



HHS Public Access

Author manuscript

J Neurochem. Author manuscript; available in PMC 2022 May 01.

Published in final edited form as:

J Neurochem. 2021 May ; 157(3): 413–428. doi:10.1111/jnc.15288.

Exosomes in Parkinson Disease

Jennifer R. Pinnell^{1,2}, Mei Cui^{3,4}, Kim Tieu^{1,4}

¹Department of Environmental Health Sciences, Florida International University, Miami, USA

²Peninsula Schools of Medicine and Dentistry, Plymouth University, Plymouth, UK

³Department of Neurology, Huashan hospital, Fudan University, Shanghai, China

Abstract

Parkinson disease (PD) is a prevalent neurodegenerative disease, in which the formation of misfolded and aggregated α -synuclein is a key neuropathological hallmark. Recent studies reveal that extracellular vesicles such as exosomes present a potential mechanism for propagation of pathological α -synuclein throughout the brain. The ability of exosomes to transport proteins and genetic material between cells, including mRNA and microRNAs which have been implicated in PD pathology, provides critical insights as to how exosomes may contribute to pathological progression in PD. Advances have also been made in the investigation of exosomes as potential tools for the modulation of Parkinson's pathology; their detection extracellularly may facilitate their use as biomarkers, whilst their small size could be utilised as vectors for the delivery of therapeutics. The aim of this review is to highlight our current knowledge of the role of exosomes in PD and potential clinical application.

Keywords

Parkinson disease; exosomes; extracellular vesicles; neurodegeneration; alpha-synuclein; protein aggregation

⁴To whom correspondence may be addressed: Kim Tieu (ktieu@fiu.edu, Phone: 305 348 0371, Fax: 62481088).

--Human subjects --

Involves human subjects:

If yes: Informed consent & ethics approval achieved:

=> if yes, please ensure that the info "Informed consent was achieved for all subjects, and the experiments were approved by the local ethics committee." is included in the Methods.

ARRIVE guidelines have been followed:

No

=> if it is a Review or Editorial, skip complete sentence => if No, include a statement in the "Conflict of interest disclosure" section:

"ARRIVE guidelines were not followed for the following reason:

Not applicable "

(edit phrasing to form a complete sentence as necessary).

=> if Yes, insert in the "Conflict of interest disclosure" section:

"All experiments were conducted in compliance with the ARRIVE guidelines." unless it is a Review or Editorial

Conflicts of interest: None

=> if 'none', insert "The authors have no conflict of interest to declare."

=> otherwise insert info unless it is already included

1. INTRODUCTION

As the second most prevalent neurodegenerative disease worldwide (Dorsey *et al.* 2018), Parkinson disease (PD) is a debilitating, progressive motor disorder characterised by degeneration of the nigrostriatal dopaminergic pathway. A hypokinetic movement disorder, its symptomology arises from the loss of dopaminergic innervation of motor nuclei in the basal ganglia which results in the characteristic tetrad of motor symptoms; bradykinesia (slowness of movement), rigidity, postural instability, and resting tremor. Parkinson's patients may also present with non-motor symptoms, some of which arise in the prodromal phase before onset of motor abnormalities. These early symptomatic changes may include olfactory dysfunction, depression, sleep disturbances, gastrointestinal changes and pain (Schrage *et al.* 2019). Recent estimates suggest that PD affects approximately ten million individuals worldwide (Dorsey *et al.* 2007; Marras *et al.* 2018). The underlying aetiology of PD is multifactorial, with complex interplay of genetic and environmental risk factors converging to confer increased disease risk.

The first genetic risk factor discovered for PD was the *SNCA* gene (Polymeropoulos *et al.* 1997). Since its discovery, *SNCA* and the α -synuclein (α -syn) protein which it encodes have been extensively researched, with over 12,000 research papers published (as of 24th September 2020). α -syn is a major protein constituent of Lewy bodies, distinctive intracellular protein aggregates which are found in the surviving nigrostriatal dopaminergic neurons of PD patient brains (Arima *et al.* 1999; Mezey *et al.* 1998; Spillantini *et al.* 1997). Their presence in the brains of both familial and sporadic cases of PD supports a central pathological role of α -syn in PD. α -syn has been shown to exist in multiple forms in the brain, including soluble unfolded mono-, oligo- and polymeric forms, as well as insoluble β -sheet-containing fibrils (Mor *et al.* 2016). Although still a topic of debate (Surmeier *et al.* 2017; Brundin & Melki 2017), there is increasing experimental evidence indicating that α -synuclein is capable of intercellular transmission, which is consistent with the proposed Braak scheme of pathological PD propagation (Braak *et al.* 2003; Dickson *et al.* 2010). This hypothesis suggests that the olfactory bulb and the gut are initial spreading sites for misfolded α -syn protein (Klingelhoefer & Reichmann 2015).

Whilst multiple mechanisms of cell-to-cell α -syn transmission have been proposed, including exocytosis, endocytosis, extracellular vesicles (EVs) and tunnelling nanotubules (Peng *et al.* 2020). EVs such as exosomes are currently of great interest as they can shuttle genetic material and protein between cells, with the capacity to impact proximal and distal to their cells of origin. Multiple cell types, including neurons, astrocytes and microglia, have been found to release exosomes in the brain; their ability to carry material which can influence gene expression and protein activity in recipient cells (Chistiakov & Chistiakov 2017) makes them prime possibilities for the modulation of protein misfolding in PD.

This review will discuss the current understanding of the role of exosomes and α -syn transmission in PD, including the potential role in disease propagation. Furthermore, we will highlight the current evidence for targeting exosomes as potential a therapeutic intervention and diagnostic tool in PD. Please note that although the term exosomes is used in this review, as pointed out by the International Society for Extracellular Vesicles (Théry *et al.*

2018), techniques used in most studies to isolate exosomes also contain other exosome-like extracellular vesicles.

2. INTERCELLULAR α -SYNUCLEIN SPREAD

Within the growing evidence that α -syn is capable of cell to cell transmission, there are multiple proposed cellular mechanisms which facilitate its propagation: non-classical exocytosis, exosomal release, endocytosis and transport *via* nanotubules, which provide physical bridges between distinct cells, have all been proposed (Peng *et al.* 2020). The discovery of α -syn's transmissibility provides a key insight into the development and progression of PD, which may lead to the advancement of future therapies through the provision of additional mechanistic targets. According to the Braak staging hypothesis of PD pathology development (Braak *et al.* 2003; Dickson *et al.* 2010; Hawkes *et al.* 2007; Parkkinen *et al.* 2008), the olfactory bulb and the gut are the initial source sites for α -syn spread. Consistent with this hypothesis, injection of preformed fibrillar α -syn into the olfactory bulb (Rey *et al.* 2016) and the gut (Kim *et al.* 2019) of mice produces trans-neuronal propagation of pathological α -syn from these respective peripheral regions to other brain regions, including the substantia nigra, in a stereotypical manner. Furthermore, mice with truncal vagotomy and α -syn deficiency prevent this gut-to-brain protein transmission, supporting the gut as an initial source of α -syn transmission (Kim *et al.* 2019). A recent study injecting Lewy body extracts containing α -syn from PD patients to the gut of non-human primates also demonstrated the spread of toxic α -syn from the gut to the brain (Arotcarena *et al.* 2020). Misfolded α -syn protein has been identified in early stage PD patient enteric nervous systems (Hilton *et al.* 2014; Killinger *et al.* 2018; Wakabayashi *et al.* 1990), further supporting the gut as an initial site where α -syn propagates from. However, other studies have demonstrated that intramuscular (Sacino *et al.* 2014) and peritoneal (Sargent *et al.* 2017) injections of α -syn also induce widespread brain pathology. Taken together, these studies indicate that α -syn can propagate from multiple peripheral sites to the central nervous system.

