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PORT-TO-PORT DELIVERY: MOBILIZATION OF TOXIC SPHINGOLIPIDS VIA EXTRACELLULAR VESICLES

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Abstract

The discovery that most cells produce extracellular vesicles (EVs) and release them in the extracellular milieu has spurred the idea that these membranous cargoes spread pathogenic mechanisms. In the brain, EVs may have multifold and important physiological functions, from deregulating synaptic activity, to promoting demyelination, to changes in microglial activity. The finding that small EVs (exosomes) contain α -synuclein and β -amyloid, among other pathogenic proteins, is an example of this notion, underlining their potential role in the brain of patients with Parkinson's and Alzheimer's diseases. Being membranous-vesicles, we speculate that EVs also have an intrinsic capacity to incorporate sphingolipids. In conditions where these lipids are elevated to toxic levels such as in Krabbe's disease and Metachromatic leukodystrophy, EVs may contribute to spread disease from sick to healthy cells. In this essay, we discuss a working hypothesis that brain cells in sphingolipidoses clear some of the accumulated lipid material to attempt restoring cell homeostasis via EV secretion. We hypothesize that secreted sphingolipid-loaded EVs shuttle pathogenic lipids to cells that are not intrinsically affected, contributing to establishing non-cell autonomous defects.

Keywords

extracellular vesicles; exosomes; β -amyloid; α -synuclein; tau; lysosomes; autophagy; sphingolipidosis

INTRODUCTION AND HYPOTHESIS

The traditional view of pathogenesis in sphingolipidoses is that cellular defects arise from signaling deregulation produced by the local accumulation of lipid material and lysosomal defects in the cell where the deficiency manifests (*i.e.* cell autonomous defects). However, a growing body of evidence is showing that non-cell autonomous defects also occur, stemming

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All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ; Study concept and design: GS, ALM, ERB and MIG; Acquisition of data: GS, ALM, ERB and MIG; Analysis and interpretation of data: GS, ALM, ERB and MIG; Drafting of the manuscript: GS, ALM, ERB and MIG; Critical revision of the manuscript for important intellectual content: GS, ALM, ERB and MIG; Obtained funding: ERB and MIG; Study supervision: ERB and MIG

from aberrant environmental signaling and/or abnormal cell-to-cell interactions. The release of molecules to the extracellular milieu via vesicular secretion is an exciting and underestimated mechanism to elicit non-cell autonomous alterations on the homeostasis or physiological state of the receiving cell (Tkach and Thery 2016). The finding that many of the accumulated lipid substrates in sphingolipidoses circulate in the plasma and cerebrospinal fluid (CSF) of affected patients underlines the possibility of alternative and potentially complementing pathogenic mechanisms in the context of disease. In general, lipids circulate bound to carrier proteins such as serum albumin and various lipoproteins (Green and Glickman 1981). However, lipids are key components of the and lumen of EVs with a particular distribution according to their biogenesis (Record et al. 2011). EVs are known to contain an array of different signals from microRNAs, mRNA, to proteins, and are thought to participate in basic cellular functions without the need of cell-to-cell contact (Record et al. 2014). EVs are produced by direct budding from the plasma membrane or via the formation of membranous multivesicular bodies (MVB). Their secretion into the extracellular milieu occurs as intact nanosized vesicles. This property may add an additional and relevant role for EVs in the pathogenesis of sphingolipidoses, leading us to *hypothesize that in sphingolipidoses, neural cells promote EVs release to clear some of the accumulated lipids to restore cell homeostasis. Under these pathological conditions, sphingolipid-loaded EVs become shuttles that spread and transfer pathogenic lipids to cells that are not intrinsically affected, contributing to non-cell autonomous deficits.*

EVs: MOLECULAR VEHICLES FOR CELL-TO-CELL COMMUNICATION

Due to the current limited consensus on the techniques to isolate microvesicles and exosomes, in this commentary we will refer to these types of vesicles as EVs.

Biogenesis

Various types of EVs have been described depending on their biogenesis, intracellular origin, biochemical and biophysical properties (Colombo et al. 2014; Stoorvogel et al. 2002; Thery et al. 2002). Microvesicles (MVs) are a heterogeneous class of EVs sized 100–1000 nm, originated by outward budding of the plasma membrane (Colombo et al. 2014). Their biogenesis occurs in lipid rafts or lipid rafts-like portions of the plasma membrane although their synthesis still remains unclear (Del Conde et al. 2005). MVS are loaded with different kind of molecules, including receptors, proteins, genomic material and lipids, being able to shuttle to different target cells (Basso and Bonetto 2016).

Intriguingly, the release of MVs can be trigger by a direct stimulus received by the donor cells, including Ca^{2+} , phorbol ester and ATP (Baj-Krzyworzeka et al. 2006; Bianco et al. 2005). This suggests that the process of MVs release is not stochastic but rather subjected to regulation. This regulation may be key to control intercellular trafficking, with potentially important effects during development.

