Exosomes as Intercellular Messengers in Neurodegeneration

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Exosomes of endosomal origin are one class of extracellular vesicles that are important in intercellular communication. Exosomes are released by all cells inbody and their cargo consisting of lipids, proteins and nucleic acids has a footprint reflective of their parental origin. The exosomal cargo has the power to modulate the physiology of recipient cells in the vicinity of the releasing cells or cells at a distance. Harnessing the potential of exosomes relies upon the purity of exosome preparation. Exosomes have an intercellular communicator role in the spread of misfolded proteins aiding the propagation of pathology.

Keywords: exosomes ; neurons ; glial cells ; central nervous system (CNS) ; neurodegenerative diseases ; Alzheimer's disease ; Parkinson's disease ; amyotrophic lateral sclerosis

1. Introduction

Exosomes are a subtype of extracellular vesicles of endocytic origin with a size range of 40–160 nm in diameter [1]. Tracer studies demonstrated that macromolecules are endocytosed in bristle-coated (i.e., clathrin-coated) invaginations or pits on the cell surface. These invaginations eventually separate from the plasma membrane and are detected in the cytoplasm. After losing bristles (i.e., clathrin), endocytosed coated vesicles become smooth and fuse with early endosomes for either recycling rapidly back to the cell surface (recycling endosomes in **Figure 1**) [2] or undergo maturation to form late endosomes. Early and late endosomes act as "sorting hubs" to appropriately direct their content: (i) for retrograde transport to the *trans*-Golgi network (i.e., endosome-to-TGN retrieval) [3][4]; (ii) to lysosomes for degradation of macromolecules to simple products for reuse within the cell [5][6]; and/or (iii) for recycling to the cell surface through multivesicular bodies [7]. During maturation of early endosomes into late endosomes, the internal endosome membrane undergoes invagination, resulting in the formation of intraluminal vesicles (now designated as exosomes). Specific macromolecules such as cytosolic proteins, nucleic acids, and lipids are sorted into developing exosomes. Late endosomes packed with exosomes are identified as multivesicular bodies that migrate towards the plasma membrane. Upon fusion with the plasma membrane, multivesicular bodies release exosomes into the extracellular space through the process of exocytosis (**Figure 1**) [8][9]. A large subset of proteins that are sequentially recruited play a decisive role from the process of endocytosis to early endosome, endosome maturation to late endosomes, and their subsequent intracellular fate [7][8][10]. The importance of these proteins is highlighted by their loss and/or mutation(s) that cause various diseases. For instance, Strumpellin and SWIP proteins are a part of the WASH complex that participates in actin polymerization, as well as endosome fission and sorting. Mutations in the *Strumpellin* gene causes hereditary spastic paraplegia, characterized by degeneration of motor neurons [11]. A single point mutation (Pro1019Arg) in the SWIP protein destabilizes the WASH complex. The destabilized WASH complex inhibits endolysosomal trafficking, causing clumping of endosomes. Defective endosomal traffic impacts cognition and motor neuron function, symptoms described as nonsyndromic mental retardation [12][13].