α -syn host to graft cell transmission was reported in two studies investigating the potential symptomatic relief provided by grafting foetal ventral mesencephalic tissue into the nigrostriatal system of PD patients (Kordower *et al.* 2008; Li *et al.* 2008). Functional dopamine production persisted for over a decade post-transplant, with some long-surviving neurons displaying characteristic α -syn- and ubiquitin-positive Lewy bodies, which were positive for Serine-129 phosphorylated α -syn (Li *et al.* 2008), strongly suggesting the presence of disease-related, post-translationally modified and potentially aggregated α -syn. Increase of α -syn-containing tyrosine hydroxylase-positive neurons correlated with increasing age of the graft, supportive of progressive, gradual α -syn infiltration from host to graft neurons, consistent with increasing age as a major PD risk factor (Bridgeman & Arsham 2017). This host-to-graft protein transmission was identified as a potentially limiting factor of cell replacement therapy, as increasing α -syn abundance during aging may detrimentally impact the long-term graft function and survival. In one patient who died 14 years post-transplant, reduced dopamine transporter expression was evident in the grafted region, suggestive that α -syn infiltration may also impact neuronal function and viability (Kordower *et al.* 2008). Similar examples of α -syn spread from host to engrafted neurons

have been recapitulated in pre-clinical models (Desplats *et al.* 2009; Hansen *et al.* 2011; Kordower *et al.* 2011), including α -syn pre-formed fibrils in a non-human primate model (Chu *et al.* 2019) and human embryonic stem cells (hESC) transplanted into a humanized- α -syn rat model of PD (Hoban *et al.* 2020).

α -syn transmission has been shown to occur in neuron to neuron, neuron to astrocyte, neuron to microglia and microglia to neurons (Desplats *et al.* 2009; Guo *et al.* 2020; Kim *et al.* 2013; Lee *et al.* 2010; Luk *et al.* 2012; Harischandra *et al.* 2019; Xu *et al.* 2018; Fan *et al.* 2019). This protein transmission between cell types highlights a limitation of current PD treatments, which focus on DA neurons alone; future interventions will likely hold greater therapeutic potential if they can target or protect multiple cell types from α -syn infiltration and the resultant pathology. Interestingly, in contrast to microglia, astrocytes do not appear to readily spread α -syn to neurons due to their efficient degradation of intracellular α -syn (Loria *et al.* 2017; Rappold & Tieu 2010)

There are multiple regulated cellular mechanisms of α -syn release and uptake, which are currently reported as probable contributors to its intercellular transmission (Figure. 1). Free-floating α -syn protein seeds (Fig. 1A) have been found to transmit across the extracellular space, where they may then pass through the plasma membrane or be up taken by endocytic mechanisms for infiltration of recipient cells, as demonstrated *in vitro* by the downstream initiation of protein pathology following cell exposure to wild type α -syn from cell culture media (Nonaka *et al.* 2010). Endocytosis likely plays a role in the cellular uptake of α -syn once it has traversed the extracellular space; there is evidence to support the classical endocytic uptake of higher order α -syn structures, which may have evolved as a protective mechanism to regulate extracellular α -syn levels by facilitating protein uptake for degradation (Masaracchia *et al.* 2018; Rodriguez *et al.* 2018). This mechanism, however, would rely on the continued effectiveness of intracellular protein degradation pathways, such as autophagy, which have been shown to be restricted by excessive α -syn levels (Arotcarena *et al.* 2019; Fan *et al.* 2019; Tanik *et al.* 2013). Accumulation of α -syn species may therefore lead to cytotoxic saturation of the cells, interfering with protein degradation and contributing to increased cellular death which may release further α -syn into the extracellular space. Endocytosis may also facilitate the uptake of α -syn-containing vesicles, however for reactivity of α -syn in these cases the protein would somehow need to leave the vesicles to enable interactions with other intracellular proteins, to act as a seed for intracellular α -syn aggregation or to exert other cytotoxic changes. The potential mechanism of intracellular vesicular release of α -syn remains unknown. Inhibition of endocytosis with monodansylcavdaverine and dynasore has been shown to decrease α -syn uptake *in vitro* and *in vivo* (Hansen *et al.* 2011).

Exocytosis (Fig. 1B) is a potential mechanism of α -syn release from donor cells, or cells of origin, in the intracellular α -syn transmission cascade; overexpression of α -syn was found to result in the secretion of monomers and aggregated α -syn from neuroblastoma and primary rat cortical neurons (Lee *et al.* 2005; Lee *et al.* 2013; Logan *et al.* 2017). These changes were inhibited by reduced temperature, a classical test for blockade of vesicular exocytosis, which suggests exocytosis as a likely facilitatory mechanism (Lee *et al.* 2005). Inhibition of the autophagy-lysosome pathway in α -syn-expressing cells resulted

in increased exocytosis of α -syn protein (Lee *et al.* 2013). Further work is still required to elucidate the exact mechanisms by which α -syn induces exocytic release, however the implication of autophagic dysfunction further supports the ineffective or incomplete clearance of α -syn as a contributing factor to its transcellular transmission.

Secretory lysosomes (Fig. 1C), also known as the process of lysosomal exocytosis, is a mechanism of protein transmission from organelles of the autophagic- and endo-lysosomal system (Medina *et al.* 2011). Lysosomes can release their contents to the extracellular space following fusion with the cellular membrane (Blott & Griffiths 2002; Lettau *et al.* 2007), contributing to cellular clearance and cell-to-cell communication. Recent work in iPSC-derived dopaminergic neurons from *ATP13A2/PARK9* mutated PD patients implicates lysosomal exocytosis in the clearance of intracellular α -syn in human dopaminergic neurons, with *ATP13A2/PARK9* mutations shown to contribute to α -syn storage (Tsunemi *et al.* 2019). As lysosomal exocytosis has been previously implicated in mediation of Ca^{2+} levels, the authors investigated the impact of pharmacologically increasing lysosomal Ca^{2+} , which restored this defective secretory mechanism to alleviate intracellular α -syn accumulation (Tsunemi *et al.* 2019). This observation has led to therapeutic interest in targeting secretory lysosomes, however enhancing this α -syn release could exacerbate its cellular transmission to detrimentally impact neighbouring cells.

Tunnelling nanotubes (TNTs, Fig. 1E) are versatile structures composed of F-actin-based tubular connections which allow direct communication and transport between distal cells (Abounit & Zurzolo 2012). TNTs have been implicated in the transmission of organelles, plasma membrane components, pathogens, Ca^{2+} and even electrical signals between cells. Evidence suggests that rather than acting as passive conduits for transmission, TNTs may be regulated by gap junctions and gating mechanisms. TNTs provide continuity between the cytoplasm of remote cells, with a dynamic nature that can lead to *de novo* formation of TNTs within minutes, and exhibiting lifetimes which may range from minutes to several hours *in vitro* (Rustom *et al.* 2004). Investigation of α -syn spread via TNTs demonstrated the effective transfer of α -syn fibrils from donor to acceptor cells inside lysosomal vesicles (Abounit *et al.* 2016). These fibrils acted as seeds in the acceptor cells, to promote aggregation of soluble α -syn within the cytosol of the recipient cells. Abounit *et al.* (2016) proposed that the transfer of these α -syn-containing vesicles may result from α -syn overload in the cells of origin, which dispose of the excess protein through use of the TNT transfer system. This results in progression of the pathology if these lysosomes are not degraded by the recipient cells. Research suggests that as well as the identification of α -syn in nanotubule structures, the addition of aggregated α -syn to cell cultures can increase the number of TNTs (Abounit *et al.* 2016), which would further promote intercellular protein transmission. This mode of transmission is not limited to neurons alone as intercellular transmission of α -syn has also been demonstrated in cultured astrocytes (Rostami *et al.* 2017). Further investigation of how TNT-transmitted vesicles may release their contents into cells is required to assist our understanding of how this transmitted α -syn may promote intracellular α -syn aggregation.