At the other end, exosomes are small EVs with a diameter between 20 to 150 nm that are actively secreted by most cell types. They are generated in the endosomal pathway from intraluminal vesicles (ILV) formed by inward budding of endosomes membranes. During this process, ILVs can acquire various types of molecules including receptors, proteins,

miRNA, RNA, DNAs, and lipids, becoming true shuttles for multiple signaling molecules. The release of ILVs inside endosomes generates MVBs, which can be routed to lysosomes for degradation or fused with the plasma membrane for the release of their content (*i.e.* exosomes) to the extracellular milieu. MVBs are components of the lysosomal-endosomal system and therefore lysosomal dysfunction in LSDs could promote an increase in exosomal secretion. Once secreted, it is believed that exosomes can be taken up by neighboring cells or mobilized to circulating fluids (Caby et al. 2005; Colombo et al. 2014; Fevrier and Raposo 2004; Thery et al. 2006; Valadi et al. 2007). The mechanism(s) of exosomes import into cells is still unclear, but it appears to include membrane fusion and receptor-assisted endocytosis (Feng et al. 2010; Plebanek et al. 2015; Soares et al. 2015). Exosomes exert biological functions for significant periods of time (Fevrier and Raposo 2004; Hurley and Odorizzi 2012; Raposo and Stoorvogel 2013; Record et al. 2014; Stoorvogel et al. 2002; Valadi et al. 2007; Wood et al. 2011).

Composition

EVs are structurally and chemically heterogeneous and their formation is a complex process that involves enrichment in proteins and lipids. Certain proteins such as Alix, Rabs and CD63 are highly enriched in small EVs and considered exosomal markers (Duijvesz et al. 2011; Hurley and Odorizzi 2012). Many proteins are sorted into exosomes via the endosomal-sorting complex required for transport (ESCRT), which is fundamental to recognize specific proteins and catalyze their loading into ILVs (Record et al. 2014). ESCRT-independent protein sorting mechanisms have been also described for incorporation of detergent resistant-protein complexes (Record et al. 2014). Other proteins are sorted by lipid affinity, which involves the clustering of tetraspanins into specialized domains of the endosomal membrane. These clusters, named Tetraspanin-Enriched Membrane Domains (TEMs), act as a sorting machinery to selectively load proteins like integrins, growth factors and MHC class II molecules into exosomes (Perez-Hernandez et al. 2013). TEMs are characterized by the presence of gangliosides and cholesterol, and share some analogies with lipid rafts (Le Naour et al. 2006; Yanez-Mo et al. 2009).

During their biogenesis, exosomes also undergo lipid changes. For example, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, and cholesterol are enriched and their molar content increases during maturation of the exosomes membrane in MVBs (Carayon et al. 2011; Record et al. 2014). Cholesterol also contributes in the selection of cargo proteins by affecting the sorting of tetraspanin CD82 to exosomes (Xu et al. 2009) and facilitates their release (Strauss et al. 2010). Many lipids are important not only for exosomes biogenesis, but also for sorting cargoes. For example, ceramides act synergistically with sphingomyelin and cholesterol in a raft-like manner, promoting the release of exosomes and affecting their functions (Trajkovic et al. 2008; Wang et al. 2012). This correlation was clearly shown by Wang and co-workers in an *in vitro* model of Alzheimer's disease. In this work, the exogenous administration of ceramide and amyloid peptides in nSMase2 deficient astrocytes caused an increase in exosome release, able to induce apoptosis in recipient cells (Wang et al. 2012).

Function

EVs were originally considered as debris vesicles for the clearance of unwanted plasma membrane components during reticulocyte maturation (Johnstone and Ahn 1990; Johnstone et al. 1991). However, EVs are currently considered functional vesicles that mediate intercellular communication without the need of cell-to-cell contact. Their high stability in the extracellular space makes EVs important players in paracrine and endocrine communication (Chen et al. 2012; Ramachandran and Palanisamy 2012). Moreover, the presence of mRNA and miRNA inside exosomes cargo confers them the ability to regulate transcriptional activity and even lead to reprogramming of recipient cells (Ratajczak et al. 2006). Due to their properties to convey information between cells (Figure 1A), EVs are also involved in regulating complex processes including inflammation, where they exhibit both pro- and anti-inflammatory properties (Thery et al. 2009) angiogenesis (Gai et al. 2016), metastasis (Bobrie et al. 2012; Hoshino et al. 2013; Peinado et al. 2012; Skog et al. 2008), and axonal regeneration (Lopez-Leal and Court 2016; Rajendran et al. 2014). Moreover, the fact that EVs contain bioactive lipids positions them as capable to affect the lipid metabolism of recipient cells (*i.e.* the cells that take up exosomes from the extracellular milieu), causing secondary non-cell autonomous alterations (Prinetti et al. 2011; Record et al. 2014).

Toxicity from EVs release might not only affect the tissue(s) of origin, but also be exerted on several other tissues. In order for this long-range toxicity to be accomplished, EVs from nerve tissue need to overcome the blood-brain barrier (BBB) and then reach the circulatory system. EVs are well known to cross BBB, thus potentially increasing the number of target tissues. As shown by several studies, exosomes can reach and successfully deliver their content in the central nervous system after systemic administration (Alvarez-Erviti et al. 2011; Haney et al. 2015; Zhuang et al. 2011). This ability, which could be usefully used to deliver drugs to sensitive targets like the brain, might also represent a pharmacological target to reduce the long-range effect of the accumulation of toxic molecules.

EVs: SHUTTLING TOXINS WITHIN THE NERVOUS SYSTEM?

The physiological role of EVs in the normal CNS is still largely unclear, primarily due to the difficulties to measure *in vivo* dynamics parameters for these vesicles in the living brain. The finding of myelin components like proteolipid protein, galactocerebrosides, sulfatides, and cholesterol in EVs has led to propose their participation in coordinating myelination (Kramer-Albers et al. 2007), and in communication with other neural cell types including microglia and neurons (Bakhti et al. 2011; Budnik et al. 2016; Fruhbeis et al. 2012; Fruhbeis et al. 2013; Kramer-Albers et al. 2007). Furthermore, MVBs were identified in the adaxonal cytoplasmic compartment of myelinated axons supporting a role in transferring of information between myelinating cells and axons, and potentially during demyelination (Fruhbeis et al. 2013). EVs may play similar functions communicating different neural types in the healthy brain without the need of cell-to-cell contact (Figure 1A).

