective exosome loading of CD81 tetraspanin-associated molecules. Protein lipidation, including the GPI (glycosylphosphatidylinositol) anchor and saturated fatty acid modification, serve as tags for targeting proteins to exosomes (35, 36). GPI-anchored proteins, such as CD55, CD58, and CD59, and palmitoylated proteins, such as Lyn, are selectively incorporated into exosomes during reticulocyte maturation (32, 37). Proteins with these lipid modifications are preferentially recruited into lipid rafts, which are microdomains of cell membranes (35, 38). The lipid composition of exosome membranes is distinct from that of cellular membranes, and the major components of lipid rafts, namely SM and cholesterol, concentrate in exosome membranes (25, 39). Just how these lipids are more preferentially packed into the exosomes than in parental cells remains unclear. However, ILV formation induced by SM conversion might result in the high loading of raft lipids into the generated vesicles. Indeed, other components of lipid rafts, glycosphingolipids (GSLs), are abundant in exosomes (40, 41). Various raft-resident proteins have also been reported to be abundant in exosomes (42). As another mode of targeting into exosomes, oligomerized membrane-anchored proteins, such as HIV Gag proteins, have been reported to be sorted in exosomes (43–45). In addition to proteins and lipids, exosome cargos include genetic materials such as mRNA and miRNA (46). They can be translated or regulate gene expression in recipient cells (47–49). To catalog accumulated data of exosome cargos, a manually curated web-based database, ExoCarta (http://www.exocarta.org) has records of proteins, lipids, and RNAs that have been identified in different studies (50).

Physiological function of exosomes in nervous systems

Exosomes are released constitutively or stimulus-dependently from almost all cell types in the nervous system, including neurons (51), astrocytes (52), oligodendrocytes (53), and microglia (54). The resultant exosomes can be collected from CSF in several animal species and humans (10, 55). Exosomes are capable of transferring their cargos between cells either homologous or heterologous (Figure 1). Here, we summarize the recent studies on the physiological roles of exosomes in neuronal development, transmission, and regeneration.

Development

During development in the central nervous system (CNS), oligodendrocytes change their morphology as they enwrap neuronal axons as the myelin sheath for rapid

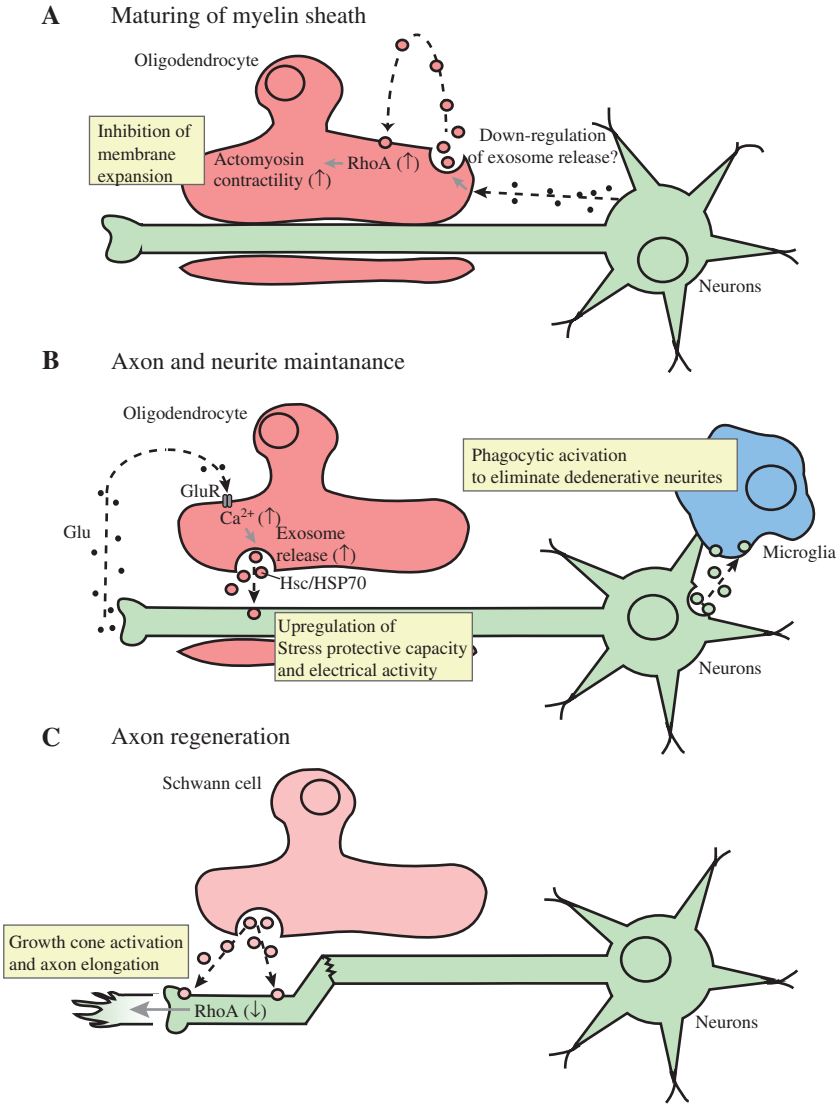

Figure 1: Roles of exosomes via neuron-glia communication.
(A) Oligodendroglial exosomes autonomously inhibit myelin sheath formation through RhoA-actomyosin signaling. As yet unknown secreted molecules from activated neurons inhibit oligodendroglial exosome release. (B) Electrically active neurons release glutamate (Glu). Glu stimulates oligodendrocytes to release exosomes through the Glu receptor (GluR)-mediated Ca^{2+} influx. The exosomes are taken up into neurons in where the effects of their cargos such as Hcp/HSP70 are exerted. Neuronal exosomes accelerate phagocytic activity in microglia. (C) Exosomes released from Schwann cells are selectively incorporated into axons. The exosomes activate growth cones, which leads to the elongation of injured axons.

impulse conduction. Extension of the myelin membrane sheath is regulated by signals from both the oligodendrocytes themselves and targeted neurons (56). The exosomes released from primary cultured oligodendrocytes have the ability to prevent membrane expansion (57). The feedback inhibitory effect of the exosomes is dependent on src-family kinase Fyn and RhoA-ROCK-myosin signaling. The molecules in the exosomes that are involved in actomyosin contractility have not been identified yet. It has been reported that the conditioned medium from primary cultured neurons inhibits the exosome release from oligodendrocytes. The signals from neurons that regulate glial exosome secretion are also as yet unknown. However, these signals could provide a novel mode of neuron-glia communication, in which neurons control myelin sheath formation by modulating the inhibitory activity of exosomes against oligodendrocytes.

The Wnt signaling pathway provides crucial positional information that regulates a number of developmental processes such as pattern formation, cell proliferation, and migration (58). Wnt proteins play important roles in axon guidance and synaptogenesis in the nervous system. Exosomes are found to be involved in intracellular transmission of Wnt proteins in the neuromuscular junction (59). Moreover, Wnt signaling plays a pivotal role in neuromuscular development in *Drosophila* (60). Fly Wingless (Wg), a Wnt homolog, is secreted from neurons with exosomes in an activity-dependent manner (61). The secreted Wg protein recognizes the Wg receptor Frizzled-2 (Fz2) at the postsynaptic surface and transduces signals in muscle cells. The neuronal protein Evi is of the protein required for the intracellular transport of the Wg protein and the subsequent exosome loading. Knockdown of YKT6, a SNARE protein, prevents the exosome-associated Wnt/Evi secretion; thus, it effectively blocks Wnt secretion with the exosomes and simultaneously induces adult wing notching in *Drosophila* (62). To date, the exosome-associated Wnt secretion has been confirmed in many other cell cultures including rat microglia and human HEK cells (62, 63). These findings have important implications regarding a universal role of exosomes in Wnt morphogenic signaling during nervous system development.