Extracellular membrane-bound vesicles (Fig. 1D & F), play a prominent role in the mechanism of protein transmission proposed for the intercellular spread of α -syn. The most

widely investigated EVs are exosomes, and to a lesser extent, microvesicles. As described in the next section, exosomes are derived from endosomes. Microvesicles are formed by outward budding from the plasma membrane. Other terms for microvesicles include microparticles and budding vesicles. Since some of the protein cargo of microvesicles overlaps with those of exosomes, it is challenging to make definitive distinction between microvesicles and exosomes. However, the sizes of exosomes are typically between ~40–160 nm in diameter whereas those of microvesicles can range from 100 nm to 1 μ m. Although exosomes were first described in 1983 (Harding *et al.* 1983; Pan & Johnstone 1983), they have become an increasingly popular structure for research over the past two decades following the 2007 discovery that cells can utilise these small structures for the transfer of messenger and micro RNAs between cells (Valadi *et al.* 2007). Since then, our understanding of their role in physiological and pathological pathways is growing rapidly, with investigation of their function in disease states and as potential drug delivery vectors.

3. EXOSOME FORMATION AND FUNCTION

As illustrated in Figure 2, exosomes are produced in the endosomal compartment of most eukaryotic cells (Théry *et al.* 2018; Yáñez-Mó *et al.* 2015); multiple mechanisms of exosome formation have been described, with the most studied being the endosomal sorting complex required for transport (ESCRT)-dependent pathway (Wollert & Hurley 2010). The ESCRT machinery mediates a ubiquitinated pathway which recognises and retains ubiquitinated proteins marked for packaging in the late endosomal membrane (Wollert & Hurley 2010). The ESCRT-0 subunit retains ubiquitinated proteins, with downstream recognition of ESCRT-0 by ESCRT I/II which creates an endosomal membrane invagination to begin the formation of intraluminal vesicles (ILVs) (Hessvik & Llorente 2018; Wollert & Hurley 2010). ESCRT III forms a spiral structure to constrict the ILV neck, whilst ATPase VPS4 is responsible for scission of the membrane (Wollert & Hurley 2010). This results in formation of multivesicular bodies (MVBs), which can fuse with the cellular plasma membrane to release these ILVs into the extracellular space as exosomes (Gruenberg & van der Goot 2006). There are also ESCRT-independent non-canonical pathways of exosome formation (Baietti *et al.* 2012).

Exosomes can shuttle genetic and protein constituents between cells, facilitating cell-to-cell communication and regulating information transfer, which may influence the cells function. Exosomes are typically between ~40–160 nm in diameter (Kalluri & LeBleu 2020) and they have been demonstrated to play communicative roles in immune responses, with exosomes from B lymphocytes bearing MHC class II molecules found to activate T lymphocytes *in vitro* (Raposo *et al.* 1996), suggestive of regulation in a manner similar to that of parent B cells. Further work found that exosomes from dendritic cells, which are specialised to activate T cells in immune response initiation, promoted anti-tumour responses in mice (Zitvogel *et al.* 1998). Outside of their immunoreactive functions, exosomal functions are still being explored and they have been found to transmit pathogenic prion proteins and amyloid-beta aggregates (Berrone *et al.* 2015; Cheng *et al.* 2018; Guix *et al.* 2018; Pérez *et al.* 2019). The remainder of this review will focus on the implication of exosomes in PD, α -syn transmission and whether they may represent therapeutic targets to modulate PD pathology and prognosis.

4. EXOSOMES IN PD

Exosomes have two major roles in the pathogenesis of PD; the first and most investigated is their role as important mediators of α -syn transmission between cells, and the second is their capacity to transport RNAs, such as PD-implicated microRNAs (miRNA) between cells.

4.1 α -synuclein, autophagy and exosomes

As α -syn accumulation is a prominent feature of PD pathology, understanding the mechanisms underlying its aggregation and intercellular transmission may improve our understanding of disease pathology and progression to facilitate the development of more effective therapies. Recent work implicating exosomes in these pathological processes suggests that targeting exosomes may hold therapeutic potential. Exosomes contribute to non-cell autonomous-mediated neurotoxicity and can mediate α -syn transmission over greater distances than some other mechanisms of cell-to-cell transmission. Exosomes have been demonstrated to transfer α -syn protein to normal neuronal cells (Alvarez-Erviti *et al.* 2011a), where it can form aggregates and induce death of the recipient cell (Desplats *et al.* 2009; Hansen *et al.* 2011; Fan *et al.* 2019; Guo *et al.* 2020; Harischandra *et al.* 2019). α -syn-oligomers associated with exosomes have been found to increase the likelihood of cellular uptake, with greater subsequent neurotoxicity than free α -syn oligomers (Danzer *et al.* 2012). The study of exosomes in PD models has included investigation of their spread between glia and neurons, with inter-neuronal α -syn transmission the most studied interaction.

In SH-SY5Y neuroblastoma cells, overexpression of wild-type α -syn led to its exosomal release, with both monomeric and oligomeric α -syn structures detectable in the conditioned media collected (Emmanouilidou *et al.* 2010; Fan *et al.* 2019; Guo *et al.* 2020). This is indicative that both small and larger α -syn structures are capable of intercellular transmission within exosomes, which may result in different downstream processes to mediate toxicity. α -syn has been found in brain- and CSF-derived exosomes from both PD and dementia with Lewy bodies (DLB) patients (Ngolab *et al.* 2017; Stuenkel *et al.* 2016; Guo *et al.* 2020; Harischandra *et al.* 2019), as α -syn is a common feature of both neurodegenerative pathologies. DLB-patient CSF-derived exosomes have been utilised *in vivo* to investigate α -syn transmission, with intracerebral delivery of patient-derived exosomes to mouse brains resulting in the generation of pathological inclusions (Minakaki *et al.* 2018). DLB-patient brain-tissue-derived exosomes have also been found to induce α -syn aggregate seeding in cultured cells and mouse brains (Ngolab *et al.* 2017).

Recent work has implicated the PD risk factor Manganese, to which individuals may be occupationally exposed, in the upregulation of α -syn-containing exosome release *in vitro* (Harischandra *et al.* 2019). These exosomes were endocytosed through caveolae upon application to primary microglial cells, triggering neuroinflammatory changes which support the role of α -syn transmission in microglial activation. These exosomes also exerted neurotoxic effects in a human dopaminergic cell model and manganese was demonstrated to accelerate α -syn transmission, resulting in dopaminergic neurotoxicity, in a mouse model of manganese exposure (Harischandra *et al.* 2019).

The autophagy-lysosomal pathway has also been implicated in exosomal α -syn transmission, with lysosomal inhibition of donor cells found to increase both exosome-mediated release and transfer of α -syn (Alvarez-Erviti *et al.* 2011a). This observation is most likely due to the effect that when autophagy flux is impaired, the MVB and amphisome pathways are compensatorily enhanced to reduce intracellular α -syn levels by releasing exosomes (Figure 2). Concurrent reduction in intracellular α -syn aggregation has been reported to accompany the increased exosomal α -syn release in instances of autophagy inhibition (Lee *et al.* 2013); Furthermore, Fussi *et al.* (2018) found that inhibition of autophagosome formation, via *Atg5* inhibition, resulted in protection from α -syn toxicity, which was thought to be the result of increased exosomal α -syn secretion; inhibition of exosome secretion was found to enhance α -syn-induced cell death. This study supports the proposal of α -syn excretion as a protective compensatory mechanism of cells to avoid their intracellular protein accumulation. Although the reduction of intracellular aggregation may initially demonstrate cellular protection as a result of enhanced removal of α -syn from the cell via exosomal release, this effect propagates PD pathology and may overload the extracellular space with toxic α -syn (Poehler *et al.* 2014).