The role of EVs in neurodegenerative conditions is far less clear. LSDs, mitochondrial disorders, and late onset disorders such as Alzheimer's and Parkinson's diseases may have involvement of EVs. LSDs arise from genetic mutations impairing key enzymes for

lysosomal degradation of lipids (Schulze and Sandhoff 2011). Despite their genetic origins, LSDs show a large spectrum of clinical phenotypes, depending on the kind of substrate(s) and the type of cells in which they accumulate. In sphingolipidoses, in general a lysosomal enzyme responsible for the degradation of a particular sphingolipid is deficient, causing the accumulation of the lipid over time, lysosomal dysfunction and eventually cell death (Futerman and van Meer 2004; Platt 2014). In various LSDs pathologies the storage of one particular lipid might cause a secondary accumulation of others lipids (Futerman and van Meer 2004), resulting in additional toxic effects. Furthermore, alterations in lipids profile caused by LSDs could affect raft-dependent signalling and endocytic pathway, reflected in alterations of cellular homeostasis (Simons and Gruenberg 2000).

In addition to classic LSDs, mitochondrial dysfunction deeply affects lipid trafficking and lysosomes activity, altering lysosomal calcium levels and autophagosome-lysosome fusion leading to LSD-phenotypes (Baixauli et al. 2015). Alterations in mitochondrial activity contribute to peripheral neuropathies, with pain, sensory loss, muscle weakness such as in diabetes (Ferryhough et al. 2010) or to Charcot Marie Tooth neuropathy (Niemann et al. 2006) and to maladaptive responses in myelinating cells such as Schwann cells, via the endoplasmic reticulum and unfolded protein response (UPR) (Lin and Popko 2009; Pennuto et al. 2008). These conditions lead to demyelination and axonal degeneration, with alteration in lipid metabolism such as acylcarnitines (Viader et al. 2013).

The role of EVs in these disorders has received limited attention. Our interest in EVs as pathogenic contributors in LSDs arose from recent work on late onset pathologies. Most of the evidence favoring a pathogenic role for EVs stems from studies of toxic amyloid proteins in late onset (adult) neurodegenerative disorders, including Alzheimer's and Parkinson's disease. Many of these adult neurodegenerative disorders are characterized by the progressive accumulation of intracellular and extracellular deposits of insoluble material (β -amyloid, α -synuclein, tau) (Cai et al. 1993; Murakami et al. 2002; Serpell et al. 2000). The mechanism(s) involved for the deposition of these proteins is still controversial, since most of these pathologies are largely idiopathic (Chin-Chan et al. 2015; Volta et al. 2015). These conditions are almost invariably affected by a progressive spreading of insoluble material, with spreading patterns remarkably similar among patients (Braak et al. 1993; Braak et al. 2003; Guo and Lee 2014). Various possible mechanisms have been discussed to explain the spreading pattern including axonal transport, fluid-phase, and EV mobilization (Roy et al. 2005). In support of the latter, β -amyloid, α -synuclein, tau, and PrPsc have been found in EVs derived from cellular models of Alzheimer, Parkinson and prion diseases (Danzer et al. 2012; Emmanouilidou et al. 2010; Loov et al. 2016; Rajendran et al. 2006; Saman et al. 2012). The release of α -synuclein from induced pluripotent stem cell-derived neurons from Parkinson's disease patients carrying a deficiency for glucocerebrosidase (Fernandes et al. 2016) has been linked to the inefficient autophagosome activity to degrade this protein, reinforcing the potential link between deficient degradation pathways, accumulation of amyloid-like proteins, and an increased in EVs release.

Even though EVs cargoes are representative of the metabolome status of the parental cell, the mechanism by which such pathogenic proteins are sorted into EVs is still unclear. Although solid evidence is still missing, ESCRT-independent sorting of high-order

oligomers (*i.e.* amyloid-like proteins) might be a contributing mechanism (Fang et al. 2007). Even less known is how EVs carrying toxic amyloids can diffuse in the brain and be internalized to other neurons following a specific pattern. If this process contributes to spreading of disease, there are a number of important aspects that need to be investigated, including the heterogeneity of EVs, their internalization mechanism(s), and how EVs may recognize specific cell types for internalization. We speculate that EVs secretion of amyloidogenic proteins may be part of a neuronal mechanism to reduce and isolate toxic molecules within affected amyloid-producing neurons, by promoting their removal through secretion to the extracellular space. Therefore, EVs mobilization of toxic proteins to the extracellular milieu and eventual uptake by other cells may function as a “prion-like” spreading mechanism, promoting both short and long-range toxicity, protein deregulation and/or changes in cellular homeostasis in neighboring cells. Growing evidence has shown that amyloid aggregates induce neurodegeneration in mice by promoting the formation of neurofibrillary tangles (Gotz et al. 2001), supporting the general idea of “disease spreading” mechanisms.