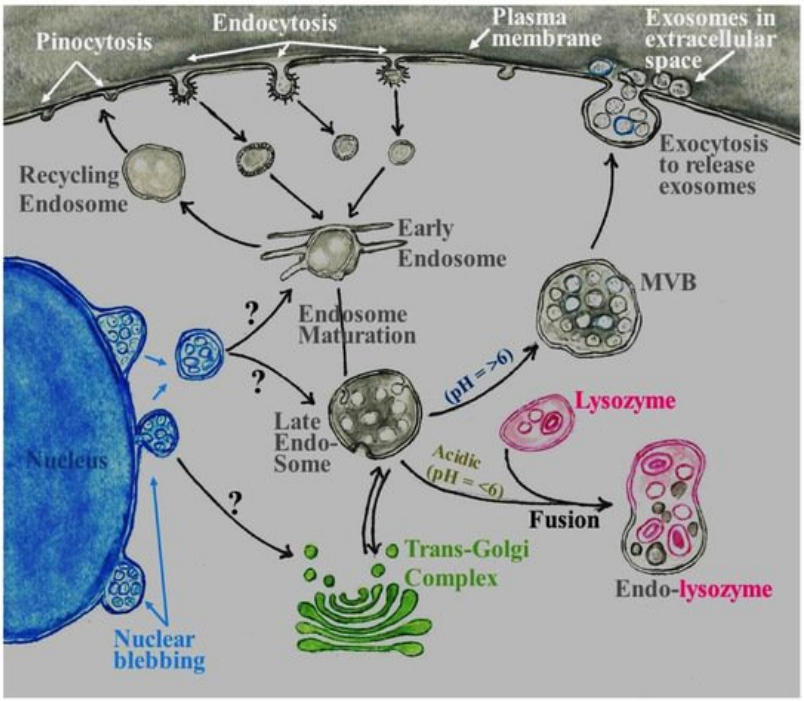

Figure 1. Cellular events related to biogenesis and release of exosomes from the cell. Pinocytosis, clathrin (bristle-coated)-mediated and clathrin-independent endocytosis phosphate) from the *trans*-Golgi network and phagosomes (not shown here). The lumen of late endosomes becomes acidic due to the progressive concentration of ATP-driven H+ pumps in the vesicle membrane. Acidification is a complex phenomenon not completely understood. If the pH in the lumen is below 6, then late endosomes fuse with lysosomes for degradation and recycling of their content. However, if the pH in the lumen of late endosome is above 6, then they escape fusion with lysosomes and subsequent hydrolysis. During maturation of early endosomes to late endosomes, intraluminal vesicles arise from inward invaginations of the internal membrane. RNAs, proteins, and lipids are incorporated into developing intraluminal vesicles through ESCRT-dependent and ESCRT-independent mechanisms. Late endosomes packed with intraluminal vesicles have a multivesicular appearance and are identified as multivesicular bodies. They migrate towards and fuse with the plasma membrane to release their intraluminal vesicles, i.e., exosomes into the extracellular space. Nuclear blebbing appears to generate structures similar to multivesicular bodies (here referred to as MVB type II) that eventually detach from the nuclear membrane and become cytosolic. The MVB type II packed with RNA and/or genomic DNA may receive RNA-binding proteins from the nucleus and/or the *trans*-Golgi network and fuse with early/late endosomes to export their intraluminal vesicles into the extracellular space. **Figure 1** was drawn by authors based upon [10][14][15][16][17][18].

In the cytoplasm, exosomes originate from endosomes using the endosomal sorting complexes required for transport (ESCRT), also known as the ESCRT-dependent mechanism of exosome biogenesis [8][10]. Two groups independently reported lipid-mediated biogenesis of exosomes, an ESCRT-independent mechanism. Two lipids identified were bis(monoacylglycero)phosphate/lysobisphos phatidic acid (BMP/LBPA) and ceramide [19][20]. Rab31 is another ESCRT-independent mechanism for exosome biogenesis in the cytoplasm. Activation of Rab31 stimulates formation of intraluminal vesicles [21]. There is another mechanism that is currently under-discussed and under-explored. This mechanism is based upon ultrastructural observations made in the late 1950s to 1970s that showed cell nuclei can be a site for origin multivesicular bodies packed with small vesicles. Small vesicles described in these studies were perhaps exosomes but the term exosome was not coined at that time. Kilarski and Jasinski observed a process termed nuclear blebbing or nuclear extrusions in cells of the swim bladder of the perch fish, *Perca fluviatilis* L. [14] with content similar to nucleolar karyoplasm [22]. These authors noted nuclear blebbing can be stimulated by removal of gas from the swim bladder of the perch fish [14]. The process of nuclear blebbing appears to have merit as the site of origin of exosomes enriched with nucleic acids and/or ribonucleoproteins. Nuclear blebs were filled with small vesicles that arose from single in-foldings of the internal membrane of the nuclear blebs, a process akin to the formation of intraluminal vesicles in endosomes. Nuclear blebs filled with ‘small vesicles’ eventually separated from nuclei to become part of the cytoplasm. Kilarski and Jasinski referred to nuclear blebs as multivesicular bodies in their manuscript due to lack of a better terminology. Inadvertently, these authors might have been right as subsequent studies reported the presence of multivesicular bodies in nuclei of cells [23]. The nuclear blebs filled with small vesicles have morphology similar to multivesicular bodies originating from endosomes in the cytoplasm (**Figure 1**). Because of the similarity of nuclear multivesicular bodies with viral nucleocapsid, researchers focused their attention on virology and the phenomenon of nuclear blebbing and its cellular function remained underexplored [24]. Irrespective of the mode of biogenesis, exosomes are composed of a lipid bilayer and contain macromolecular content consisting of lipids, proteins, and nucleic acids. Some

of the macromolecules are common to exosomes originating from all cells and hence are classified as exosome markers and are used as a confirmatory tool to distinguish exosomes from other extracellular vesicles. Unique macromolecules present in exosomes often reflect their parental origin and hence are likely to be designated as biomarker(s).