Degradation of extracellular α -syn species, including exosome-contained protein, is a major function of microglia, which phagocytose dead cells and help clear misfolded protein aggregates in the brain (Brück *et al.* 2016). α -syn has also been found to activate microglia by stimulating an immune response which includes the release of pro-inflammatory cytokines; microglia-mediated neuroinflammation has been previously linked to PD pathogenesis (Hirsch *et al.* 2012). A recent study demonstrates that aggregated α -syn activates microglia through the Kv1.3 voltage-gated potassium channel (Sarkar *et al.* 2020). Blocking Kv1.3 function genetically or pharmacologically was found to significantly reduce neuroinflammation and neurodegeneration in animal models of PD (Sarkar *et al.* 2020). Studies of isolated microglia and monocytes from mice found that in young mice there was increased phagocytosis of exosomal α -syn oligomers, with a concomitant reduction in microglial activation as indicated by decreased secretion of pro-inflammatory cytokines (Bliederhaeuser *et al.* 2016). This increase in microglial α -syn phagocytosis was not sustained in microglia isolated from older mice, suggesting that microglia in aged brains may have reduced phagocytic capacity to modulate extracellular α -syn levels. In the BV-2 microglial cell line, exosome release was found to cause apoptotic cell death in co-cultured cortical neurons (Chang *et al.* 2013). Our recent work supports the role of microglia in exosomal α -syn transmission; in primary microglial cell cultures, application of human α -syn preformed fibrils (PFFs) stimulated release of α -syn-containing exosomes, which were fully capable of inducing protein aggregation in recipient neurons (Guo *et al.* 2020); this release of α -syn following PFF treatment may be an attempt to modulate intracellular levels of abnormal protein. Co-treatment with PFFs and pro-inflammatory cytokine treatment increased α -syn aggregation *in vitro*, implicating microglial activation with enhanced α -syn transmission and toxicity. The importance of exosomes in this protein transmission was verified by inhibition of exosome synthesis 24 hours prior to PFF treatment, which robustly reduced exosome secretion from microglia without inducing microglial cell death. Labelling of isolated exosomes from these inhibited microglia subsequently demonstrated that neuronal uptake of these exosomes was also reduced, with a consistent reduction in

α -syn aggregation found in these neurons (Guo *et al.* 2020). This finding supports the potential targeting of exosomes as an intervention to limit α -syn transmission between cells.

Stereotaxic delivery of PFFs to the mouse dorsal striatum resulted in increased phospho- α -syn in the striatum and anatomically associated brain regions after 30 days, along with time-dependent degeneration of TH-positive neurons in the SNpc and TH-positive terminals in the striatum (Guo *et al.* 2020). Enhanced microglial activation in the SNpc and striatum supports the implication of microglia in these neuropathological changes. Microglial depletion via inhibition of colony stimulating factor 1 receptor (CSF1R), with subsequent PFF delivery as before reduced microglial activation and α -syn aggregation to demonstrate that partial depletion of microglia may protect against α -syn transmission and aggregation in the brain (Guo *et al.* 2020). Further to the study of PFF-induced microglial activation, stereotaxic injection of primary microglial culture-derived exosomes to the mouse dorsal striatum resulted in a time-dependent increase in phospho- α -syn, confirming the transmissibility of microglial-derived α -syn-containing exosomes *in vivo*. Concurrent loss of TH-positive neurons and terminals in the SNpc and striatum, with concomitant striatal dopamine depletion at 180 days post-surgery is consistent with progressive neuropathological changes. These neurotoxic changes were not detectable at 30 days post-surgery, supportive of microglial-derived exosome-mediated time-dependent retrograde degeneration of the nigrostriatal pathway, with accompanying development of motor dysfunction (Guo *et al.* 2020). Similarly, Alzheimer's disease-related tau protein has been found to spread via microglial exosomes, with depletion of microglia and inhibition of microglial exosome synthesis found to attenuate tau propagation (Asai *et al.* 2015).

Mechanistically, autophagy has been implicated in pathological α -syn transmission; microglial activation has been found to impair autophagy (Du *et al.* 2017) and α -syn PFFs were found to impair autophagy flux via PELI1 upregulation, which in turn resulted in LAMP1 degradation in activated microglia (Guo *et al.* 2020). Lysosomal impairment has been previously found to promote exosome-mediated protein secretion (Vella *et al.* 2007; Vella *et al.* 2008) and autophagosomes have been suggested to affect exosomes (Fader *et al.* 2008). Overall, exosomes are strongly implicated as contributors to α -syn intercellular transmission in PD, with autophagy and apoptosis as two suggested mechanisms of cellular damage.

Interestingly, exosomal transmission in PD has also been linked to the genetic risk factor *ATP13A2*, which is also involved in the juvenile parkinsonian disorder Kufor-Rakeb syndrome. Loss of function mutations in this gene cause lysosomal dysfunction and α -syn accumulation, whereas overexpression was found to suppress α -syn toxicity (Gitler *et al.* 2009; Kong *et al.* 2014). *ATP13A2* overexpression was associated with enhanced exosome release in H4 neuroglia cells, which suggests that these cells may utilise exosomal removal of α -syn to suppress toxicity (Tsunemi *et al.* 2014).

4.2 Exosomes and miRNAs

Further to their function in mediating intercellular α -syn transmission in PD, exosomes are additionally implicated in PD pathogenesis by their capacity to transfer genetic material between cells. MiRNAs can be packaged into exosomes and have previously been

implicated in PD. Some miRNAs target PD-related genes, such as miR-7 which can combine with the 3'-untranslated region (UTR) of *SNCA* mRNA to inhibit its transcription; thus loss of miR-7 contributed to increased α -syn upregulation, aggregation and dopaminergic neuron loss in PD-patient brains (McMillan *et al.* 2017). Significant upregulation of miR-4639-5p, which negatively regulated the post-transcription of DJ-1, has been found to result in severe oxidative stress and neuronal death in PD patients (Chen *et al.* 2017). Exosome-associated miR-137 has been found to be upregulated in neurons in PD, where it plays a vital role in neuronal oxidative stress induction. MiR-137 directly targets oxidation resistance-1 (OXR1) to negatively regulate its expression, thereby inducing oxidative stress. The levels of miRNAs have also been investigated in some PD models, such as in a manganese model where 12 miRNAs were significantly increased in exosomes; these miRNAs were shown to regulate key PD pathogenesis pathways including autophagy, inflammation and protein aggregation (Harischandra *et al.* 2018).

Overall, research to date strongly supports exosomes as important multi-functional contributors to PD pathogenesis, through transmission of abnormal protein, propagation of protein pathology across brain regions and transfer of genetic material between cells.

5. CLINICAL POTENTIAL OF EXOSOMES

Exosomes may hold clinical potential for PD, including therapeutic potential as treatment vehicles and diagnostic tools for the monitoring of specific protein and miRNA levels in serum exosomes. The ability of exosomes to transport toxic proteins, such as α -synuclein, to propagate PD pathology through the brain suggests they may be suitable targets to reduce protein pathology if their cellular release or uptake can be targeted. They have also been identified as potential vectors for the delivery of drug or genetic therapies, as it may be possible to hijack their native capacity for the transport of genetic and protein materials between cells for therapeutic benefit. Additionally, because exosomes are components of native cellular transport mechanisms, they may also avoid activating cellular immunogenic responses.

5.1 Limiting protein propagation

Impairment in autophagic activity and lysosomal function has been documented to increase the release of exosomes in neuronal cells (Alvarez-Erviti *et al.* 2011a; Danzer *et al.* 2012; Minakaki *et al.* 2018). Previous work has also reported that α -syn levels in exosomes increase when autophagy flux is inhibited in α -syn over-expressing neurons by enhancing the MVB and amphisome pathways as a compensatory response to reduce intracellular α -syn levels through releasing exosomes. (Alvarez-Erviti *et al.* 2011a; Minakaki *et al.* 2018; Poehler *et al.* 2014). It has been reported that exosome-associated α -syn oligomers are internalised by recipient cells whereas free α -syn oligomers are not, and remain bound to cell surface (Delenclos *et al.* 2017). As our understanding of the intercellular transport of α -syn has highlighted the multi-cell type involvement of neurons, microglia and astrocytes, we have come to recognise the importance of targeting exosome release from all cell types as a potential therapeutic strategy for PD, not just neuronal exosomes as glial cells also participate in the process of intercellular protein transmission.