On the other hand, a pathogenic role of EVs in sphingolipidoses is largely unknown. Under physiological conditions, most cells, and neurons in particular, overcome toxic accumulation of substances by increasing the rate of lysosomal degradation and autophagy (Rothenberg et al. 2010). LSDs show an increase in lysosomal exocytosis (Klein et al. 2005; Park et al. 2016) and altered calcium homeostasis (Segatori 2014), conditions that favor EVs secretion. Release of EVs from defective cells could facilitate the relocation and concentration of undegraded lipid material into the extracellular milieu, and subsequently, promote long-range toxicity via bystander cellular stress mechanisms (Platt 2014) (Figure 1B).

Although very little is known about the presence of specific sphingolipids in EVs, recent studies from our laboratories are starting to reveal aspects associated to EVs secretion in sphingolipidoses. Sphingolipids such as sulfatides and psychosine, which are accumulated in Metachromatic leukodystrophy (MLD) and Krabbe’s disease, respectively, and highly enriched in brain lipid rafts (Moyano et al. 2014; White et al. 2009), are good examples of potentially EVs-released neurotoxic lipids. For example, we showed that increased sulfatides in MLD neural precursors parallels the secretion of higher levels of PDGFR α , a key regulator of oligodendroglial proliferation and survival (Pituch et al. 2015). A direct role for sulfatides in this effect is under investigation (Moyano and Givogri, unpublished) but sulfatides have been found in EVs secreted by mature oligodendrocytes (Kramer-Albers et al. 2007). Ongoing studies on psychosine secretion also underline a role for EVs in the metabolism of this lipid in the Krabbe brain (Scesa and Bongarzone, in preparation).

Transfer of toxic sphingolipids such as sulfatides and psychosine to unaffected (*i.e.* cells that do not synthesize these lipids) may lead to aberrant pathogenic effects such as axonal death (Smith et al. 2011), transport deficiencies (Cantuti Castelvetti et al. 2013), or microglial activation (Ijichi et al. 2013)(Figure 1B). The finding that sulfatides and psychosine are present in human plasma, serum, and CSF (Fredman et al. 1992; Haghghi et al. 2013; Li et al. 2007; Moyano et al. 2013; Sugiyama et al. 1999; Tarkowski et al. 2003; Zanfini et al. 2013; Zhu et al. 2012) reinforces the possibility of long-range spreading of toxins via mobilized EVs.

Assessing the validity of this hypothesis will require not only to detect the presence of the candidate toxic lipid in EVs but also –and critically- to confirm its ability to generate a toxic response after EV uptake by recipient cells. Such approach provided evidence for the transfer of superoxide dismutase and catalase from oligodendrocytes to neurons through EVs, in an *in vitro* model of cerebral ischemia (Frohlich et al. 2014). The availability of labeling techniques to report the localization of specific molecules may facilitate these analyses. Labeling lipids using fluorophores such as Bodipy or fluorescent lipid analogs may allow for imaging analyses of EV dynamics, with the caveat that such derivatives may introduce alterations in membrane behavior and affecting the release and/or uptake of EVs (Carquin et al. 2016). The use of reporter genes may also help in tracking EVs delivery. Zomer and coworkers (Zomer et al. 2016) developed a protocol that relies on the expression of Cre recombinase by exosomes-donor cells, facilitating the identification of secreting vs receiving cells.

FINAL REMARKS

EVs have the intrinsic capacity for cell-to-cell communication. We speculate that EVs may be important mediators of brain toxicity by shuttling undegraded material in conditions where lysosomal degradation is deficient such as in sphingolipidoses and other LSDs. EVs secretion may play important roles in relevant pathophysiological processes, including synaptic dysfunction, demyelination, axonal damage, microglial activation, and/or astrogliosis (Figure 1B). Advancing the study of these vesicles is needed to better understand their role in neurodegenerative diseases and in the healthy nervous system, and to determine whether they represent a therapeutic target (Baixauli et al. 2015).

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References

- 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. *Nature biotechnology*. 2011; 29(4):341–345.
- Baixauli F, Acin-Perez R, Villarroja-Beltri C, Mazzeo C, Nunez-Andrade N, Gabande-Rodriguez E, Ledesma MD, Blazquez A, Martin MA, Falcon-Perez JM, Redondo JM, Enriquez JA, Mittelbrunn M. Mitochondrial Respiration Controls Lysosomal Function during Inflammatory T Cell Responses. *Cell metabolism*. 2015; 22(3):485–498. [PubMed: 26299452]
- Baj-Krzyworzeka M, Szatanek R, Weglarczyk K, Baran J, Urbanowicz B, Branski P, Ratajczak MZ, Zembala M. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. *Cancer immunology, immunotherapy* : CII. 2006; 55(7):808–818. [PubMed: 16283305]
- Bakhti M, Winter C, Simons M. Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. *The Journal of biological chemistry*. 2011; 286(1):787–796. [PubMed: 20978131]
- Basso M, Bonetto V. Extracellular Vesicles and a Novel Form of Communication in the Brain. *Frontiers in neuroscience*. 2016; 10:127. [PubMed: 27065789]