All cells release exosomes into the extracellular space. From the extracellular space, exosomes find their way into various biological fluids and reach cells locally and at a distance. Cells cultured in vitro release their exosomes into culture media. Continuous release of exosomes into the extracellular space makes culture medium and biological fluids a rich source for the isolation of exosomes.

Exosomes contain a substantial amount of coding and non-coding RNAs, double-stranded genomic DNA fragments, and mitochondrial DNA. Lipids are an important part of exosome membranes and are distributed asymmetrically between the two exosome membrane leaflets. Exosomal proteins are comprised of those common to most exosomes as well as unique proteins specific to a certain parent cell. The protein content of all exosomes is generally enriched for targeting and fusion proteins such as tetraspanins, intergrins, chaperone proteins, e.g., heat shock proteins (Hsp60, Hsp70, and Hsp90), the small HSP membrane trafficking proteins, Rab proteins, ARF GTPases, annexins, and proteins involved in the formation of multivesicular bodies such as ALIX and TSG101 [25].

2. Role of Exosomes in the Central Nervous System (CNS)

Neuron-Derived Exosomes: Cultured neurons of mouse and human origin, and neural stem cells release exosomes [26][27][28]. An interesting observation was made by Chivet and colleagues while studying exosomes released by neuroblastoma cells. They observed cell-specific internalization of exosomes. Exosomes from neuroblastoma cells were readily endocytosed by astrocytes and oligodendrocytes but to a much lesser extent by hippocampal neurons. Exosomes released by neurons in response to synaptic activity, on the other hand, were taken up only by neurons suggesting high specificity of exosome-mediated communications among brain cells [29]. Neuron-derived exosomes have been speculated to participate in synaptic plasticity by removing obsolete proteins and RNA. This is achieved by activating phagocytic function of microglia [30]. Potassium-induced depolarization of cultured human neuroblastoma cells releases exosomes enriched in a set of activity-related miRNAs and the synaptic protein MAP1b. The MAP1b protein is abundantly present in axon growth cones and dendritic spines, and is associated with synaptic plasticity [31]. Depolarization stimulates uptake of soma-dendritic exosomes by neighboring synapses. Internalized exosomes facilitate synaptic strength by rapidly translating mRNAs relevant to synaptic activity in the post-synaptic region [31]. Additional support for the role of exosomes in modulating neurotransmission is provided by studies using primary embryonic cortical neurons that release exosomes containing two microRNAs, Let-7c and miR-21. These microRNAs are ligands for the Toll-like receptor 7 (Tlr7). Activation of the Toll-like receptor 7 plays a negative role in axonal and dendritic growth. Incubation of neurons with Let-7c and miR-21 had a negative impact on dendritic growth in normal neurons and this effect was obliterated in neurons from $Tlr7^{(-/-)}$ knockout mice [32]. It is thus reasonable to assume that neuronal exosomes may modulate neurotransmission by regulating neurite growth during neurodevelopment. Synaptogenesis and synapse maintenance are also influenced by exosomes. The proline-rich transmembrane protein, PRR7 is released by neurons in an activity-dependent manner via exosomes. Internalization of PRR7-containing exosomes by neighboring neurons reduces their number of excitatory synapses. Reduction of excitatory synapses is achieved by activating GSk3β, stimulating proteasomal degradation of PSD proteins, and simultaneously inhibiting exosomal secretion of Wnts [33]. Exosomes are thus influential players for neuron–neuron and neuron–glial communications.

Astrocyte-Derived Exosomes: Astrocytes represent the most abundant glial cell type in the brain. They are critical for maintenance of brain homeostasis and provide vital functions such as neurotrophic support, modulation of synaptic activity, and plasticity [34][35]. Astrocytic processes maintain contact with blood capillaries in the brain contributing to the maintenance of the blood–brain barrier [36]. In addition to performing these vital roles, astrocytes function as immunocompetent cells in the CNS. Depending upon the context, astrocyte activity can exacerbate inflammatory reactions or promote immunosuppression and tissue repair. At sites of CNS tissue damage, reactive astrocytes form scars that effectively serve as functional barriers to inflammatory cells, thereby controlling CNS inflammation [37]. Reactive astrocytes release neurotoxic substances and inflammatory molecules such as cytokines and chemokines. Some of these functions of astrocytes are partly facilitated by releasing exosomes. In addition, accumulating evidence lends credence to the idea that neuroprotective role of astrocytes are mediated via exosomes. Rat astrocytes cultured in vitro release exosomes containing neuroprotective and neurotrophic factors. In particular, astrocytic exosomes were enriched with fibroblast growth factor 2 and vascular endothelial growth factor [38].