Neuronal activity

Neuronal exosome release was firstly observed in primary cultured cortical neurons (51). Proteomic analysis revealed that the neuronal exosomes contain the glutamate receptor subunit GluR2/3 and the adhesion molecule L1CAM, which is mainly expressed in neurons (64), together with exosomal marker proteins such as Alix, Tsg101, and flotillin-1. The exosomes are released from both soma and dendrites of neurons. Exosome secretion from neurons is promoted by Ca^{2+} influx or treatment with GABA receptor antagonists. In contrast, it is prevented by treatment with GluR antagonists (65), implicating that neuronal exosomes are released in a synaptic-activity-dependent manner. Neuroblastoma-derived exosomes are preferentially incorporated into glial cells rather than to neurons, whereas the exosomes released from stimulated cortical neurons are selectively bound and endocytosed into neurons (66). The machinery for exosome targeting selectivity remains unclear; however, these findings suggest a novel role of exosomes in interneuronal transport such as recycling of synaptic components.

Oligodendrocytes play critical roles not only in axon myelination but also in supporting the maintenance of long-term axonal integrity and control of nervous activity. Recent studies have shown that oligodendroglial exosomes have broad effects on neuronal physiology (53, 67, 68). The exosomes released from oligodendrocytes are triggered by Ca^{2+} entry or glutamate receptor stimulation similarly to those released from neurons (67). Hence, the glutamate that is released from the stimulated neurons might affect oligodendrocytes to promote exosome generation. The secreted oligodendroglial exosomes are in turn incorporated into neurons either from axons or somata. The protein and RNA cargos that are enclosed in exosomes are functionally retrieved and increase cell viability under the conditions of oxidative stress, nutrient deprivation, and ischemia (67, 69). The nature of the neuroprotective effect of oligodendrocytic exosomes remains obscure; however, multiple sets of signaling alterations in oligodendrocytes have been identified after exosome treatment using phosphorylation arrays (68). These include Akt, Erk1/2, CREB, GSK-3a/β, and JNK, which may contribute to the neuroprotective function. Another cargo protein, Hsc/Hsp70, might also be involved in cell robustness. The protein is derived from glial cells and shows neuroprotection (70). Hsc/Hsp70 is reported to be loaded onto the oligodendroglial exosomes and then taken up by neurons (67). In addition, the transferred oligodendroglial exosomes also enhance spontaneous electrical neuronal activity, as revealed by electrophysiological analysis (68). On the other hand, the exosomes derived from neurons support the microglia-dependent removal of degenerating neurites (71). In this case, serum depletion induces neurite degeneration in differentiated neuroblastoma, and cocultured microglial cells in turn remove the degenerative neurites by phagocytosis. It was found that neurite removal is accelerated when microglial cells are preincubated with the exosomes

derived from stimulated neuroblastoma, indicating a role of neuronal exosomes as a regulator of neuritic elimination through microglial activation. The removal of degenerative neurites and inactivated synapses is required for the reorganization of complex neuronal circuits in the brain. A future work will be necessary to elucidate the exosome functions *in vivo*; nevertheless, it is obvious that exosomes act as a crucial player in the physiological state of the nervous system.

Neuronal regeneration

In the peripheral nervous system, Schwann cells (SCs) are the principal glial cells that wrap motor and sensory neurons to form the myelin sheath and provide these neurons trophic support. SCs are also involved in neuronal regeneration after nerve injury. Nerve repair proceeds through the interaction between neurons and glia, which is not under the control of neuronal soma (72). SC-derived exosomes were found to promote axonal regeneration (73, 74). The exosomes derived from rat primary SC cultures are internalized into the axons of dorsal root ganglion neurons, not fibroblasts, and are especially concentrated in the axon terminal growth cones. The SC-exosomes facilitate axonal elongation after mechanical transection of dorsal root ganglion explant cultures. Following the exosome treatment, growth cones morphologically represent the active state, and the activity of RhoA, a GTPase whose activation inhibits axonal elongation, is significantly reduced. The effect of SCs-exosomes on axonal elongation is further confirmed in an *in vivo* study (73). Administration of SC-exosomes to rats results in the acceleration of nerve fiber regeneration after sciatic nerve crush.

Exosomes in neurodegenerative diseases and aging

It has been apparent that several pathogenic proteins that are involved in neurodegenerative diseases, including prion disease, Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), are loaded into ILVs in MVBs then subsequently secreted extracellulary via exosomes. Endocytic disturbances such as abnormalities of MVB formation commonly found in these diseases suggest that impairment of exosome generation is associated with the development of such diseases (Table 1). In the following, we summarize the

Table 1: Neurological disease proteins found in exosomes and MVBs/endocytic disturbance.

Human disease	Exosome-asscosiated protein	References	MVB/endocytic impairment	References
Creutzfeldt-Jakob disease	PrPc, PrPsc	(10, 75–79)	MVB and endosome enlargement	((80), (81) [BSE in cattle])
Alzheimer's disese	Aβ	(82–86)	Endosome enlargement	(81, 87–89)
	APP	(12, 82–84)	Overexpression of RabGTPases	
	BACE	(83, 84)	CHMP2B high immunoreactivity	
	Presenilin	(83)	PICALM, BIN1 mutation	
Alzheimer's disese, frontal temporal dementia	Tau	(55, 86, 90, 91)	Same as above	Same as above
Parkinson's disease	α-synuclein	(92–98)	CHMP2B mutation	(100, 101)
	LRRK2	(99)	CHMP2B-positive inclusions	
Amyotrophic lateral sclerosis	SOD1	(102–105)	CHMP2 mutation	(108–110)
	TDP-43	(106, 107)	[2)Axonal exdosome transport deficits	
Polyglutamine diseae (Huntington disease)	Heat shock protein (HSP40, HSP70, HSP90)	(111)		
Schizophrenia	Dysbindin-1B	(112)	Mutations in BLOC-1 subunits, dysbindin and muted	(113, 114)

accumulated lines of evidence on the bidirectional roles of exosomes, that is, they function in the clearance of intracellular pathogens as well as the intercellular spread of their toxicity in disease pathogenesis.

Prion disease

Exosomes in the nervous system were first studied in prion diseases, in which exosomes contained prion proteins (75). Prion diseases include scrapie in sheep, bovine spongiform encephalopathy in cattle, and Creutzfeldt-Jakob disease in humans. They are transmissible neurodegenerative disorders in which the cellular prion protein PrPc is abnormally folded into the neurotoxic pathogenic form PrPsc. In an infectious prion disease, when PrPsc proteins enter the host through the gastrointestinal tract, they replicate themselves in peripheral organs and then invade into CNS. Approximately 80% of all cases of prion diseases are sporadic and are characterized by the propagation of endogenously generated PrPsc into the brain. Aging is reported as one of the risk factors for a sporadic prion disease, since the onset of the disease is usually at the ages of 45–75 years, with the average of approximately 65 years of age. From recent *in vitro* studies, exosomes have been suggested to contribute to the release of membrane-bound PrP from host cells and to the incorporation of the pathogen into other cells. Both PrPc and PrPsc have been observed in MVBs and secreted into the culture medium with exosomes (75–78). PrP was also detected in the exosomes isolated from ovine CSF and PrP-inoculated mouse plasma (10, 79). The mechanism by which PrP is loaded into exosomes is still obscure. Recent findings suggest that the GPI membrane anchor of PrP might facilitate PrP sorting. PrP is predominantly localized at lipid rafts in the plasma membrane of culture cells (115). Inhibition of nSMase activity induces in turn inhibits both exosome release and PrP packaging (77, 116). These suggest that PrP might attach to lipid rafts through its GPI membrane anchor and then subsequently directly enter into the exosomes. Furthermore, it has been demonstrated that the exosome-bound PrP is infectious *in vitro* and *in vivo*. Exosomes released from infected cells can be transferred to other cells including neurons (76). Infectious PrPsc, which is contained in the exosomes, induces the conversion of PrPc into PrPsc in recipient cells. Intracerebral inoculation of infected cell-derived exosomes into normal mice for 3 months produced several symptoms of prion disease such as weight loss and locomotor disturbance in the inoculated mice (76).

If PrP in the exosomes spreads via body fluid, there might be no distance restriction in its propagation. Thus, exosomes could play an important role in transmitting the pathogenic protein from the peripheral to CNS. Several types of cell in the peripheral tissues have been reported to secrete PrP associated with exosomes as well as neurons (78). However, normal membrane vesicles including exosomes are generally considered to be not capable of passing through the blood-brain barrier (BBB). Recent studies shown that malignant alternation can change the miRNA profiles in the exosomes derived from the brain metastatic cancer cells, and the exosomes that hold specific miRNAs can promote the destruction of BBB (117). PrP infection has also been shown to cause changes in miRNA ultrastructural features and signature in exosomes (118, 119). The study of the efficiency of exosomes to target the CNS might be one of the challenging issues in the future.