- Bianco F, Pravettoni E, Colombo A, Schenk U, Moller T, Matteoli M, Verderio C. Astrocyte-derived ATP induces vesicle shedding and IL-1 beta release from microglia. *Journal of immunology* (Baltimore, Md : 1950). 2005; 174(11):7268–7277.
- Bobrie A, Krumeich S, Reyat F, Recchi C, Moita LF, Seabra MC, Ostrowski M, Thery C. Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression. *Cancer Res.* 2012; 72(19):4920–4930. [PubMed: 22865453]
- Braak H, Braak E, Bohl J. Staging of Alzheimer-related cortical destruction. *Eur Neurol.* 1993; 33(6): 403–408. [PubMed: 8307060]
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging.* 2003; 24(2):197–211. [PubMed: 12498954]
- Budnik V, Ruiz-Canada C, Wendler F. Extracellular vesicles round off communication in the nervous system. *Nature reviews Neuroscience.* 2016; 17(3):160–172. [PubMed: 26891626]
- Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. *Int Immunol.* 2005; 17(7):879–887. [PubMed: 15908444]
- Cai XD, Golde TE, Younkin SG. Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. *Science (New York, NY).* 1993; 259(5094):514–516.
- Cantuti Castelvetti L, Givogri MI, Hebert A, Smith B, Song Y, Kaminska A, Lopez-Rosas A, Morfini G, Pigino G, Sands M, Brady ST, Bongarzone ER. The sphingolipid psychosine inhibits fast axonal transport in Krabbe disease by activation of GSK3beta and deregulation of molecular motors. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2013; 33(24):10048–10056. [PubMed: 23761900]
- Carayon K, Chaoui K, Ronzier E, Lazar I, Bertrand-Michel J, Roques V, Balor S, Terce F, Lopez A, Salome L, Joly E. Proteolipidic composition of exosomes changes during reticulocyte maturation. *The Journal of biological chemistry.* 2011; 286(39):34426–34439. [PubMed: 21828046]
- Carquin M, D'Auria L, Pollet H, Bongarzone ER, Tyteca D. Recent progress on lipid lateral heterogeneity in plasma membranes: From rafts to submicrometric domains. *Progress in lipid research.* 2016; 62:1–24. [PubMed: 26738447]
- Chen X, Liang H, Zhang J, Zen K, Zhang CY. Horizontal transfer of microRNAs: molecular mechanisms and clinical applications. *Protein Cell.* 2012; 3(1):28–37. [PubMed: 22314808]
- Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci.* 2015; 9:124. [PubMed: 25914621]
- Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol.* 2014; 30:255–289. [PubMed: 25288114]
- Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, Vanderburg CR, McLean PJ. Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Molecular neurodegeneration.* 2012; 7:42. [PubMed: 22920859]
- Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood.* 2005; 106(5):1604–1611. [PubMed: 15741221]
- Duijvesz D, Luider T, Bangma CH, Jenster G. Exosomes as biomarker treasure chests for prostate cancer. *Eur Urol.* 2011; 59(5):823–831. [PubMed: 21196075]
- Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, Stefanis L, Vekrellis K. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2010; 30(20):6838–6851. [PubMed: 20484626]
- 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 biology.* 2007; 5(6):e158. [PubMed: 17550307]
- Feng D, Zhao WL, Ye YY, Bai XC, Liu RQ, Chang LF, Zhou Q, Sui SF. Cellular internalization of exosomes occurs through phagocytosis. *Traffic.* 2010; 11(5):675–687. [PubMed: 20136776]

- Fernandes HJ, Hartfield EM, Christian HC, Emmanouilidou E, Zheng Y, Booth H, Bogetofte H, Lang C, Ryan BJ, Sardi SP, Badger J, Vowles J, Evetts S, Tofaris GK, Vekrellis K, Talbot K, Hu MT, James W, Cowley SA, Wade-Martins R. ER Stress and Autophagic Perturbations Lead to Elevated Extracellular alpha-Synuclein in GBA-N370S Parkinson's iPSC-Derived Dopamine Neurons. *Stem cell reports*. 2016; 6(3):342–356. [PubMed: 26905200]
- Fernyhough P, Roy Chowdhury SK, Schmidt RE. Mitochondrial stress and the pathogenesis of diabetic neuropathy. *Expert review of endocrinology & metabolism*. 2010; 5(1):39–49. [PubMed: 20729997]
- Fevrier B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol*. 2004; 16(4):415–421. [PubMed: 15261674]
- Fredman P, Wallin A, Blennow K, Davidsson P, Gottfries CG, Svennerholm L. Sulfatide as a biochemical marker in cerebrospinal fluid of patients with vascular dementia. *Acta neurologica Scandinavica*. 1992; 85(2):103–106. [PubMed: 1574982]
- Frohlich D, Kuo WP, Fruhbeis C, Sun JJ, Zehendner CM, Luhmann HJ, Pinto S, Toedling J, Trotter J, Kramer-Albers EM. Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction and gene regulation. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2014; 369(1652)
- Fruhbeis C, Frohlich D, Kramer-Albers EM. Emerging roles of exosomes in neuron-glia communication. *Front Physiol*. 2012; 3:119. [PubMed: 22557979]
- Fruhbeis C, Frohlich D, Kuo WP, Amphornrat J, Thilemann S, Saab AS, Kirchhoff F, Mobius W, Goebbels S, Nave KA, Schneider A, Simons M, Klugmann M, Trotter J, Kramer-Albers EM. Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. *PLoS biology*. 2013; 11(7):e1001604. [PubMed: 23874151]
- Futerman AH, van Meer G. The cell biology of lysosomal storage disorders. *Nature reviews Molecular cell biology*. 2004; 5(7):554–565. [PubMed: 15232573]
- Gai C, Carpanetto A, Deregibus MC, Camussi G. Extracellular vesicle-mediated modulation of angiogenesis. *Histol Histopathol*. 2016; 31(4):379–391. [PubMed: 26662176]
- Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301 tau transgenic mice induced by Aβ42 fibrils. *Science (New York, NY)*. 2001; 293(5534):1491–1495.
- Green PH, Glickman RM. Intestinal lipoprotein metabolism. *J Lipid Res*. 1981; 22(8):1153–1173. [PubMed: 7033425]
- Guo JL, Lee VM. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nature medicine*. 2014; 20(2):130–138.
- Haghighi S, Lekman A, Nilsson S, Blomqvist M, Andersen O. Increased CSF sulfatide levels and serum glycosphingolipid antibody levels in healthy siblings of multiple sclerosis patients. *Journal of the neurological sciences*. 2013; 326(1–2):35–39. [PubMed: 23385003]
- Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, Patel T, Piroyan A, Sokolsky M, Kabanov AV, Batrakova EV. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *Journal of controlled release : official journal of the Controlled Release Society*. 2015; 207:18–30. [PubMed: 25836593]
- Hoshino D, Kirkbride KC, Costello K, Clark ES, Sinha S, Grega-Larson N, Tyska MJ, Weaver AM. Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell reports*. 2013; 5(5):1159–1168. [PubMed: 24290760]
- Hurley JH, Odorizzi G. Get on the exosome bus with ALIX. *Nat Cell Biol*. 2012; 14(7):654–655. [PubMed: 22743708]
- Ijichi K, Brown GD, Moore CS, Lee JP, Winokur PN, Pagarigan R, Snyder EY, Bongarzone ER, Crocker SJ. MMP-3 mediates psychosine-induced globose cell formation: implications for leukodystrophy pathology. *Glia*. 2013; 61(5):765–777. [PubMed: 23404611]
- Johnstone RM, Ahn J. A common mechanism may be involved in the selective loss of plasma membrane functions during reticulocyte maturation. *Biomed Biochim Acta*. 1990; 49(2–3):S70–75. [PubMed: 2386531]
- Johnstone RM, Mathew A, Mason AB, Teng K. Exosome formation during maturation of mammalian and avian reticulocytes: evidence that exosome release is a major route for externalization of obsolete membrane proteins. *J Cell Physiol*. 1991; 147(1):27–36. [PubMed: 2037622]