Oligodendrocyte-Derived Exosomes: Fast and reliable propagation of action potential along axons depends upon myelination of neurons. Oligodendrocytes ensheath multiple axons and have the capacity to renew myelin sheaths three

times in 24 h [39]. Myelin is particularly enriched in lipids but does contain a number of proteins integral to formation of myelin. Major myelin proteins include proteolipid protein (PLP), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) [40]. Two proteins, PLP and MBP, are distributed within the myelin sheath and are instrumental in the process of compaction that results in the closely apposed multilayered structure of myelin. In addition to their important function in myelin production, oligodendrocytes provide trophic support to axons and hence are essential for axonal integrity [41]. Communication between oligodendrocytes and neurons is critical to achieve this important function. Recent studies show that oligodendrocyte exosomes are internalized by neurons to enable trophic support to axons. Similar to neurons, exosome release from oligodendrocytes is activity dependent. The neurotransmitter glutamate released by active neurons stimulates an increase in intracellular calcium in oligodendroglia via NMDA and AMPA receptors.

Microglia-Derived Exosomes: Microglial cells are the macrophages of the brain and spinal cord. They account for approximately 10% of glia. Although their number is comparatively low, they have important functions of maintaining homeostasis by monitoring the presence of tissue infection and damage in the CNS. Under homeostatic conditions, microglia cells are maintained in a quiescent state, but they are constantly scanning their environment. When activated, they exert their phagocytic activity and have the ability to release inflammatory molecules such as cytokines, chemokines. Microglia can acquire two phenotypes, the M1 (inflammatory type) and M2 subtypes (pro-regenerative type), depending upon the stimuli and have the ability to switch between these two opposing phenotypes [42], exemplifying their remarkable plasticity. Similar to neurons and other glial cells, microglia release exosomes to communicate with neighboring cells and cells at a distance. The bioactive cargo of microglial exosomes depends upon the phenotype of the releasing microglia, either pro-inflammatory or pro-regenerative. Microglial exosomes contain all enzymes essential for anaerobic glycolysis and lactate production. Therefore, it is proposed that lactate packaged in exosomes could function as a supplementary energy source for neurons during synaptic activity [43]. Microglial exosomes regulate synaptic transmission by promoting ceramide and sphingolipid production in neurons. Enhanced sphingolipid metabolism positively affects excitatory neurotransmission presenting a novel way by which microglia influence synaptic activity [44].