Alzheimer's disease

In addition to prion disease, the exosomes in relation to the pathogenesis of AD have been intensively investigated. Although the exact pathological significance of exosomes remains to be conclusively resolved, several fascinating schemes have recently been proposed. AD is a neurological disorder with progressive loss of memory and cognitive function, and is characterized by extracellular deposits of amyloid-β peptide (Aβ), called senile plaques, mainly in the hippocampus and cortex. Aβ is a short peptide composed of about 40 amino acids and is generated mainly in neurons and released extracellulary. The levels of Aβ are maintained at a steady state in normal brains. However, impairment of the balance of Aβ metabolism is attributed to the formation of its toxic assemblies, which are connected to the AD pathology. In neurons, Aβ is derived from the sequential processing of amyloid precursor protein (APP) with β- and γ-secretases. Both of these enzymes are localized in endocytic compartments; hence, Aβ generation mainly occurs in the endocytic pathway (120). Neuronal endocytic dysfunction such as MVB enlargement occurs in the early stage of AD, suggesting that it might contribute to pathological Aβ accumulation (121–123). Aβ is found to accumulate in MVBs in AD brains (124). This is similarly found in APP-transfected neuroblastoma in which Aβ is localized in MVBs and secreted in association with the exosomes (82). APP and its metabolite CTFs are loaded into the exosomes(83). Exosomal proteins such as Alix and flotillin-1 are abundant in senile plaques in AD patients, suggesting a potential role of the exosomes in the formation of Aβ deposits (82).

Accumulated lines of evidence indicate that glycan-linked sphingolipids, especially sialic acid-containing GSLs, serve as sites for Aβ attachment (125, 126). Aβ-bound GM1, a GSL with sialic acid, has been found in brains exhibiting early pathological changes of AD (127). In neuroblastoma cell cultures, we found that synthetic Aβ binds to exosomes and the degradation of GSL-glycans prevents the binding between Aβ and exosomes (128). We next examined the GSLs-derived glycans in exosomes by quantitative GSLs-glycomics and found that GSLs are more highly abundant in the exosomes than in the parental cells (41). In particular, the levels of sialylated GSLs are much higher in the exosomes. These indicate that Aβ can associate with accumulated and clustered GSL-glycans, which are localized at the exosomal surface and exposed to the outer space. Later, the exosomes that are released from primary cultured neurons are also found to have much high levels of GSLs than those from primary microglia and astrocytes (85). Accordingly, Aβ can binds to only neuronal exosomes, not glial exosomes. It remains to be clarified how high levels of GSLs are packed into exosomes; however, it is reasonable to consider that GSLs might be integrated into the exosome membrane as components of lipid rafts through ceramide-dependent ILV formation. Indeed, the exosome release from neurons is almost prevented by SMase inhibition (128). Intriguingly, Aβ cannot only attach to exosome, but can also form nontoxic fibril assemblies at the exosomal surface in a GSLs-dependent manner. Microglia are brain-resident phagocytes, which contribute to the removal of dead cells and debris. Several studies including ours showed that neuronal and glial exosomes are preferentially internalized by microglia (128, 129). Aβ-bound exosomes are also actively taken up into microglia and then Aβ is degraded within microglia by the lysosomal system (128). These suggest that exosomes support Aβ clearance by trapping Aβ with their surface GSL and transporting it into microglia (Figure 2). An *in vivo* study showed that the neuron-derived exosomes injected into APP transgenic mouse brains also associate with endogenous Aβ in the brains and are incorporated into microglia (41). Furthermore, continuous infusion of the exosomes for two weeks decreases Aβ, inhibits amyloid depositions, and attenuates synaptic toxicity (41, 85). These studies clearly demonstrated that intracerebral exosome administration ameliorates Aβ-related AD pathogenesis. Improvement of Aβ clearance by exosome supplementation or enhancement of exosome generation might provide a novel therapeutic approach for AD. However, these indicate that normal phagocytic function is important for exosome-dependent Aβ clearance. If the clearance function of microglia is impaired or absent, the exosome-hoboing Aβ assemblies would

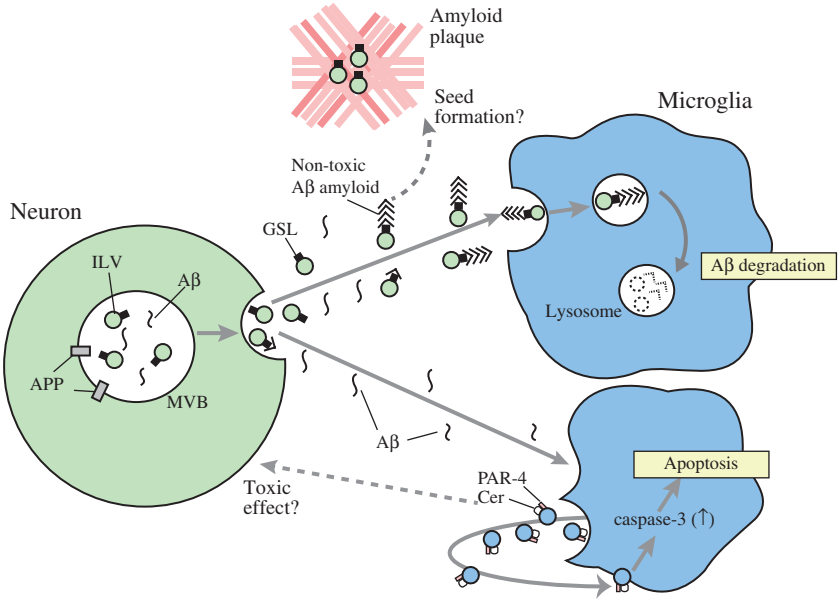

Figure 2: Roles of neuronal and microglial exosomes related to Aβ in Alzheimer's disease.
Neuronal exosomes are associated with intracellular or extracellular Aβ through GSLs abundant on the exosomal surface. Exosomes promote the assembly of nontoxic Aβ. Aβ-exosome complex is subsequently taken up into microglia to degrade Aβ. Neuronal exosomes likely promote Aβ clearance. In the absence of microglial phagocytic activity, exosome-associated Aβ might act as a seed for Aβ amyloid plaque. In turn, exosomes that are released from Aβ-stimulated microglia are autonomously incorporated into microglia and induces caspase-3-dependent apoptosis. Enriched Cer and PAR-4 in the microglial exosomes are responsible for the toxic effect.

trigger pathological events (i.e. senile plaque formation and interneuronal transfer of Aβ). Further careful *in vivo* studies are required to clarify the roles of exosomes in AD pathogenesis.

On the other hand, recent findings in primary astrocyte cultures indicated that synthetic Aβ accelerates exosome release. The exosomes derived from Aβ-treated-astrocytes, which contain prostate apoptosis response-4 (PAR-4) and abundant Cer, especially C18:0 Cer, cause apoptosis of normal astrocytes (130). Cotreatment with a specific antibody against PAR-4 is found to prevent the exosome-induced apoptosis. In addition, exosomes collected from nSMase-2-deficient (fro/fro) astrocytes do not show astroglial toxicity. These indicate that enriched Cer and PAR-4 in the exosomes are involved in the apoptosis of astrocytes. In addition to neuronal impairment, astrocytic dysfunction has been reported to be associated with AD pathogenesis, e.g. the elevated number of senile plaques (131). Thus, the astrocyte-derived exosomes might contribute to AD development. As challenging issues in the future, we need to confirm whether the toxic exosomes are generated within brains and examine how the exosomes affect other types of cell including neurons.