- Klein D, Bussow H, Fewou SN, Gieselmann V. Exocytosis of storage material in a lysosomal disorder. *Biochemical and biophysical research communications*. 2005; 327(3):663–667. [PubMed: 15649398]
- Kramer-Albers EM, Bretz N, Tenzer S, Winterstein C, Mobius W, Berger H, Nave KA, Schild H, Trotter J. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics Clin Appl*. 2007; 1(11):1446–1461. [PubMed: 21136642]
- Le Naour F, Andre M, Boucheix C, Rubinstein E. Membrane microdomains and proteomics: lessons from tetraspanin microdomains and comparison with lipid rafts. *Proteomics*. 2006; 6(24):6447–6454. [PubMed: 17109380]
- Li G, Hu R, Kamijo Y, Nakajima T, Aoyama T, Inoue T, Node K, Kannagi R, Kyogashima M, Hara A. Establishment of a quantitative, qualitative, and high-throughput analysis of sulfatides from small amounts of sera by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Analytical biochemistry*. 2007; 362(1):1–7. [PubMed: 17254536]
- Lin W, Popko B. Endoplasmic reticulum stress in disorders of myelinating cells. *Nature neuroscience*. 2009; 12(4):379–385. [PubMed: 19287390]
- Loov C, Scherzer CR, Hyman BT, Breakefield XO, Ingelsson M. *alpha-Synuclein in Extracellular Vesicles: Functional Implications and Diagnostic Opportunities*. Cellular and molecular neurobiology. 2016
- Lopez-Leal R, Court FA. Schwann Cell Exosomes Mediate Neuron-Glia Communication and Enhance Axonal Regeneration. *Cellular and molecular neurobiology*. 2016
- Moyano AL, Li G, Lopez-Rosas A, Mansson JE, van Breemen RB, Givogri MI. Distribution of C16:0, C18:0, C24:1, and C24:0 sulfatides in central nervous system lipid rafts by quantitative ultra-high-pressure liquid chromatography tandem mass spectrometry. *Analytical biochemistry*. 2014; 467:31–39. [PubMed: 25205652]
- Moyano AL, Pituch K, Li G, van Breemen R, Mansson JE, Givogri MI. Levels of plasma sulfatides C18 : 0 and C24 : 1 correlate with disease status in relapsing-remitting multiple sclerosis. *Journal of neurochemistry*. 2013; 127(5):600–604. [PubMed: 23777394]
- Murakami K, Irie K, Morimoto A, Ohigashi H, Shindo M, Nagao M, Shimizu T, Shirasawa T. Synthesis, aggregation, neurotoxicity, and secondary structure of various A beta 1–42 mutants of familial Alzheimer's disease at positions 21–23. *Biochemical and biophysical research communications*. 2002; 294(1):5–10. [PubMed: 12054732]
- Niemann A, Berger P, Suter U. Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. *Neuromolecular medicine*. 2006; 8(1–2):217–242. [PubMed: 16775378]
- Park S, Ahuja M, Kim MS, Brailoiu GC, Jha A, Zeng M, Baydyuk M, Wu LG, Wassif CA, Porter FD, Zerfas PM, Eckhaus MA, Brailoiu E, Shin DM, Muallem S. Fusion of lysosomes with secretory organelles leads to uncontrolled exocytosis in the lysosomal storage disease mucopolidosis type IV. *EMBO reports*. 2016; 17(2):266–278. [PubMed: 26682800]
- Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nature medicine*. 2012; 18(6):883–891.
- Pennuto M, Tinelli E, Malaguti M, Del Carro U, D'Antonio M, Ron D, Quattrini A, Feltri ML, Wrabetz L. Ablation of the UPR-mediator CHOP restores motor function and reduces demyelination in Charcot-Marie-Tooth 1B mice. *Neuron*. 2008; 57(3):393–405. [PubMed: 18255032]
- Perez-Hernandez D, Gutierrez-Vazquez C, Jorge I, Lopez-Martin S, Urs A, Sanchez-Madrid F, Vazquez J, Yanez-Mo M. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. *The Journal of biological chemistry*. 2013; 288(17):11649–11661. [PubMed: 23463506]
- Pituch KC, Moyano AL, Lopez-Rosas A, Marottoli FM, Li G, Hu C, van Breemen R, Mansson JE, Givogri MI. Dysfunction of platelet-derived growth factor receptor alpha (PDGFRalpha) represses the production of oligodendrocytes from arylsulfatase A-deficient multipotential neural precursor cells. *The Journal of biological chemistry*. 2015; 290(11):7040–7053. [PubMed: 25605750]