3. Role of Exosomes in Neurodegenerative Diseases

Alzheimer’s Disease: Alzheimer’s disease is the most common form of neurodegenerative dementia characterized by a progressive loss of memory and cognitive abilities. Central to the pathology of Alzheimer’s disease is formation of extracellular aggregates of β-amyloid (Aβ) known as amyloid plaques combined with neurofibrillary tangles of tau. The (Aβ) peptides are derived from sequential proteolytic processing of amyloid precursor protein (APP) by β- and γ-secretases. As APP is an intracellular protein, a hypothesis was formulated that the pathological lesions of neurodegenerative disease involves the physical spread of the misfolded protein from neuron to neuron [45]. However, mechanism(s) for transmission of misfolded proteins remained an intriguing question. One of the earliest reports that started to shed light on the possible mechanisms how Aβ is shed into the extracellular space came from the study conducted by Rajendran and Colleagues [46]. While investigating the location of APP cleavage, they observed that β-secretase cleavage of APP occurs in a subset of early endosomes with subsequent trafficking of Aβ peptide to multivesicular bodies. A small fraction of Aβ peptide associated with exosome membrane was secreted into the extracellular space. Exosomes containing amyloid plaques had exosomal marker proteins, flotillin-1 and Alix. Thus, exosome membrane-associated Aβ peptide may represent a novel mechanism that contributes to amyloid plaque formation in the extracellular space [46]. Since this initial observation, full-length APP and many of its metabolites and several members of secretase family of proteases involved in APP processing were detected in exosomes [47]. Aβ can exist in different conformational states that have different properties and intermediate products of fibril formation. Of these, low-molecular-weight Aβ and protofibrils have been suggested to be particularly neurotoxic and act as seeds for protein aggregation [48][49]. Exosomes isolated from postmortem brains of Alzheimer’s disease patients were shown to have increased levels of Aβ oligomers. These exosomes were internalized when incubated with cultured neurons. Most importantly, they were able to spread Aβ oligomers to other neurons, causing cytotoxicity [50]. The initial observations were confirmed by incubating exosomes containing APP with primary cultures of normal neurons in vitro [51] and in vivo [52]. The question is why are Aβ or Aβ oligomers packaged into exosomes? Do neurons sense the toxic nature of Aβ or Aβ oligomers and hence try to clear toxic proteins from intracellularly present Aβ or Aβ oligomers similar to transferrin receptor in reticulocytes [53][54]? Monoubiquitination is required for sorting into MVB/exosomes. That raises a second question as to whether Aβ undergoes ubiquitination to be sorted into MVB/exosomes. The amyloid precursor protein (APP) has five lysine residues (Lys-724, Lys-725, Lys-726, Lys-751, and Lys-763) at its C-terminal end [55]. These residues have been mutated individually or in combination to examine effect on APP processing to form Aβ peptide. Ubiquitination of APP at Lys-726 mediated by the F-box and leucine-rich repeat protein2 (FBL2), a component of the E3 ubiquitin ligase, reduced Aβ generation [56]. On the other hand, APP ubiquitination at Lys-763 sequestered APP in the

Golgi complex and prevented APP maturation [57]. Inhibition of ubiquitination by substitution of all five lysine residues to arginine in C-terminal fragment of APP (C99) prevented efficient degradation of APP and accumulation of protein in structures with Golgi-like appearance. This was attributed to a deficiency in endoplasmic reticulum-associated degradation. The C99 undergoes cleavage by γ-secretase to produce Aβ [58]. Mutation of three lysine residues (Lys-724, Lys-725, and Lys-726) simultaneously caused the protein to be retained in the limiting membrane of endosomes instead of becoming internalized into intraluminal vesicles of MVBs [59]. When all five lysine residues were mutated to prevent ubiquitination, the protein did not efficiently sort to MVB/exosomes and a selective increase in Aβ40 was observed [60]. This finding is comparable to the presence of Aβ40 in amyloid deposits in cerebral amyloid angiopathy [61]. While it is evident that ubiquitination of APP may direct the protein to MVB/exosomes, direct evidence using neuronal cultures or in vivo models is required to prove that APP, Aβ, or Aβ oligomers are monoubiquitinated for their targeting to MVB/exosomes and that they are not ubiquitinated to be targeted for endoplasmic reticulum-associated degradation (ERAD).

Another pathological hallmark of Alzheimer's disease is abnormally phosphorylated tau protein in neurofibrillary tangles (NFT). Tau is a cytoplasmic protein known to stabilize microtubules. Increasing evidence suggests that the pathological tau protein can spread between cells, recruiting native tau to form aggregates. Microglia phagocytose tau-containing cytopathic neurons and recycle tau through exosomes thus incriminating exosomes in propagation of Alzheimer's disease. In healthy brains, a number of protein kinases and phosphatases are responsible for phosphorylation and de-phosphorylation of tau, respectively. Dysregulation of these important enzymes may lead to abnormal phosphorylation pattern of tau in AD.

Exosomes released by neurons, astrocytes, and microglia act as scavengers and soak up seed-free soluble Aβ to promote Aβ aggregation that is internalized by microglia for degradation [62][63][64].

Parkinson's Disease: Parkinson's disease is one of the most common age-related brain disorders—it is primarily considered a movement disorder, with typical symptoms of a resting tremor, rigidity, bradykinesia and motor instability [65]. Additionally associated with this disease are cognitive decline, depression, and psychosis [66]. Pathologically, it is characterized by degeneration of nigrostriatal dopaminergic neurons and the presence of Lewy bodies which contain misfolded α-synuclein protein in surviving neurons. Alpha synuclein is detected in many body fluids including cerebrospinal fluid and plasma [67][68]. Alpha synuclein is found in culture medium when cells expressing α-synuclein are cultured in vitro [69].