Recent work by our group found that exosome release was increased in circumstances of impaired autophagy flux and that targeting dynamin related protein 1 (Drp1), commonly known for its mitochondrial fission function, was effective at reducing α -syn-containing exosome release from both neurons and microglia (Fan *et al.* 2019). We found that exosomes released from α -syn PFF-treated neurons conferred α -syn toxicity to neurons. Inhibition of Drp1 enhanced autophagy flux, reduced α -syn aggregation and reduced exosome release; this work demonstrates that indirect modulation of exosome levels through restoration of autophagy flux attenuated α -syn propagation and aggregation. Both siRNA knockdown of Drp1 and inhibition with a pharmacological agent, mitochondrial division inhibitor 1 (Mdivi-1), were effective at reducing α -syn transmission between SH-SY5Y cells. Further investigation in primary mouse microglia treated with PFFs in conjunction with LPS demonstrated enhanced exosome release in co-treated cells. Application of microglial-derived exosomal fractions to SH-SY5Y cultures confirmed the capacity of exosomes to mediate microglia-to-neuron α -syn transmission, with Drp1 inhibition effective at reducing this protein transmission and aggregation. Combined assessment of the data from primary microglia and BV2 cells indicates that microglia are capable of releasing α -syn-containing vesicles through which they propagate the spread of synuclein to neurons, with Drp1 blockade found to significantly reduce these pathological changes. This work supports the potential therapeutic application of limiting exosome release to mitigate α -syn transmission and aggregation in PD, with efficacy demonstrated in modulation of both neuronal and microglial derived exosomes.

5.2 Exosomes as therapeutic vehicles

The current methods of PD treatment focus on the abrogation of symptomatic presentation, with the primary emphasis on the control of motor dysfunctions. There are currently no disease modifying or curative treatments, so there is a need to develop novel treatment strategies which alter disease progression. A major barrier to the development of new drug treatments for neurodegenerative diseases is the capacity of drugs to cross the blood-brain-barrier (BBB), which many are unable to do (Pardridge & Boado 2012). As described elsewhere in this review, exosomes are nano-scaled extracellular vesicles (EV) which can easily permeate membranes including the BBB, suggesting that they may be effective vehicles for the delivery of drugs to the brain (Lai & Breakefield 2012; Zhuang *et al.* 2011). Another benefit of using exosomes to deliver therapeutics is that they can evade immunogenicity. As artificial exosomes can be loaded with therapeutically active molecules, such as medicinal compounds, siRNAs and proteins for delivery to the brain, it may be possible to mediate site-specific targeting of exosomes to the appropriate recipient cells. Native exosomes are known to transport nucleic acids to target cells, it is therefore reasonable to consider their potential as delivery mechanisms for modified, therapeutic genetic materials which could regulate gene expression to alter PD prognosis or progression.

Research *in vivo* and *in vitro* has found that human exosomes isolated from the blood and loaded with a saturated dopamine solution can cross the BBB to deliver dopamine into the brain via interaction between transferrin and the transferrin receptor (Qu *et al.* 2018). In a mouse model, the dopamine-loaded exosomes demonstrated greater therapeutic efficacy and lower toxicity than systemic intravenously delivered free dopamine. Further research into the

attenuation of neuroinflammation in PD utilised catalase-loaded exosomes which were taken up by neurons. The antioxidant effects of catalase, which was released into the neurons following exosome uptake, was found to increase neuronal survival and ameliorate neural inflammation in PD models *in vitro* and *in vivo* (Haney *et al.* 2015). Kojima *et al.* (2018) also reported a set of devices for exosomal transfer into cells which produce designer exosomes in engineered mammalian cells to deliver therapeutic catalase mRNA to the brain.

There is therapeutic potential for exosomes carrying small interfering RNAs (siRNAs) for PD. A study in the S129D α -syn transgenic mice utilised systemic injection of modified α -syn-siRNA-containing exosomes, which reduced the amount of α -syn mRNA transcription and translation in the brain (Cooper *et al.* 2014). Another group reported that exosome delivery of hydrophobically modified siRNA to the brain efficiently targeted *mHtt* mRNA in a Huntington's disease model, which is encouraging for the potential use of siRNAs to target α -syn in PD (Didiot *et al.* 2016). Alvarez-Erviti *et al.* (2011b) demonstrated that siRNA could be transported to mouse brains via exosomes following systemic injection. However, siRNAs may be of limited effectiveness in PD treatment due to their short efficacy and poor bioavailability in systemic circulation. As such, short hairpin RNAs (shRNAs) have also been investigated. Izco *et al.* (2019) designed shRNA minicircles which were delivered by RVG-exosomes to target synuclein in a PD mouse model induced by preformed synuclein fibrils. Treatment with exosomal anti-synuclein shRNA reduced synuclein aggregation, decreased dopaminergic neuron death and ameliorated the clinical symptoms of PD in this mouse model (Izco *et al.* 2019). This work supports the potential of exosomes as transport vehicles for drugs, such as dopamine, or genetic modulators, such as shRNA, into the brain for therapeutic benefit. Exosomes may prove to be useful vehicles for the delivery of various RNA-based therapies for PD, as they may protect the RNAs from degradation in circulation (Maheshwari *et al.* 2017) and could provide an RNA delivery method to improve clinical practice.

Mesenchymal stem cell (MSC)-derived exosomes have been identified as effective treatment tools, with beneficial effects shown in a number of pathologies including PD, multiple sclerosis and osteoarthritis (Li *et al.* 2019; Mianehsaz *et al.* 2019). MSC-derived exosomes proved effective at rescuing dopaminergic neurons in the 6-OHDA mouse model of PD (Vilaça-Faria *et al.* 2019) and they can also carry miRNAs and interact with neuronal cells to reduce neuroinflammation and promote neurogenesis in mouse PD models. Whilst further investigations and clinical trials are required to confirm the benefits of therapeutic application of exosomes in PD, mounting evidence supports that the separation of exosomes from various cell types and their modification to target specific brain regions may hold therapeutic benefits for PD, amongst other disorders (Batrakova & Kim 2015).

5.3 Exosomes as diagnostic tools

PD is primarily diagnosed by the clinical manifestation of disease symptoms at present (Postuma *et al.* 2015). However, prodromal non-motor symptoms are commonly reported, which can begin decades prior to the onset of motor degeneration (Chaudhuri *et al.* 2006; Drolet *et al.* 2009; Schrag *et al.* 2019). There is currently no accurate method for the diagnosis of PD in the earlier stages, which is when therapeutic interventions may be

more beneficial if they can slow or reduce the nigrostriatal dopaminergic degeneration which initiates motor dysfunction. Therefore, development of an early diagnostic test and identification of novel PD biomarkers would be an important breakthrough.

α -syn is considered an inefficient PD biomarker despite its presence in patient blood and CSF samples due to its low abundance. For example, in PD patient CSF α -syn has been shown to be present at lower levels than in healthy controls (Hall *et al.* 2012; Hong *et al.* 2010; Kang *et al.* 2013; Mollenhauer *et al.* 2013). Stuendl *et al.* (2016) reported that levels of EV- α -syn are also lower in PD patient CSF samples, consistent with the low overall α -syn levels reported. Detection of serum and plasma α -syn has also proven ineffective as a biomarker, as peripheral blood cells can produce α -syn. Previous analysis of extracellular vesicle constituents, including exosomes, isolated from the blood or CSF of PD patients suggested that extracellular vesicles (EVs) may be efficient biomarkers for PD (Vella *et al.* 2016). Shi *et al.* (2014) found that α -syn can be transported from the CSF to blood and that some is packaged into EVs expressing the CNS-specific neural cell adhesion molecule L1 (L1CAM). The authors demonstrated that CNS-derived levels of EV- α -syn in plasma were significantly higher in PD patients, with levels relating to the severity of the disease presentation, leading to the suggestion that CNS-derived EV- α -syn in plasma can be used as a biomarker for PD with high specificity and sensitivity. It was also recently reported that salivary EVs isolated from PD patients demonstrated higher absolute levels of α -syn oligomers, and also the α -syn oligomers/total α -syn ratio was increased when compared with healthy controls (Rani *et al.* 2019). Increased levels of Tau, which is a microtubule-associated protein primarily implicated in Alzheimer's disease pathogenesis, have also been detected in CNS-derived EVs isolated from human plasma, with significantly higher levels in PD patients compared with AD patients (Shi *et al.* 2016). There has also been investigation of proteins related to familial and sporadic PD in EVs. DJ-1 levels in CNS-derived EVs, and the ratio of EV-DJ-1 to total DJ-1 derived from the CNS were substantially higher in PD patient plasma compared to controls (Zhao *et al.* 2018). Fraser *et al.* (2016) investigated whether the levels of auto-phosphorylated Ser(P)-1292 LRRK2 in urine EVs could predict LRRK2 mutation carriers and non-carriers with or without PD; the authors found that these levels could predict LRRK2 mutation status using the elevated ratio of Ser(P)-1292 LRRK2 to total LRRK2 in urine EVs. Furthermore, patients with PD demonstrated a higher ratio than LRRK2 carriers without PD. This study utilised 79 idiopathic PD patients and 79 healthy controls, identifying higher Ser(P)-1292 LRRK2 in men than women, and increased Ser(P)-1292 LRRK2 in idiopathic PD patients in contrast to the healthy controls. Ser(P)-1292 LRRK2 levels were also shown to relate to the severity of cognitive impairment and may therefore serve as a useful biomarker for both familial and idiopathic PD, providing an indication as to the severity of anticipated cognitive symptoms.