AD is a tauopathy which is characterized by the presence of neurofibrillar tangles composed of phosphorylated tau. Exosome-associated tau has been detected in culture media and CSF (55, 86, 91). Tau has been found in the exosomes derived from human tau-expressed neuroblastoma cells and to possess phosphoepitopes such as AT180, AT8, and PHF1, associated with AD (55). The levels of exosomal phosphorylated tau (AT270) increase in the CSF at an early stage of AD. The mechanism by which tau is load into exosomes remains unresolved. Electron microscopy showed that tau appears to attach to the surface of CSF exosomes. However, from the exosome topology, cytosolic protein tau would be expected to reside in the exosomal lumen. Further additional examination is required under conditions that tau leaked from degenerated cells is excluded. Recently, tau transmissibility between neurons and microglia has been proposed to be based on the propagation of tau during AD development (91). In a tau propagation mouse model, microglia, which phagocyte tau-containing neuronal debris, release tau with exosomes, which efficiently transmit tau to neurons. Depletion of microglia in the mice suppressed tau propagation.

As AD-related agents have been found to be included in blood exosomes, the neuron-derived exosomes are isolated by L1CAM immunoabsorption from human serum from the subjects of healthy control and mild cognitive impairment (MCI), and patients with sporadic AD (69, 86). As a result, higher levels of $A\beta_{1\text{-}42}$ and phosphorylated tau (S396, T181) are demonstrated in the neuronal exosomes from MCI subjects and AD patients than in healthy control. In addition, several lysosome-associated proteins, such as cathepsin D and lysosome-associated membrane protein-1 (LAMP-1), also show significantly increased levels in the exosomes in AD compared with the control, indicating that impairment of the endosome-lysosome pathway occurs in neurons in early AD brains. The findings implicate that the above proteins may be potential biomarkers for predicting AD development before clinical diagnosis.

Parkinson's disease

PD is a progressive neurological disorder pathologically characterized by dopaminergic neuronal loss and presence of intraneuronal inclusions called Lewy bodies (LB) in the midbrain. LB is formed from abnormally folded aggregates of α-synuclein (α-syn), which also forms toxic oligomeric intermediates that are directly linked to neuronal damage (132). α-Syn is generally considered to exert its toxic effect only inside cells; however, recent findings showed that α-syn is secreted extracellulary and transmits its toxicity to neighboring cells both *in vitro* and *in vivo* (133, 134). In α-syn-transfected neuroblastoma cells, either monomeric or oligomeric α-syn is released from the cells in association with exosomes (92). The exosome-bound α-syn induces the cell death upon their exposure of differentiated neuroblastoma cells to this α-syn. Moreover, the exosome-associated α-syn oligomers are more prone to be uptaken into neuroblastoma cells, exerting greater toxic effects to the recipient cells than free α-syn (94). The report that the microglial capacity for uptaking exosome-associated α-syn oligomers decreases age-dependently suggests the enhancement of α-syn toxicity against neurons at an old age (135). The exosomes might function in α-syn oligomerization, not only in transporting preformed α-syn aggregates (136). The aggregation of α-syn was accelerated by either the exosomes derived from neuroblastoma cells or the synthetic liposomes formed from extracted exosomal lipids, both of which contain exosomal GSLs, such as GM1 and GM3. These raise the possibility that glycolipids on the exosome surface have the potential to transform monomeric α-syn into toxic assemblies, opposing the effect of Aβ that forms nontoxic fibrils (128). α-Syn has been detected in human plasma and CSF (137). Part of plasma α-syn is reported to associate with exosomes (96, 137). Radiolabeled α-syn included in exosomes is transported to blood as soon as it is intracerebrally injected into mouse brains. α-Syn is

also detected in the exosomes of human plasma and there is a significantly higher levels of exosomal α-syn in the blood of PD patients than in that of age-matched control. Elevated levels of exosomal α-syn in blood might reflect the enhancement of α-syn-bound exosome generation in PD brains. Dysfunction of several proteins that are involved in exosome generation has been reported to be linked to PD pathogenesis both in cellular models and PD patients. Rab11 GTPase and CHMP2B, two components of ESCRTIII, colocalize with α-syn-positive intracellular inclusions, and Rab11 activity modulates α-syn secretion and aggregation (98, 100). Loss of the expression of the ATPase ion pump ATP13A2 (PARK9) decreases the number of ILVs in MVB. Inversely, elevated ATP13A2 expression levels, enhance exosome-related α-syn secretion and decrease α-syn levels inside cells, which consequently attenuate intracellular α-syn toxicity in neuroblastoma cells (95, 97). The above findings suggest the dual roles of exosomes, that is, protective and pathological effects on PD development. Thus, the promotion of exosome generation combined with the prevention of exosome incorporation into neighboring neurons might be necessary for exosome-mediated therapeutic strategies for PD.

Amyotrophic lateral sclerosis

ALS is an adult-onset neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons mainly in the spinal cord and brainstem. Mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) are predominantly associated with familial ALS (138). Misfolded SOD1 has been observed in neurons in sporadic and familial ALS (105). Recent findings suggest that misfolded SOD1 converts other SOD1 proteins including the WT to misfolded forms that are released into the extracellular space similar to pathogenic prion proteins (103). Exosome- associated SOD1 secretion has been reported in either WT or mutant SOD1 (G93A)-overexpressed motor neurons (102). Mutant SOD1 (G93A)-overexpressing primary astrocytes also release SOD1 extracellulary with exosomes (104). The mutant SOD1-associated astrocytic exosomes potentiate neuronal death in a coculture system of primary spinal neurons and astrocytes, suggesting that the exosomes transfer toxic molecules including G93A SOD1 to neighboring neurons, which might contribute to the spatiotemporal propagation of the ALS pathology. The mechanism by which SOD1 is loaded into exosomes remains unclear. Mutant SOD1 (G127X, G85R) is detected on the exosome surface (105), which is different from WT SOD1, which is usually localized inside exosomes (139). Thus, treatment of the exosomes with specific antibodies against mutant SOD1, which could recognize their epitopes on the exosomes and then block their toxicity, inhibits the conversion of SOD1 into its misfolded form in the recipient cells (105). TAR DNA-binding protein of 43 kDa (TDP-43) has been reported to be included in the exosomes (106). TDP-43 is a protein composed of an intraneuronal inclusion found in ALS patients. Pathogenic TDP-43 such as hyperphosphorylated and ubiquitinated species, tends to aggregate and represent cellular toxicity. Neurons containing TDP-43-positive inclusions are deficient in normal TDP-43 functions. Loss of normal function and gain of toxic function are both proposed to contribute to the ALS pathology (140). Phosphorylated TDP-43 aggregates are released from neuroblastoma cells and incorporated into recipient cells, in which the toxic assemblies operate as templates for new aggregate formation (106). Extracellular TDP-43 is observed in exosomes, which suggests the roles of exosomes against toxic aggregates similar to those in AD and PD, in which the exosomes are exploited to decrease the intracellular toxicity of SOD1 and TDP-43 by facilitating their release from cells and concomitantly supporting their intraneuronal propagation in development of ALS pathology.

Aging

Aging has been considered as the most common risk factor among neurodegenerative disorders such as AD, PD, ALS, and sporadic prion disease, which all show rising rates of morbidity with aging. Several groups including ours analyzed the exosomes derived from CSF in animal models and human subjects in relation to aging. Such studies provide clues to clarifying the roles of exosomes in disease pathogenesis. We isolated CSF exosomes from APP transgenic mice during aging from 2 to 23 months of age and measured the exosome concentration by nanoparticle analysis (85). We found that the number of CSF exosomes markedly declined during aging. Similarly, we purified the exosomes from CSF of cymologus monkeys and found significant reductions in the levels of the exosome marker proteins Alix and Tsg101 in the aged monkeys compared with the young (our unpublished observation). Furthermore, extracellular vesicles including the exosomes have been reported to undergo temporal reduction in levels with age in human CSF (141). In accordance with reductions in the levels of mouse and monkey CSF exosomes, Aβ in the CSF exosomes keep decreasing with age (85). However, the Aβ levels continue to increase from 6 to 23 months of age in the brain tissues of both APP transgenic mice and

cymologus monkeys. The data raise the possibility that the generation of endogenous exosomes from brain cells might modulate Aβ metabolism in the brain.