A first indication that exosomes could indeed be involved in the pathogenesis came from an in vitro study employing SH-SY5Y cells expressing α-synuclein. The reserch demonstrated extracellular secretion of α-synuclein via exosomes in a calcium-dependent manner and suggested their involvement in spread of Parkinson's disease pathology [70]. Following this study, α-synuclein-containing exosomes have been identified from different cells, cerebrospinal fluid, and plasma of Parkinson's disease patients [71][72][73].

A fundamental question to understand the pathogenesis of Parkinson's disease is how do exosomes relay the toxic effects of α-synuclein? Exosomes may aid Parkinson's disease pathogenesis by promoting aggregation of α-synuclein due to their lipid and/or protein composition thus facilitating uptake of α-synuclein by cells. Several studies pointed out that exosomes contain α-synuclein as oligomers. Several cellular processes and enrichment of certain molecules within cells cause α-synuclein oligomerization and often in combination with other proteins. Exosomal ganglioside lipids GM1 or GM3 accelerate α-synuclein aggregation [74]. A combination of ceramides and neurodegeneration-linked proteins including α-synuclein and tau in exosomes is capable of inducing aggregation of wild-type α-synuclein [75]. Oxidation of two adjacent amino acids, methionine [Met(38)] and tyrosine [Tyr(39)], results in aggregation of γ-synuclein and seed aggregation of α-synuclein. Neuronal exosomes containing γ-synuclein upon internalization can cause aggregation of intracellular proteins in astrocytes, resulting in synucleinopathies [76]. Levels of Golgi complex localized the gamma adaptin ear-containing, ARF-binding protein 3 (GGA3) were downregulated in postmortem substantia nigra of PD patients as compared to controls. GGA3 induces oligomerization of α-synuclein in endosomes, resulting in secretion of α-synuclein oligomers [77]. In another study, researchers reported interaction between α-synuclein and the autophagy protein, LC3B that resulted in formation of detergent-insoluble oligomeric aggregates. Alpha-synuclein oligomers are deposited on the surface of late endosomes and are eventually secreted out of human pluripotent stem cells through exosomes [78].

Microglia are a double-edge sword in the CNS as they can be either neuroprotective or neurotoxic. Incubation of microglial cell line BV2 with α-synuclein released an increased number of exosomes enriched with MHC class II molecules and membrane TNFα. Internalization of these exosomes by neurons was neurotoxic suggesting a role for microglia in α-synuclein-induced neurodegeneration [79]. The question is how α-synuclein is internalized by microglia. Microglial cells selectively express Toll-like receptor 2 (TLR2) that acts as a ligand of α-synuclein. Binding of α-synuclein to TLR2 activates microglia. Since α-synuclein is present on the surface of exosomes, they are internalized by microglia.

The excessive exosome uptake by microglia causes an inflammatory response [80], inhibition of autophagy, and reduced scavenger activity. Reduced phagocytosis of α-synuclein-containing exosomes was seen in mouse microglia and human monocytes from aged donors. This observation suggests an age-dependent predisposition to incidence of misfolded proteins in Parkinson's disease [81], which is in line with the onset of Parkinson's disease occurring predominantly after 60 years of age.

In summary, there has been substantial interest in exosome research in the context of Parkinson's disease. It is apparent that exosomes are important mediators of α-synuclein transmission among brain cells. In addition, the ability of exosomes to transfer proteins and miRNAs contributes to pathogenesis.