The identification of increased levels of specific miRNAs in serum EVs from PD patients compared to controls (Cao *et al.* 2017) suggests that monitoring of miRNA in EVs could serve as useful tools in the diagnosis of PD. MicroRNAs, as discussed in the previous section, are known post-transcriptional regulators which can be transported intercellularly by exosomes. They inhibit via sequence-specific targeting of the 3' untranslated region of mRNAs, regulating cell proliferation, cell differentiation and apoptosis (Chang *et al.* 2016). Abnormal miRNA expression is linked to major features and risk factors of PD,

including α -syn overexpression, LB formation and neuronal apoptosis (Doxakis 2010; Leggio *et al.* 2017; McMillan *et al.* 2017; Wang *et al.* 2016). Gui *et al.* (2015) established a profiling method for exosomal miRNAs, which were differentially expressed in exosomes derived from CSF of healthy vs PD patients; data suggested 11 downregulated miRNAs and 16 upregulated miRNAs in PD, with further analysis identifying significantly up or downregulation in 6 miRNAs. A further 24 exosomal miRNAs were evaluated in serum samples from patients with PD, confirming some (such as miRNA19b, miRNA195 and miRNA24) as potential candidates for PD diagnosis with specificity and sensitivity to distinguish PD patients from healthy individuals using exosomal miRNAs (Cao *et al.* 2017). Prediction of gene-target sites of three exosomal miRNAs using the Targetscan tool found LRRK2-miRNA19b and ATP13A2-miRNA24/miRNA195 correlated with neurodegenerative processes in PD (Cao *et al.* 2017; Heman-Ackah *et al.* 2013). This work revealed the potential of miRNAs to act as biomarkers for PD diagnosis in the early stages of the disease, which could allow for improved monitoring and earlier treatment interventions. Further work is needed to investigate how miRNAs could be monitored to deliver therapeutic nucleic acids for regulation of abnormal expression of these miRNAs in PD pathology process.

Exosomes have also been investigated for their potential as success biomarkers in clinical trials; for example, in a single-centre Exenatide-PD trial, 60 idiopathic PD patients were randomly assigned to subcutaneous administration of 2mg exenatide or placebo once weekly for 48 weeks, followed by a 12 week drug withdrawal period. Blood samples were collected at weeks 0, 24, 48 and 60, with neuronal-derived EVs with LICAM selectively isolated for quantification of insulin signalling proteins (Athauda *et al.* 2019). Patients receiving exenatide were found to have enhanced brain insulin signalling with increased phosphorylation of insulin receptor substrate 1 (IRS-1) at 48 weeks and 60 weeks, compared with placebo controls. Furthermore, expression of downstream pathway substrates was increased, including total Akt and mTOR levels. The implication of mTOR in this drugs effects suggests it may impact autophagy flux, which is known to alter exosomal production. Changes in the levels of EV biomarkers, including IRS-1 p-S616, t-mTOR and p-mTOR S2488, correlated with changes in Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 3 scores at 48 weeks. At 60 weeks, the MDS-UPDRS part 3 scores were closely associated with changes in t-mTOR. Overall, these findings suggest that exenatide can improve MDS-UPDRS part 3 scores, which were significantly related to changes in these biomarkers in EVs (Athauda *et al.* 2019). This study also supports the use of EV biomarkers as potential markers for the evaluation of treatment responses and prognosis in clinical trials. However, despite the clinical potential of using exosomes as drug deliveral vehicles or diagnostic tools, as discussed below, there are major limitations and concerns that must be taken into consideration.

6. CONCLUSION AND FUTURE DIRECTIONS

Overall, the field of exosome research has advanced rapidly over the past decade. As illustrated in Fig. 3, because exosomes can influence gene expression and protein activity in recipient cells by carrying cargos such as proteins, mRNA and microRNA (Chistiakov & Chistiakov 2017; Valadi *et al.* 2007), they may contribute to the spread of misfolded proteins

such as α -syn. Exosomes also present potential attractive targets for novel therapeutics and diagnostic tools.

As discussed in this review, there is strong evidence to support exosomal transmission as a mechanism of α -syn propagation through the brain, implicating exosomes in the development and progression of PD neuropathology. The identified interplay between autophagic flux, α -syn levels and exosomes has provided clues as to the relationship between cellular mechanisms and Parkinson's pathology. Enhancing autophagy has proven effective at reducing α -syn transmission via exosomes, supporting the potential benefit of targeting exosomes, even indirectly, to limit α -syn transmission between cells. Whether changes in autophagic functionality impact the transmission of other exosome-encompassed cargo, such as RNAs and other proteins, remains to be established; this link will be important if exosome-contained proteins are to be utilised as PD biomarkers, as alteration of autophagy flux could otherwise impact the interpretation of biomarker data for diagnostics.

There is a strong interest in targeting exosomes as diagnostic tools for detection of encapsulated biomarkers, and as prospective vehicles for the delivery of pharmacological or genetic therapeutic agents to specific brain regions or cell types for PD treatment. However, despite the apparent advantages which exosomes present, their limitations must also be considered. For example, the procedures for isolating and purifying exosomes need to be improved and standardized. It is not uncommon that the isolated fraction of exosomes is also contaminated with other small exosome-like extracellular vesicles or proteins such as α -syn. A lack of general consensus on a universal exosome marker is another challenge. Additional research into the differential reactivity of exosomes isolated from different sources, to identify any adverse effects, should be undertaken to identify the best cellular exosome source for therapeutic application (Sarko & McKinney 2017). Additional research is required to determine how membrane markers may target exosomes to specific cell types and how the content of exosomes may confer influence over the terminal destination. Understanding how exosomes are targeted between cells may offer additional considerations for the development of exosome inhibitors to modulate disease. In addition to exosomes, other EVs such as the recently described arrestin domain containing protein 1-mediated microvesicles (Wang *et al.* 2018) should be investigated as another delivery platform for therapeutic molecules.

In summary, there has been a significant interest in exosome research in the context of PD and other neurodegenerative disorders in recent year; however, there remain major technical issues and unanswered questions in exosome regulation and pathological contributions to disease. Further investigation of exosomes and other EVs, along with refinement of experimental techniques and improvement in our ability to manipulate them for delivery of therapeutics, would advance understanding of PD pathology, treatment and diagnostic approaches.

ACKNOWLEDGEMENTS

This work was supported in part by the NIH/NIEHS grant R35-ES030523 to KT; Robert Stempel College of Public Health & Social Work, Florida International University, and Peninsula Schools of Medicine and Dentistry,

Plymouth University, UK (JP). Figures were re-drawn by Victoria Morus and Laura Hausmann in BioRender (<https://biorender.com/>) on the basis of a draft provided by the author.”