Accumulating evidence demonstrated that the interaction of the two axonal transport proteins, dynactin and dynein, is impaired in the axons in the brains of aged monkeys (142). Dysfunction of the motor protein complex leads to axonal transport impairment and endocytic pathologies including Rab GTPases overexpressions and endosome enlargements (143). Endocytic impairment has been found in dynein-siRNA-treated neuroblastoma cells, which are used as a model of aged neurons, concomitant with a significant inhibition of exosome release from the cells. Intracellular accumulations of Aβ and tau have been identified in neurons in the brains of aged monkeys, suggesting that endocytic disturbance might prevent the exosome-mediated secretion of pathogenic proteins in aged brain cells. However, it has been reported that aging accelerates exosome release in other tissues except those in the nervous system. In the retina pigment epithelium (RPE), decrease in lysosome activity during aging results in the impairment of intracellular organelles such as mitochondria, with damaged DNA accumulating inside cells. Thus, exocytic activity is elevated in the cells to discard obsolete components, which promotes exosome secretion (144). The exosomes form extracellular, amorphous deposits called drusen on Bruch's membrane in the macula of the retina, which leads to the development of age-related macular degeneration (AMD) (145). Age-related changes in the endosome-lysosome pathway might modulate exosome secretion differently in individual cells. Further studies would contribute to better understanding of the endocytic machinery for modulating exosome generation according to age-dependent endocytic alternations.

Conclusions and perspectives

In this review we have illustrated that new roles of exosomes are emerging, which cover a broad range of neuronal physiological functions and diseases. It appears that exosomes in the nervous systems act more than just in the disposal of cellular contents, but also in intercellular exchanges of their functional cargos. These would enable development to proceed normally and maintain normal function of the nervous systems; on the other hand, these exacerbate the pathologies through the spread of pathogenic agents over time throughout the nervous systems. Exosome studies in this area have been carried out mostly with cell cultures; thus, their degree of contribution to elucidating the *in vivo* functions has not been fully clarified. Further studies are required including the development of techniques to analyze exosomes in small volumes of fluids and the generation of animal models with the altered levels of exosome secretion. Deepening our understanding of the fundamental process such as biogenesis, cargo sorting and recipient targeting and the identification of specific markers of each origin would open the way to solving the problems.

Potential values of exosomal miRNA have been validated as the biomarkers of many types of cancer (146). Alterations of exosomal miRNA profiling have been reported in neurodegenerative diseases such as AD and PD, and also in some mental disorders such as schizophrenia and bipolar disorder (147, 148). Although the involvement of these identified exosomal miRNAs in the pathogenesis of these diseases is unclear, the miRNA profiles of CSF- or blood-derived exosomes might be available as novel diagnostic or prognostic biomarkers for the diseases. Understanding of the significant pathological roles of exosomes would lead to the development of novel therapeutic approaches for the diseases. For example, with neuronal exosomes found to clear Aβ, exosome administration might provide a new approach to attenuating the AD pathology. Exosomes have been used as a delivery platform for targeting encapsulated reagents or siRNAs into the brain (149, 150). Peripheral injection of exosomes carrying siRNA succeeded in brain targeting and specific gene knockdown in AD and PD mouse models (149, 151). Development of engineered nanovesicles in combination with the understanding of the pathological roles of exosomes could lead to the exploitation of exosomes as a clinical tool.

List of abbreviations

Aβ	amyloid-β peptide
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
α-syn	α-synuclein
BBB	blood-brain barrier
Cer	ceramide
CSF	cerebrospinal fluids
CNS	the central nervous system
ESCRT	endosomal sorting complex required for transporter
Fz2	Frizzled-2
GPI	glycosylphosphatidylinositol
GSLs	glycosphingolipids
ILVs	intraluminal vesicles
LAMP-1	lysosome-associated membrane protein-1
LB	Lewy bodies

MCI	mild cognitive impairment
MVBs	multivesicular bodies
NPC1	Niemann-Pick type C1 disease
nSMase	neutral sphingomyelinase
PA	phosphatidic acid
PAR-4	prostate apoptosis response-4
PD	Parkinson's disease
PC	phosphatidylcholine
SCs	Schwann cells
SM	sphingomyelin
SOD	Cu/Zn superoxide dismutase
TDP-43	TAR DNA-binding protein of 43 kDa
Wg	Wingless

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References

1. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014; 30: 255–89.
2. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002; 2: 569–79.
3. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200: 373–83.
4. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983; 97: 329–39.
5. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell 1983; 33: 967–78.
6. Harding CV, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. J Cell Biol 2013; 200: 367–71.
7. Caby M-P, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. Int Immunol 2005; 17: 879–87.
8. Ogawa Y, Kanai-Azuma M, Akimoto Y, Kawakami H, Yanoshita R. Exosome-like vesicles with dipeptidyl peptidase IV in human saliva. Biol Pharm Bull 2008; 31: 1059–62.
9. Admyre C, Johansson SM, Qazi KR, Filén J-J, Lahesmaa R, Norman M, Neve EP, Scheynius A, Gabrielsson S. Exosomes with immune modulatory features are present in human breast milk. J Immunol 2007; 179: 1969–78.
10. Vella LJ, Greenwood DL, Cappai R, Scheerlinck J-P, Hill AF. Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. Vet Immunol Immunopathol 2008; 124: 385–93.
11. Yuyama K, Yamamoto N, Yanagisawa K. Accelerated release of exosome-associated GM1 ganglioside (GM1) by endocytic pathway abnormality: another putative pathway for GM1-induced amyloid fibril formation. J Neurochem 2008; 105: 217–24.
12. Vingtdeux V, Hamdane M, Loyens A, Gelé P, Drobeck H, Bégard S, Galas M-C, Delacourte A, Beauvillain J-C, Buée L, Sergeant N. Alkalizing drugs induce accumulation of amyloid precursor protein by-products in luminal vesicles of multivesicular bodies. J Biol Chem 2007; 282: 18197–205.
13. Liscum L. Niemann-Pick type C mutations cause lipid traffic jam. Traffic 2000; 1: 218–25.
14. Strauss K, Goebel C, Runz H, Möbius W, Weiss S, Feussner I, Simons M, Schneider A. Exosome secretion ameliorates lysosomal storage of cholesterol in Niemann-Pick type C disease. J Biol Chem 2010; 285: 26279–88.
15. Fader CM, Sánchez D, Furlán M, Colombo MI. Induction of autophagy promotes fusion of multivesicular bodies with autophagic vacuoles in k562 cells. Traffic 2008; 9: 230–50.
16. Fader CM, Colombo MI. Multivesicular bodies and autophagy in erythrocyte maturation. Autophagy 2006; 2: 122–5.
17. Baixauli F, López-Otín C, Mittelbrunn M. Exosomes and autophagy: coordinated mechanisms for the maintenance of cellular fitness. Front Immunol Frontiers 2014; 5: 403.
18. Mittelbrunn M, Sánchez-Madrid F. Intercellular communication: diverse structures for exchange of genetic information. Nat Rev Mol Cell Biol 2012; 13: 328–35.
19. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzás EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Krämer-Albers E-M, Laitinen S, Lässer C, Lener T, Ligeti E, Linē A, Lipps G, Llorente A, Lötvall J, Manček-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'t Hoen EN, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pállinger é, del Portillo HA, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarém N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Wauben MH, De Wever O. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015; 4: 27066.
20. Hurley JH. ESCRTs are everywhere. EMBO J 2015; 34: 2398–407.
21. Bissig C, Gruenberg J. ALIX and the multivesicular endosome: ALIX in Wonderland. Trends Cell Biol 2014; 24: 19–25.
22. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 2008; 319: 1244–7.
23. Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S-I. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. Nat Commun 2013; 4: 2712.
24. Laulagnier K, Grand D, Dujardin A, Hamdi S, Vincent-Schneider H, Lankar D, Salles J-P, Bonnerot C, Perret B, Record M. PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. FEBS Lett 2004; 572: 11–4.
25. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim Biophys Acta 2014; 1841: 108–20.
26. van Niel G, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, Marks MS, Rubinstein E, Raposo G. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. Dev Cell 2011; 21: 708–21.
27. Edgar JR, Eden ER, Futter CE. Hrs- and CD63-dependent competing mechanisms make different sized endosomal intraluminal vesicles. Traffic 2014; 15: 197–211.