Amyotrophic Lateral Sclerosis: Amyotrophic lateral sclerosis (ALS) is a late-onset, fatal neurodegenerative disease with a median survival of only 2–5 years—it affects upper motor neurons which project from the cortex to brain stem and spinal cord, as well as lower motor neurons that project from the spinal cord to muscles. Patients develop progressive muscle paralysis and death usually occurs due to respiratory failure. Most cases are sporadic but some are familial cases. ALS is characterized by misfolding of Cu/Zn dismutase (SOD-1) [82] and TAR DNA-binding protein 43 (TDP-43) [83]. SOD 1 is a cytosolic mitochondrial enzyme involved in clearance of superoxide molecule, while TDP-43 is a highly conserved nuclear RNA/DNA-binding protein involved in RNA processing. Post-translational modifications such as cleavage, hyper-phosphorylation and ubiquitination of TDP-43 can lead to cytoplasmic accumulation and aggregation of TDP-43. Both SOD-1 and TDP-43 are packaged in exosomes [84][85]. By overexpressing both wild-type and mutated SOD-1 in NSC-34 motor neuron-like cells, Grad and colleagues observed that misfolded SOD-1 protein was transferred from cell to cell via exosomes in addition to direct uptake of SOD-1 protein aggregates by micropinocytosis [85]. Studies have suggested that astrocytes may play a role in pathogenesis of ALS. Exosomes released by primary astrocyte cultures expressing mutant SOD-1 efficiently transferred mutant SOD-1 protein to spinal neurons, causing selective motor neuron death [86]. A study utilizing a SOD-1 transgenic mouse model demonstrated that mutant SOD-1 was enriched in exosomes derived from both neurons and astrocytes, suggesting that these two cell types may contribute to spread of pathology in ALS [87]. TDP-43, another protein involved in pathogenesis of ALS, was detected in exosomes purified from cerebrospinal fluid of ALS patients [88], supporting the idea that exosomes contribute to disease propagation. Indeed, cerebrospinal fluid enriched with TDP-43-containing exosomes was able to promote accumulation of toxic TDP-43 in human glioma U251 cells [89]. Furthermore, TDP-43 oligomers present in exosomes were transmitted intercellularly [90]. Interestingly, levels of exosomal TDP-43 (full-length protein and C-terminal fragments) are upregulated in brains of ALS patients. When Neuro2a cells were exposed to exosomes from ALS brains, TDP-43 was redistributed in the cytoplasm of Neuro2a cells [91]. Compared to other neurodegenerative diseases, research into the pathogenesis of this devastating fatal disease is much more limited. Much of the research has been performed in vitro. With refinement of exosome isolation techniques from brain tissue it is hoped that scholars will have a clearer picture of the role-played by exosomes in spread of ALS.

Exosomes and the blood–brain barrier: The blood–brain barrier is a physical barrier between brain and the peripheral circulation, controlling a strict influx and efflux of molecules to maintain the homeostasis. Accumulating evidence suggests that exosomes have the remarkable ability to cross the blood–brain barrier from both directions. Exosomes carry cargos of membrane and cytosolic proteins and genetic material such as mRNAs, non-coding RNAs including miRNAs that otherwise generally do not cross plasma membrane. Exosomes released from cancer cells have been shown to destroy the blood–brain barrier through action of microRNA-181c, leading to actin mislocalization and perhaps, resulting in breakdown of blood–brain barrier integrity. Such leakiness of the blood–brain barrier is also seen in cases of neurodegeneration often as a result of neuroinflammation. Furthermore, glioblastoma-specific mRNAs have been detected in exosomes in the peripheral circulation [92]. Experimental evidence suggests that exosomes can cross the blood–brain barrier from the periphery and localize in the brain. Analyses of fluorescent or luciferase-labeled exosomes demonstrated that they have the ability to accumulate in the brain from the periphery [93][94]. Exosomes loaded with siRNA were able to deliver their cargo to neurons, microglia and oligodendrocytes in brain when administered intravenously [95]. Exosomes derived from hematopoietic cells can be transferred to Purkinje cells in the brain and importantly were able to modulate gene expression in these cells. This observation suggests that transfer of exosomes via the blood–brain barrier can have functional implications. The ability of exosomes to cross the blood–brain barrier presents a great potential for exosomes as a drug delivery system. Equally important is that uptake of the exosomal cargo by recipient cells can have profound functional impacts on the CNS. Thus, understanding how exosomes traverse the blood–brain barrier bidirectionally can have great therapeutic potential and diagnostic utility.

As the exosome field is witnessing exponential growth, it is perhaps an understatement to say that there is a requirement for more uniformity in exosome isolation and characterization methods. Refinement of exosome isolation in an in vivo setting will definitely enable the discovery of novel biological functions of exosomes. Many exosome studies have been performed using cells cultured in vitro. Future studies involving animal and clinical research will be a key to unlocking the

potential of exosome biology. Particularly, a better understanding of the role played by exosomes in pathogenesis of neurodegeneration will pave the way for new therapeutic avenues. This is specifically significant as the aging population increases and with it a growing incidence of neurodegenerative diseases. The biological content of exosomes can be harnessed for biomarker discovery aiding in diagnosis and prognostic follow up studies. This is of particular importance as exosomes are present in most biological fluids and the biological cargo is stable and protected within the boundaries of exosome membranes.

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