- Platt FM. Sphingolipid lysosomal storage disorders. *Nature*. 2014; 510(7503):68–75. [PubMed: 24899306]
- Plebanek MP, Mutharasan RK, Volpert O, Matov A, Gatlin JC, Thaxton CS. Nanoparticle Targeting and Cholesterol Flux Through Scavenger Receptor Type B-1 Inhibits Cellular Exosome Uptake. *Sci Rep*. 2015; 5:15724. [PubMed: 26511855]
- Prinetti A, Prioni S, Chiricozzi E, Schuchman EH, Chigorno V, Sonnino S. Secondary alterations of sphingolipid metabolism in lysosomal storage diseases. *Neurochem Res*. 2011; 36(9):1654–1668. [PubMed: 21207141]
- Rajendran L, Bali J, Barr MM, Court FA, Kramer-Albers EM, Picou F, Raposo G, van der Vos KE, van Niel G, Wang J, Breakefield XO. Emerging roles of extracellular vesicles in the nervous system. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014; 34(46):15482–15489. [PubMed: 25392515]
- 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. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103(30):11172–11177. [PubMed: 16837572]
- Ramachandran S, Palanisamy V. Horizontal transfer of RNAs: exosomes as mediators of intercellular communication. *Wiley Interdiscip Rev RNA*. 2012; 3(2):286–293. [PubMed: 22012863]
- Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013; 200(4):373–383. [PubMed: 23420871]
- Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia*. 2006; 20(5):847–856. [PubMed: 16453000]
- Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiological. *Biochim Biophys Acta*. 2014; 1841(1):108–120. [PubMed: 24140720]
- Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem Pharmacol*. 2011; 81(10):1171–1182. [PubMed: 21371441]
- Rothenberg C, Srinivasan D, Mah L, Kaushik S, Peterhoff CM, Ugolino J, Fang S, Cuervo AM, Nixon RA, Monteiro MJ. Ubiquitin functions in autophagy and is degraded by chaperone-mediated autophagy. *Human molecular genetics*. 2010; 19(16):3219–3232. [PubMed: 20529957]
- Roy S, Zhang B, Lee VM, Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. *Acta Neuropathol*. 2005; 109(1):5–13. [PubMed: 15645263]
- Saman S, Kim W, Raya M, Visnick Y, Miro 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. *The Journal of biological chemistry*. 2012; 287(6):3842–3849. [PubMed: 22057275]
- Schulze H, Sandhoff K. Lysosomal lipid storage diseases. *Cold Spring Harbor perspectives in biology*. 2011; 3(6)
- Segatori L. Impairment of homeostasis in lysosomal storage disorders. *IUBMB life*. 2014; 66(7):472–477. [PubMed: 25044960]
- Serpell LC, Berriman J, Jakes R, Goedert M, Crowther RA. Fiber diffraction of synthetic alpha-synuclein filaments shows amyloid-like cross-beta conformation. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(9):4897–4902. [PubMed: 10781096]
- Simons K, Gruenberg J. Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends in cell biology*. 2000; 10(11):459–462. [PubMed: 11050411]
- Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008; 10(12):1470–1476. [PubMed: 19011622]
- Smith B, Galbati F, Castelvetti LC, Givogri MI, Lopez-Rosas A, Bongarzone ER. Peripheral neuropathy in the Twitcher mouse involves the activation of axonal caspase 3. *ASN neuro*. 2011; 3(4)