28. Chairoungdua A, Smith DL, Pochard P, Hull M, Caplan MJ. Exosome release of β-catenin: a novel mechanism that antagonizes Wnt signaling. J Cell Biol 2010; 190: 1079–91.
29. Savina A, Furlán M, Vidal M, Colombo MI. Exosome release is regulated by a calcium-dependent mechanism in K562 cells. J Biol Chem 2003; 278: 20083–90.
30. Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, Freitas RP, Goud B, Benaroch P, Hacohen N, Fukuda M, Desnos C, Seabra MC, Darchen F, Amigorena S, Moita LF, Théry C. Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 2010; 12(Suppl 1–13): 19–30.
31. Hsu C, Morohashi Y, Yoshimura S-I, Manrique-Hoyos N, Jung S, Lauterbach MA, Bakhti M, Grønborg M, Möbius W, Rhee J, Barr FA, Simons M. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. J Cell Biol 2010; 189: 223–32.
32. Savina A, Vidal M, Colombo MI. The exosome pathway in K562 cells is regulated by Rab11. J Cell Sci 2002; 115: 2505–15.
33. Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 2009; 458: 445–52.
34. Pérez-Hernández D, Gutiérrez-Vázquez C, Jorge I, López-Martín S, Ursa A, Sánchez-Madrid F, Vázquez J, Yáñez-Mó M. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. J Biol Chem 2013; 288: 11649–61.
35. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 1992; 68: 533–44.
36. Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA. Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many raft proteins are acylated, while few are prenylated. J Biol Chem 1999; 274: 3910–7.
37. Rabesandratana H, Toutant JP, Reggio H, Vidal M. Decay-accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59) are released within exosomes during In vitro maturation of reticulocytes. Blood 1998; 91: 2573–80.
38. Levental I, Lingwood D, Grzybek M, Coskun U, Simons K. Palmitoylation regulates raft affinity for the majority of integral raft proteins. Proc Natl Acad Sci USA 2010; 107: 22050–4.
39. Record M, Poirot M, Silvente-Poirot S. Emerging concepts on the role of exosomes in lipid metabolic diseases. Biochimie 2014; 96: 67–74.
40. Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Möbius W, Hoernschemeyer J, Slot J-W, Geuze HJ, Stoorvogel W. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003; 278: 10963–72.
41. Yuyama K, Sun H, Sakai S, Mitsutake S, Okada M, Tahara H, Furukawa J-I, Fujitani N, Shinohara Y, Igarashi Y. Decreased amyloid-β pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. J Biol Chem 2014; 289: 24488–98.
42. de Gassart A, Geminard C, Fevrier B, Raposo G, Vidal M. Lipid raft-associated protein sorting in exosomes. Blood 2003; 102: 4336–44.
43. Fang Y, Wu N, Gan X, Yan W, Morrell JC, Gould SJ. Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes. PLoS Biol 2007; 5: e158.
44. Shen B, Wu N, Yang J-M, Gould SJ. Protein targeting to exosomes/microvesicles by plasma membrane anchors. J Biol Chem 2011; 286: 14383–95.
45. Shen B, Fang Y, Wu N, Gould SJ. Biogenesis of the posterior pole is mediated by the exosome/microvesicle protein-sorting pathway. J Biol Chem 2011; 286: 44162–76.
46. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654–9.
47. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem 2010; 285: 17442–52.
48. Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MÁ, Bernad A, Sánchez-Madrid F. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun 2011; 2: 282.
49. Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, Baty CJ, Gibson GA, Erdos G, Wang Z, Milosevic J, Tkacheva OA, Divito SJ, Jordan R, Lyons-Weiler J, Watkins SC, Morelli AE. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. Blood 2012; 119: 756–66.
50. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, Samuel M, Pathan M, Jois M, Chilamkurti N, Gangoda L, Mathivanan S. ExoCarta: a web-based compendium of exosomal cargo. J Mol Biol 2015 in press.
51. Fauré J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, Grange J, Schoehn G, Goldberg Y, Boyer V, Kirchhoff F, Raposo G, Garin J, Sadoul R. Exosomes are released by cultured cortical neurones. Mol Cell Neurosci 2006; 31: 642–8.
52. Taylor AR, Robinson MB, Gifondorwa DJ, Tytell M, Milligan CE. Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. Dev Neurobiol 2007; 67: 1815–29.
53. Krämer-Albers E-M, Bretz N, Tenzer S, Winterstein C, Möbius W, Berger H, Nave K-A, Schild H, Trotter J. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: trophic support for axons? Proteomics Clin Appl 2007; 1: 1446–61.
54. Potolicchio I, Carven GJ, Xu X, Stipp C, Riese RJ, Stern LJ, Santambrogio L. Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. J Immunol 2005; 175: 2237–43.
55. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem 2012; 287: 3842–9.
56. Chong SY, Chan JR. Tapping into the glial reservoir: cells committed to remaining uncommitted. J Cell Biol 2010; 188: 305–12.
57. Bakhti M, Winter C, Simons M. Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. J Biol Chem 2011; 286: 787–96.
58. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781–810.
59. Korkut C, Ataman B, Ramachandran P, Ashley J, Barria R, Gherbesi N, Budnik V. Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. Cell 2009; 139: 393–404.

60. Korkut C, Li Y, Koles K, Brewer C, Ashley J, Yoshihara M, Budnik V. Regulation of postsynaptic retrograde signaling by presynaptic exosome release. Neuron 2013; 77: 1039–46.
61. Koles K, Nunnari J, Korkut C, Barria R, Brewer C, Li Y, Leszyk J, Zhang B, Budnik V. Mechanism of evenness interrupted (Evi)-exosome release at synaptic boutons. J Biol Chem 2012; 287: 16820–34.
62. Gross JC, Chaudhary V, Bartscherer K, Boutros M. Active Wnt proteins are secreted on exosomes. Nat Cell Biol 2012; 14: 1036–45.
63. Hooper C, Sainz-Fuertes R, Lynham S, Hye A, Killick R, Warley A, Bolondi C, Pocock J, Lovestone S. Wnt3a induces exosome secretion from primary cultured rat microglia. BMC Neurosci 2012; 13: 144.
64. Rathjen FG, Schachner M. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. EMBO J 1984; 3: 1–10.
65. Lachenal G, Pernet Gallay K, Chivet M, Hemming FJ, Belly A, Bodon G, Blot B, Haase G, Goldberg Y, Sadoul R. Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. Mol Cell Neurosci 2011; 46: 409–18.
66. Chivet M, Javalet C, Laulagnier K, Blot B, Hemming FJ, Sadoul R. Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. J Extracell Vesicles 2014; 3: 24722.
67. Frühbeis C, Fröhlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, Kirchhoff F, Möbius W, Goebbels S, Nave K-A, Schneider A, Simons M, Klugmann M, Trotter J, Krämer-Albers E-M. Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. PLoS Biol 2013; 11: e1001604.
68. Fröhlich D, Kuo WP, Frühbeis C, Sun J-J, Zehendner CM, Luhmann HJ, Pinto S, Toedling J, Trotter J, Krämer-Albers E-M. Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction and gene regulation. Philos Trans R Soc Lond B Biol Sci 2014; 369: 1652.
69. Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Kapogiannis D. Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. Neurology 2015; 85: 40–7.
70. Brown IR. Heat shock proteins and protection of the nervous system. Ann NY Acad Sci 2007; 1113: 147–58.
71. Bahrini I, Song J-H, Diez D, Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Sci Rep 2015; 5: 7989.
72. Court FA, Alvarez J. Local regulation of the axonal phenotype, a case of merotrophism. Biol Res 2005; 38: 365–74.
73. Lopez-Verrilli MA, Picou F, Court FA. Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system. Glia 2013; 61: 1795–806.
74. Lopez-Verrilli MA, Court FA. Transfer of vesicles from schwann cells to axons: a novel mechanism of communication in the peripheral nervous system. Front Physiol 2012; 3: 205.
75. Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, Laude H, Raposo G. Cells release prions in association with exosomes. Proc Natl Acad Sci USA 2004; 101: 9683–8.
76. Vella LJ, Sharples RA, Lawson VA, Masters CL, Cappai R, Hill AF. Packaging of prions into exosomes is associated with a novel pathway of PrP processing. J Pathol 2007; 211: 582–90.
77. Guo BB, Bellingham SA, Hill AF. The neutral sphingomyelinase pathway regulates packaging of the prion protein into exosomes. J Biol Chem 2015; 290: 3455–67.
78. Robertson C, Booth SA, Beniac DR, Coulthart MB, Booth TF, McNicol A. Cellular prion protein is released on exosomes from activated platelets. Blood 2006; 107: 3907–11.
79. Saá P, Yakovleva O, de Castro J, Vasilyeva I, De Paoli SH, Simak J, Cervenakova L. First demonstration of transmissible spongiform encephalopathy-associated prion protein (PrPTSE) in extracellular vesicles from plasma of mice infected with mouse-adapted variant Creutzfeldt-Jakob disease by in vitro amplification. J Biol Chem 2014; 289: 29247–60.
80. Kovács GG, Gelpi E, Ströbel T, Ricken G, Nyengaard JR, Bernheimer H, Budka H. Involvement of the endosomal-lysosomal system correlates with regional pathology in Creutzfeldt-Jakob disease. J Neuropathol Exp Neurol 2007; 66: 628–36.
81. Cataldo AM, Barnett JL, Pieroni C, Nixon RA. Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. J Neurosci 1997; 17: 6142–51.
82. Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, Simons K. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc Natl Acad Sci USA 2006; 103: 11172–7.
83. Sharples RA, Vella LJ, Nisbet RM, Naylor R, Perez K, Barnham KJ, Masters CL, Hill AF. Inhibition of gamma-secretase causes increased secretion of amyloid precursor protein C-terminal fragments in association with exosomes. FASEB J 2008; 22: 1469–78.
84. Perez-Gonzalez R, Gauthier SA, Kumar A, Levy E. The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space. J Biol Chem 2012; 287: 43108–15.
85. Yuyama K, Sun H, Usuki S, Sakai S, Hanamatsu H, Mioka T, Kimura N, Okada M, Tahara H, Furukawa J-I, Fujitani N, Shinohara Y, Igarashi Y. A potential function for neuronal exosomes: sequestering intracerebral amyloid-β peptide. FEBS Lett 2015; 589: 84–8.
86. Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL, Goetzl EJ. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dement 2015; 11: 600–1.
87. Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. Am J Pathol 2000; 157: 277–86.
88. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, Heun R, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H,

Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel K-H, Klopp N, Wichmann H-E, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009; 41: 1088–93.

89. Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, Schrijvers EM, Ramirez-Lorca R, Debette S, Longstreth WT, Janssens AC, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JI, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Campion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MM, CHARGE Consortium, GERAD1 Consortium, EADI1 Consortium. Genome-wide analysis of genetic loci associated with Alzheimer disease. J Am Med Assoc 2010; 303: 1832–40.
90. Saman S, Lee NC, Inoyo I, Jin J, Li Z, Doyle T, McKee AC, Hall GF. Proteins recruited to exosomes by tau overexpression implicate novel cellular mechanisms linking tau secretion with Alzheimer's disease. J Alzheimers Dis 2014; 40(Suppl 1): S47–70.
91. Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, Wolozin B, Butovsky O, Kügler S, Ikezu T. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci 2015; 18: 1584–93.
92. Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, Stefanis L, Vekrellis K. Cell-produced α-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J Neurosci 2010; 30: 6838–51.
93. Alvarez-Erviti L, Seow Y, Schapira AH, Gardiner C, Sargent IL, Wood MJ, Cooper JM. Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. Neurobiol Dis 2011; 42: 360–7.
94. Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, Vanderburg CR, McLean PJ. Exosomal cell-to-cell transmission of α-synuclein oligomers. Mol Neurodegener 2012; 7: 42.
95. Kong SM, Chan BK, Park J-S, Hill KJ, Aitken JB, Cottle L, Farghaian H, Cole AR, Lay PA, Sue CM, Cooper AA. Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes α-synuclein externalization via exosomes. Hum Mol Genet 2014; 23: 2816–33.
96. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, Li Y, Aro P, Dator R, He C, Hipp MJ, Zabetian CP, Peskind ER, Hu S-C, Quinn JF, Galasko DR, Banks WA, Zhang J. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol 2014; 128: 639–50.
97. Tsunemi T, Hamada K, Krainc D. ATP13A2/PARK9 regulates secretion of exosomes and α-synuclein. J Neurosci 2014; 34: 15281–7.
98. Chutna O, Gonçalves S, Villar-Piqué A, Guerreiro P, Marijanovic Z, Mendes T, Ramalho J, Emmanouilidou E, Ventura S, Klucken J, Barral DC, Giorgini F, Vekrellis K, Outeiro TF. The small GTPase Rab11 co-localizes with α-synuclein in intracellular inclusions and modulates its aggregation, secretion and toxicity. Hum Mol Genet 2014; 23: 6732–45.
99. Fraser KB, Moehle MS, Daher JP, Webber PJ, Williams JY, Stewart CA, Yacoubian TA, Cowell RM, Dokland T, Ye T, Chen D, Siegal GP, Galemmo RA, Tsika E, Moore DJ, Standaert DG, Kojima K, Mobley JA, West AB. LRRK2 secretion in exosomes is regulated by 14-3-3. Hum Mol Genet 2013; 22: 4988–5000.
100. Tanikawa S, Mori F, Tanji K, Kakita A, Takahashi H, Wakabayashi K. Endosomal sorting related protein CHMP2B is localized in Lewy bodies and glial cytoplasmic inclusions in α-synucleinopathy. Neurosci Lett 2012; 527: 16–21.
101. Kurashige T, Takahashi T, Yamazaki Y, Hiji M, Izumi Y, Yamawaki T, Matsumoto M. Localization of CHMP2B-immunoreactivity in the brainstem of Lewy body disease. Neuropathology 2012; 33: 237–45.
102. Gomes C, Keller S, Altevogt P, Costa J. Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. Neurosci Lett 2007; 428: 43–6.
103. Grad LI, Pokrishevsky E, Silverman JM, Cashman NR. Exosome-dependent and independent mechanisms are involved in prion-like transmission of propagated Cu/Zn superoxide dismutase misfolding. Prion 2014; 8: 331–5.
104. Basso M, Pozzi S, Tortarolo M, Fiordaliso F, Bisighini C, Pasetto L, Spaltro G, Lidonnici D, Gensano F, Battaglia E, Bendotti C, Bonetto V. Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis. J Biol Chem 2013; 288: 15699–711.
105. Grad LI, Yerbury JJ, Turner BJ, Guest WC, Pokrishevsky E, O'Neill MA, Yanai A, Silverman JM, Zeineddine R, Corcoran L, Kumita JR, Luheshi LM, Yousefi M, Coleman BM, Hill AF, Plotkin SS, Mackenzie IR, Cashman NR. Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. Proc Natl Acad Sci USA 2014; 111: 3620–5.
106. Nonaka T, Masuda-Suzukake M, Arai T, Hasegawa Y, Akatsu H, Obi T, Yoshida M, Murayama S, Mann DM, Akiyama H, Hasegawa M. Prion-like properties of pathological TDP-43 aggregates from diseased brains. Cell Rep 2013; 4: 124–34.
107. Ding X, Ma M, Teng J, Teng RK, Zhou S, Yin J, Fonkem E, Huang JH, Wu E, Wang X. Exposure to ALS-FTD-CSF generates TDP-43 aggregates in glioblastoma cells through exosomes and TNTs-like structure. Oncotarget 2015; 6: 24178–91.
108. Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerød L, Fisher EM, Isaacs A, Brech A, Stenmark H, Simonsen A. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol 2007; 179: 485–500.
109. van Blitterswijk M, Vlam L, van Es MA, van der Pol W-L, Hennekam EA, Dooijes D, Schelhaas HJ, van der Kooi AJ, de Visser M, Veldink JH, van den Berg LH. Genetic overlap between apparently sporadic motor neuron diseases. PLoS One 2012; 7: e48983.

110. Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM, MRC Proteomics in ALS Study, FReJA Consortium. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). Neurology 2006; 67: 1074–7.
111. Takeuchi T, Suzuki M, Fujikake N, Popiel HA, Kikuchi H, Futaki S, Wada K, Nagai Y. Intercellular chaperone transmission via exosomes contributes to maintenance of protein homeostasis at the organismal level. Proc Natl Acad Sci USA 2015; 112: 2497–506.
112. Zhu CY, Shen Y, Xu Q. Propagation of dysbindin-1B aggregates: exosome-mediated transmission of neurotoxic deposits. Neuroscience 2015; 291: 301–16.
113. Norton N, Williams HJ, Owen MJ. An update on the genetics of schizophrenia. Curr Opin Psychiatry 2006; 19: 158–64.
114. Gerrish A, Williams H, Moskvina V, Owen MJ, O'Donovan MC, Williams NM. An examination of MUTED as a schizophrenia susceptibility gene. Schizophr Res 2009; 107: 110–1.
115. Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform. Proc Natl Acad Sci USA 1997; 94: 2333–8.
116. Vilette D, Laulagnier K, Huor A, Alais S, Simoes S, Maryse R, Provansal M, Lehmann S, Andreoletti O, Schaeffer L, Raposo G, Leblanc P. Efficient inhibition of infectious prions multiplication and release by targeting the exosomal pathway. Cell Mol Life Sci 2015; 72: 4409–27.
117. Tominaga N, Kosaka N, Ono M, Katsuda T, Yoshioka Y, Tamura K, Lötvall J, Nakagama H, Ochiya T. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood-brain barrier. Nat Commun 2015; 6: 6716.
118. Coleman BM, Hanssen E, Lawson VA, Hill AF. Prion-infected cells regulate the release of exosomes with distinct ultrastructural features. FASEB J 2012; 26: 4160–73.
119. Bellingham SA, Coleman BM, Hill AF. Small RNA deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. Nucleic Acids Res 2012; 40: 10937–49.
120. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. J Biol Chem 2008; 283: 29615–9.
121. Cataldo A, Rebeck GW, Ghetri B, Hulette C, Lippa C, Van Broeckhoven C, van Duijn C, Cras P, Bogdanovic N, Bird T, Peterhoff C, Nixon R. Endocytic disturbances distinguish among subtypes of Alzheimer's disease and related disorders. Ann Neurol 2001; 50: 661–5.
122. Cataldo AM, Petanceska S, Terio NB, Peterhoff CM, Durham R, Mercken M, Mehta PD, Buxbaum J, Haroutunian V, Nixon RA. Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. Neurobiol Aging 2004; 25: 1263–72.
123. Paula-Barbosa MM, Mota Cardoso R, Faria R, Cruz C. Multivesicular bodies in cortical dendrites of two patients with Alzheimer's disease. J Neurol Sci 1978; 36: 259–64.
124. Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol 2002; 161: 1869–79.
125. Yanagisawa K, Odaka A, Suzuki N, Ihara Y. GM1 ganglioside-bound amyloid β-protein (Aβ): a possible form of preamyloid in Alzheimer's disease. Nat Med 1995; 1: 1062–6.
126. Ariga T, McDonald MP, Yu RK. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease–a review. J Lipid Res 2008; 49: 1157–75.
127. Hayashi H, Kimura N, Yamaguchi H, Hasegawa K, Yokoseki T, Shibata M, Yamamoto N, Michikawa M, Yoshikawa Y, Terao K, Matsuzaki K, Lemere CA, Selkoe DJ, Naiki H, Yanagisawa K. A seed for Alzheimer amyloid in the brain. J Neurosci 2004; 24: 4894–902.
128. Yuyama K, Sun H, Mitsutake S, Igarashi Y. Sphingolipid-modulated exosome secretion promotes clearance of amyloid-β by microglia. J Biol Chem 2012; 287: 10977–89.
129. Fitzner D, Schnaars M, van Rossum D, Krishnamoorthy G, Dibaj P, Bakhti M, Regen T, Hanisch U-K, Simons M. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. J Cell Sci 2011; 124(Pt 3): 447–58.
130. Wang G, Dinkins M, He Q, Zhu G, Poirier C, Campbell A, Mayer-Proschel M, Bieberich E. Astrocytes secrete exosomes enriched with proapoptotic ceramide and prostate apoptosis response 4 (PAR-4): potential mechanism of apoptosis induction in Alzheimer disease (AD). J Biol Chem 2012; 287: 21384–95.
131. Croisier E, Graeber MB. Glial degeneration and reactive gliosis in alpha-synucleinopathies: the emerging concept of primary gliodegeneration. Acta Neuropathol 2006; 112: 517–30.
132. Vekrellis K, Rideout HJ, Stefanis L. Neurobiology of alpha-synuclein. Mol Neurobiol 2004; 30: 1–21.
133. Hansen C, Angot E, Bergström A-L, Steiner JA, Pieri L, Paul G, Outeiro TF, Melki R, Kallunki P, Fog K, Li J-Y, Brundin P. α-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J Clin Invest 2011; 121: 715–25.
134. Hansen C, Li J-Y. Beyond α-synuclein transfer: pathology propagation in Parkinson's disease. Trends Mol Med 2012; 18: 248–55.
135. Bliederhaeuser C, Grozdanov V, Speidel A, Zondler L, Ruf WP, Bayer H, Kiechle M, Feiler MS, Freischmidt A, Brenner D, Witting A, Hengerer B, Fändrich M, Ludolph AC, Weishaupt JH, Gillardon F, Danzer KM. Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. Acta Neuropathol 2015 in press.
136. Grey M, Dunning CJ, Gaspar R, Grey C, Brundin P, Sparr E, Linse S. Acceleration of α-synuclein aggregation by exosomes. J Biol Chem 2015; 290: 2969–82.
137. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Leverenz JB, Baird G, Montine TJ, Hancock AM, Hwang H, Pan C, Bradner J, Kang UJ, Jensen PH, Zhang J. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain 2010; 133: 713–26.
138. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 2013; 14: 248–64.
139. Kim D-K, Kang B, Kim OY, Choi D-S, Lee J, Kim SR, Go G, Yoon YJ, Kim JH, Jang SC, Park K-S, Choi E-J, Kim KP, Desiderio DM, Kim Y-K, Lötvall J, Hwang D, Gho YS. EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. J Extracell Vesicles 2013; 2: 20384.

140. Arai T, Hasegawa M, Nonoka T, Kametani F, Yamashita M, Hosokawa M, Niizato K, Tsuchiya K, Kobayashi Z, Ikeda K, Yoshida M, Onaya M, Fujishiro H, Akiyama H. Phosphorylated and cleaved TDP-43 in ALS, FTLD and other neurodegenerative disorders and in cellular models of TDP-43 proteinopathy. Neuropathology 2010; 30: 170–81.
141. Tietje A, Maron KN, Wei Y, Feliciano DM. Cerebrospinal fluid extracellular vesicles undergo age dependent declines and contain known and novel non-coding RNAs. PLoS One 2014; 9: e113116.
142. Kimura N, Imamura O, Ono F, Terao K. Aging attenuates dynactin-dynein interaction: down-regulation of dynein causes accumulation of endogenous tau and amyloid precursor protein in human neuroblastoma cells. J Neurosci Res 2007; 85: 2909–16.
143. Kimura N, Inoue M, Okabayashi S, Ono F, Negishi T. Dynein dysfunction induces endocytic pathology accompanied by an increase in Rab GTPases: a potential mechanism underlying age-dependent endocytic dysfunction. J Biol Chem 2009; 284: 31291–302.
144. Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PLoS One 2009; 4: e4160.
145. Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy, exosomes and drusen formation in age-related macular degeneration. Autophagy 2009; 5: 563–4.
146. Miller IV, Grunewald TG. Tumour-derived exosomes: tiny envelopes for big stories. Biol Cell 2015; 107: 287–305.
147. Gui YX, Liu H, Zhang LS, Lv W, Hu XY. Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. Oncotarget 2015; 6: 37043–53.
148. Banigan MG, Kao PF, Kozubek JA, Winslow AR, Medina J, Costa J, Schmitt A, Schneider A, Cabral H, Cagsal-Getkin O, Vanderburg CR, Delalle I. Differential expression of exosomal microRNAs in prefrontal cortices of schizophrenia and bipolar disorder patients. PLoS One 2013; 8: e48814.
149. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011; 29: 341–5.
150. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D, Zhang H-G. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther 2011; 19: 1769–79.
151. Cooper JM, Wiklander PB, Nordin JZ, Al-Shawi R, Wood MJ, Vithlani M, Schapira AH, Simons JP, EL Andaloussi S, Alvarez-Erviti L. Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. Mov Disord 2014; 29: 1476–85.