- Soares AR, Martins-Marques T, Ribeiro-Rodrigues T, Ferreira JV, Catarino S, Pinho MJ, Zuzarte M, Isabel Anjo S, Manadas B, JPGS, Pereira P, Girao H. Gap junctional protein Cx43 is involved in the communication between extracellular vesicles and mammalian cells. *Sci Rep*. 2015; 5:13243. [PubMed: 26285688]
- Stoorvogel W, Kleijmeer MJ, Geuze HJ, Raposo G. The biogenesis and functions of exosomes. *Traffic*. 2002; 3(5):321–330. [PubMed: 11967126]
- Strauss K, Goebel C, Runz H, Mobius W, Weiss S, Feussner I, Simons M, Schneider A. Exosome secretion ameliorates lysosomal storage of cholesterol in Niemann-Pick type C disease. *The Journal of biological chemistry*. 2010; 285(34):26279–26288. [PubMed: 20554533]
- Sugiyama E, Hara A, Uemura K. A quantitative analysis of serum sulfatide by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with delayed ion extraction. *Analytical biochemistry*. 1999; 274(1):90–97. [PubMed: 10527500]
- Tarkowski E, Tullberg M, Fredman P, Wikkelso C. Correlation between intrathecal sulfatide and TNF- α levels in patients with vascular dementia. *Dementia and geriatric cognitive disorders*. 2003; 15(4):207–211. [PubMed: 12626853]
- Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. 2006; Chapter 3(Unit 3):22. [PubMed: 18228490]
- Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009; 9(8):581–593. [PubMed: 19498381]
- Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002; 2(8):569–579. [PubMed: 12154376]
- Tkach M, Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell*. 2016; 164(6):1226–1232. [PubMed: 26967288]
- Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science (New York, NY)*. 2008; 319(5867):1244–1247.
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007; 9(6):654–659. [PubMed: 17486113]
- Viader A, Sasaki Y, Kim S, Strickland A, Workman CS, Yang K, Gross RW, Milbrandt J. Aberrant Schwann cell lipid metabolism linked to mitochondrial deficits leads to axon degeneration and neuropathy. *Neuron*. 2013; 77(5):886–898. [PubMed: 23473319]
- Volta M, Milnerwood AJ, Farrer MJ. Insights from late-onset familial parkinsonism on the pathogenesis of idiopathic Parkinson's disease. *Lancet Neurol*. 2015; 14(10):1054–1064. [PubMed: 26376970]
- 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). *The Journal of biological chemistry*. 2012; 287(25):21384–21395. [PubMed: 22532571]
- White AB, Givogri MI, Lopez-Rosas A, Cao H, van Breemen R, Thinakaran G, Bongarzone ER. Psychosine accumulates in membrane microdomains in the brain of krabbe patients, disrupting the raft architecture. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009; 29(19):6068–6077. [PubMed: 19439584]
- Wood MJ, O'Loughlin AJ, Samira L. Exosomes and the blood-brain barrier: implications for neurological diseases. *Ther Deliv*. 2011; 2(9):1095–1099. [PubMed: 22833906]
- Xu C, Zhang YH, Thangavel M, Richardson MM, Liu L, Zhou B, Zheng Y, Ostrom RS, Zhang XA. CD82 endocytosis and cholesterol-dependent reorganization of tetraspanin webs and lipid rafts. *FASEB J*. 2009; 23(10):3273–3288. [PubMed: 19497983]
- Yanez-Mo M, Barreiro O, Gordon-Alonso M, Sala-Valdes M, Sanchez-Madrid F. Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes. *Trends in cell biology*. 2009; 19(9):434–446. [PubMed: 19709882]

- Zanfini A, Dreassi E, Berardi A, Governini L, Corbini G, Costantino-Ceccarini E, Balestri P, Luddi A. Quantification of psychosine in the serum of twitcher mouse by LC-ESI-tandem-MS analysis. *Journal of pharmaceutical and biomedical analysis*. 2013; 80:44–49. [PubMed: 23523865]
- Zhu H, Lopez-Rosas A, Qiu X, Van Breemen RB, Bongarzone ER. Detection of the neurotoxin psychosine in samples of peripheral blood: application in diagnostics and follow-up of Krabbe disease. *Archives of pathology & laboratory medicine*. 2012; 136(7):709–710. [PubMed: 22742542]
- Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D, Zhang HG. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2011; 19(10):1769–1779. [PubMed: 21915101]
- Zomer A, Steenbeek SC, Maynard C, van Rheenen J. Studying extracellular vesicle transfer by a Cre-loxP method. *Nature protocols*. 2016; 11(1):87–101. [PubMed: 26658469]

SIGNIFICANCE OF THIS WORK

Extracellular vesicles (EVs) are secretable membranous cargoes with important roles in cell-to-cell communication. This property underlines a potential pathogenic role for EVs as vehicles to transfer toxic molecules between cells in disease conditions. Here, we discuss this hypothesis in the context of sphingolipidoses, and propose that EVs contribute to the spreading of lipids such as sulfatides, ceramide and sphingosine-derivatives, known to be toxic in these metabolic diseases. We propose that such mobilization of toxic lipids may contribute to spreading non-cell autonomous defects in the nervous system.

Figure 1. Potential role of EVs in the spreading of toxic sphingolipids in sphingolipidoses
A) In this model of general EVs mobilization, vesicles secreted by neurons and glial cells could potentially transfer pathological molecules into the cerebrospinal fluid (CSF) and across the blood-brain barrier (BBB), into the general circulation, with the possibility to transfer information to peripheral organs and tissues. Similarly, systemic EVs produced outside of the central nervous system might potentially transfer information to resident central cells. **B)** In this model of EVs-mediated transfer of toxic sphingolipids, lysosomal dysfunction elicits the accumulation of a particular sphingolipid “X” in those cells that actively synthesize that sphingolipid. This accumulation elicits cell-autonomous defects that interfere with cellular functions/structures (*i.e.* myelin, synapses, microglia). Affected cells may also secrete some of the accumulated sphingolipid “X” to the extracellular milieu via EVs. Secreted vesicles are internalized in non-affected cells via membrane fusion, endocytosis and lipid rafts-mediated endocytosis. Transfer of these “toxic” EVs may induce a “bystander effect”, inducing non-cell autonomous dysfunction. EVs: extracellular vesicles,

LE: late endosomes, MVB: multivesicular bodies, PM: plasma membrane, PNS: peripheral nervous system and ECM: extracellular matrix.

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