Funding: This work was supported in part by the NIH/NIEHS grant R35-ES030523 to KT; Robert Stempel College of Public Health & Social Work, Florida International University, and Peninsula Schools of Medicine and Dentistry, Plymouth University, UK (JP).

Abbreviations:

α-syn	α -synuclein
BBB	blood-brain-barrier
CSF	cerebrospinal fluid
DLB	dementia with Lewy bodies
ESCRT	endosomal sorting complex required for transport
EVs	extracellular vesicles
ILVs	intraluminal vesicles
miRNA	microRNAs
MSC	mesenchymal stem cell
MVBs	multivesicular bodies
PD	Parkinson disease
PFF	α -syn preformed fibrils
shRNAs	short hairpin RNAs
SNpc	substantia nigra pars compacta
TH	tyrosine hydroxylase
TNTs	tunnelling nanotubules

REFERENCES

- Aboutit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R and Zurzolo C. (2016) Tunneling nanotubes spread fibrillar α -synuclein by intercellular trafficking of lysosomes. *EMBO J* 35, 2120–2138. [PubMed: 27550960]
- Aboutit S and Zurzolo C. (2012) Wiring through tunneling nanotubes--from electrical signals to organelle transfer. *J Cell Sci* 125, 1089–1098. [PubMed: 22399801]
- Alvarez-Erviti L, Seow Y, Schapira AH, Gardiner C, Sargent IL, Wood MJ and Cooper MJ (2011a) Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. *Neurobiology of Disease* 42, 360–367. [PubMed: 21303699]
- Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhali S and Wood MJ (2011b) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 29, 341–345. [PubMed: 21423189]
- Arima K, Hirai S, Sunohara N, Aoto K, Izumiyama Y, Ueda K, Ikeda K and Kawai M. (1999) Cellular co-localization of phosphorylated tau- and NACP/alpha-synuclein-epitopes in lewy bodies

- in sporadic Parkinson's disease and in dementia with Lewy bodies. *Brain Res* 843, 53–61. [PubMed: 10528110]
- Arotcarena ML, Dovero S, Prigent A. et al. (2020) Bidirectional gut-to-brain and brain-to-gut propagation of synucleinopathy in non-human primates. *Brain* 143, 1462–1475. [PubMed: 32380543]
- Arotcarena ML, Teil M and Dehay B. (2019) Autophagy in Synucleinopathy: The Overwhelmed and Defective Machinery. *Cells* 8.
- Asai H, Ikezu S, Tsunoda S. et al. (2015) Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat Neurosci* 18, 1584–1593. [PubMed: 26436904]
- Athauda D, Gulyani S, Karnati HK et al. (2019) Utility of Neuronal-Derived Exosomes to Examine Molecular Mechanisms That Affect Motor Function in Patients With Parkinson Disease: A Secondary Analysis of the Exenatide-PD Trial. *JAMA Neurol* 76, 420–429. [PubMed: 30640362]
- Baietti MF, Zhang Z, Mortier E. et al. (2012) Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat Cell Biol* 14, 677–685. [PubMed: 22660413]
- Batrakova EV and Kim MS (2015) Using exosomes, naturally-equipped nanocarriers, for drug delivery. *J Control Release* 219, 396–405. [PubMed: 26241750]
- Berrone E, Corona C, Mazza M. et al. (2015) Detection of cellular prion protein in exosomes derived from ovine plasma. *J Gen Virol* 96, 3698–3702. [PubMed: 26399471]
- Bliederhaeuser C, Grozdanov V, Speidel A. et al. (2016) Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. *Acta Neuropathol* 131, 379–391. [PubMed: 26576561]
- Blott EJ and Griffiths GM (2002) Secretory lysosomes. *Nat Rev Mol Cell Biol* 3, 122–131. [PubMed: 11836514]
- Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN and Braak E. (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 24, 197–211. [PubMed: 12498954]
- Bridgeman K and Arsham T. (2017) *The Comprehensive Guide to Parkinson's Disease*.
- Brück D, Wenning GK, Stefanova N and Fellner L. (2016) Glia and alpha-synuclein in neurodegeneration: A complex interaction. *Neurobiol Dis* 85, 262–274. [PubMed: 25766679]
- Brundin P and Melki R. (2017) Prying into the Prion Hypothesis for Parkinson's Disease. *J Neurosci* 37, 9808–9818. [PubMed: 29021298]
- Cao XY, Lu JM, Zhao ZQ, Li MC, Lu T, An XS and Xue LJ (2017) MicroRNA biomarkers of Parkinson's disease in serum exosome-like microvesicles. *Neurosci Lett* 644, 94–99. [PubMed: 28223160]
- Chang C, Lang H, Geng N, Wang J, Li N and Wang X. (2013) Exosomes of BV-2 cells induced by alpha-synuclein: important mediator of neurodegeneration in PD. *Neurosci Lett* 548, 190–195. [PubMed: 23792198]
- Chang H, Yi B, Ma R, Zhang X, Zhao H and Xi Y. (2016) CRISPR/cas9, a novel genomic tool to knock down microRNA in vitro and in vivo. *Sci Rep* 6, 22312. [PubMed: 26924382]
- Chaudhuri KR, Healy DG, Schapira AH and Excellence NI f. C. (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol* 5, 235–245. [PubMed: 16488379]
- Chen Y, Gao C, Sun Q, Pan H, Huang P, Ding J and Chen S. (2017) MicroRNA-4639 Is a Regulator of DJ-1 Expression and a Potential Early Diagnostic Marker for Parkinson's Disease. *Front Aging Neurosci* 9, 232. [PubMed: 28785216]
- Cheng L, Zhao W and Hill AF (2018) Exosomes and their role in the intercellular trafficking of normal and disease associated prion proteins. *Mol Aspects Med* 60, 62–68. [PubMed: 29196098]
- Chistiakov DA and Chistiakov AA (2017) α -Synuclein-carrying extracellular vesicles in Parkinson's disease: deadly transmitters. *Acta Neurol Belg* 117, 43–51. [PubMed: 27473175]
- Chu Y, Muller S, Tavares A. et al. (2019) Intraatrial alpha-synuclein fibrils in monkeys: spreading, imaging and neuropathological changes. *Brain* 142, 3565–3579. [PubMed: 31580415]
- Cooper JM, Wiklander PB, Nordin JZ et al. (2014) Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. *Mov Disord* 29, 1476–1485. [PubMed: 25112864]

- Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, Vanderburg CR and McLean PJ (2012) Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Mol Neurodegener* 7, 42. [PubMed: 22920859]
- Delenclos M, Trendafilova T, Mahesh D, Baine AM, Moussaud S, Yan IK, Patel T and McLean PJ (2017) Investigation of Endocytic Pathways for the Internalization of Exosome-Associated Oligomeric Alpha-Synuclein. *Front Neurosci* 11, 172. [PubMed: 28424577]
- Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E and Lee SJ (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A* 106, 13010–13015. [PubMed: 19651612]
- Dickson DW, Uchikado H, Fujishiro H and Tsuboi Y. (2010) Evidence in favor of Braak staging of Parkinson's disease. *Mov Disord* 25 Suppl 1, S78–82. [PubMed: 20187227]
- Didiot MC, Hall LM, Coles AH et al. (2016) Exosome-mediated Delivery of Hydrophobically Modified siRNA for Huntingtin mRNA Silencing. *Mol Ther* 24, 1836–1847. [PubMed: 27506293]
- Dorsey ER, Constantinescu R, Thompson JP et al. (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* 68, 384–386. [PubMed: 17082464]
- Dorsey ER, Sherer T, Okun MS and Bloem BR (2018) The Emerging Evidence of the Parkinson Pandemic. *J Parkinsons Dis* 8, S3–s8. [PubMed: 30584159]
- Doxakis E. (2010) Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153. *J Biol Chem* 285, 12726–12734. [PubMed: 20106983]
- Drolet RE, Cannon JR, Montero L and Greenamyre JT (2009) Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. *Neurobiology of Disease* 36, 96–102. [PubMed: 19595768]
- Du D, Hu L, Wu J. et al. (2017) Neuroinflammation contributes to autophagy flux blockage in the neurons of rostral ventrolateral medulla in stress-induced hypertension rats. *J Neuroinflammation* 14, 169. [PubMed: 28835252]
- Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, Stefanis L and Vekrellis K. (2010) Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *J Neurosci* 30, 6838–6851. [PubMed: 20484626]
- Fader CM, Sánchez D, Furlán M and Colombo MI (2008) Induction of autophagy promotes fusion of multivesicular bodies with autophagic vacuoles in k562 cells. *Traffic* 9, 230–250. [PubMed: 17999726]
- Fan RZ, Guo M, Luo S, Cui M and Tieu K. (2019) Exosome release and neuropathology induced by α -synuclein: new insights into protective mechanisms of Drp1 inhibition. *Acta Neuropathol Commun* 7, 184. [PubMed: 31744532]
- Fraser KB, Moehle MS, Alcalay RN, West AB and Consortium LC (2016) Urinary LRRK2 phosphorylation predicts parkinsonian phenotypes in G2019S LRRK2 carriers. *Neurology* 86, 994–999. [PubMed: 26865512]
- Fussi N, Höllerhage M, Chakroun T, Nykänen NP, Rösler TW, Koeglsperger T, Wurst W, Behrends C and Höglinger GU (2018) Exosomal secretion of α -synuclein as protective mechanism after upstream blockage of macroautophagy. *Cell Death Dis* 9, 757. [PubMed: 29988147]
- Gitler AD, Chesi A, Geddie ML et al. (2009) Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* 41, 308–315. [PubMed: 19182805]
- Gruenberg J and van der Goot FG (2006) Mechanisms of pathogen entry through the endosomal compartments. *Nat Rev Mol Cell Biol* 7, 495–504. [PubMed: 16773132]
- Gui Y, Liu H, Zhang L, Lv W and Hu X. (2015) Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. *Oncotarget* 6, 37043–37053. [PubMed: 26497684]
- Guix FX, Corbett GT, Cha DJ et al. (2018) Detection of Aggregation-Competent Tau in Neuron-Derived Extracellular Vesicles. *Int J Mol Sci* 19.
- Guo M, Wang J, Zhao Y, Feng Y, Han S, Dong Q, Cui M and Tieu K (2020) Microglial exosomes facilitate α -synuclein transmission in Parkinson's disease. *Brain* 143, 1476–1497. [PubMed: 32355963]

- Hall S, Öhrfelt A, Constantinescu R. et al. (2012) Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. *Arch Neurol* 69, 1445–1452. [PubMed: 22925882]
- Haney MJ, Klyachko NL, Zhao Y. et al. (2015) Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J Control Release* 207, 18–30. [PubMed: 25836593]
- Hansen C, Angot E, Bergström AL et al. (2011) α -Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest* 121, 715–725. [PubMed: 21245577]
- Harding C, Heuser J and Stahl P. (1983) Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J Cell Biol* 97, 329–339. [PubMed: 6309857]
- Harischandra DS, Ghaisas S, Rokad D, Zamanian M, Jin H, Anantharam V, Kimber M, Kanthasamy A and Kanthasamy AG (2018) Environmental neurotoxicant manganese regulates exosome-mediated extracellular miRNAs in cell culture model of Parkinson's disease: Relevance to α -synuclein misfolding in metal neurotoxicity. *Neurotoxicology* 64, 267–277. [PubMed: 28450057]
- Harischandra DS, Rokad D, Neal ML et al. (2019) Manganese promotes the aggregation and prion-like cell-to-cell exosomal transmission of α -synuclein. *Sci Signal* 12.
- Hawkes CH, Del Tredici K and Braak H. (2007) Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol* 33, 599–614. [PubMed: 17961138]
- Heman-Ackah SM, Hallegger M, Rao MS and Wood MJ (2013) RISC in PD: the impact of microRNAs in Parkinson's disease cellular and molecular pathogenesis. *Front Mol Neurosci* 6, 40. [PubMed: 24312000]
- Hessvik NP and Llorente A. (2018) Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci* 75, 193–208. [PubMed: 28733901]
- Hilton D, Stephens M, Kirk L, Edwards P, Potter R, Zajicek J, Broughton E, Hagan H and Carroll C. (2014) Accumulation of α -synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. *Acta Neuropathol* 127, 235–241. [PubMed: 24240814]
- Hirsch EC, Vyas S and Hunot S. (2012) Neuroinflammation in Parkinson's disease. *Parkinsonism Relat Disord* 18 Suppl 1, S210–212. [PubMed: 22166438]
- Hoban DB, Shrigley S, Mattsson B. et al. (2020) Impact of α -synuclein pathology on transplanted hESC-derived dopaminergic neurons in a humanized α -synuclein rat model of PD. *Proc Natl Acad Sci U S A* 117, 15209–15220. [PubMed: 32541058]
- Hong Z, Shi M, Chung KA et al. (2010) DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain* 133, 713–726. [PubMed: 20157014]
- Izco M, Blesa J, Schlee M. et al. (2019) Systemic Exosomal Delivery of shRNA Minicircles Prevents Parkinsonian Pathology. *Mol Ther* 27, 2111–2122. [PubMed: 31501034]
- Kalluri R and LeBleu VS (2020) The biology. *Science* 367.
- Kang JH, Irwin DJ, Chen-Plotkin AS et al. (2013) Association of cerebrospinal fluid β -amyloid 1–42, T-tau, P-tau181, and α -synuclein levels with clinical features of drug-naïve patients with early Parkinson disease. *JAMA Neurol* 70, 1277–1287. [PubMed: 23979011]
- Killing BA, Madaj Z, Sikora JW et al. (2018) The vermiform appendix impacts the risk of developing Parkinson's disease. *Sci Transl Med* 10.
- Kim C, Ho DH, Suk JE et al. (2013) Neuron-released oligomeric α -synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun* 4, 1562. [PubMed: 23463005]
- Kim S, Kwon SH, Kam TI et al. (2019) Transneuronal Propagation of Pathologic α -Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* 103, 627–641.e627.
- Klingelhoefer L and Reichmann H. (2015) Pathogenesis of Parkinson disease—the gut-brain axis and environmental factors. *Nat Rev Neurol* 11, 625–636. [PubMed: 26503923]
- Kojima R, Bojar D, Rizzi G, Hamri GC, El-Baba MD, Saxena P, Ausländer S, Tan KR and Fussenegger M. (2018) Designer exosomes produced by implanted cells intracerebrally deliver therapeutic cargo for Parkinson's disease treatment. *Nat Commun* 9, 1305. [PubMed: 29610454]
- Kong SM, Chan BK, Park JS et al. (2014) Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes α -Synuclein externalization via exosomes. *Hum Mol Genet* 23, 2816–2833. [PubMed: 24603074]

- Kordower JH, Chu Y, Hauser RA, Freeman TB and Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 14, 504–506. [PubMed: 18391962]
- Kordower JH, Dodiya HB, Kordower AM, Terpstra B, Paumier K, Madhavan L, Sortwell C, Steece-Collier K and Collier TJ (2011) Transfer of host-derived α synuclein to grafted dopaminergic neurons in rat. *Neurobiol Dis* 43, 552–557. [PubMed: 21600984]
- Lai CP and Breakefield XO (2012) Role of exosomes/microvesicles in the nervous system and use in emerging therapies. *Front Physiol* 3, 228. [PubMed: 22754538]
- Lee HJ, Cho ED, Lee KW, Kim JH, Cho SG and Lee SJ (2013) Autophagic failure promotes the exocytosis and intercellular transfer of α -synuclein. *Exp Mol Med* 45, e22.
- Lee HJ, Patel S and Lee SJ (2005) Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci* 25, 6016–6024. [PubMed: 15976091]
- Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, Hwang D, Masliah E and Lee SJ (2010) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J Biol Chem* 285, 9262–9272. [PubMed: 20071342]
- Leggio L, Vivarelli S, L'Episcopo F, Tirolo C, Caniglia S, Testa N, Marchetti B and Iraci N. (2017) microRNAs in Parkinson's Disease: From Pathogenesis to Novel Diagnostic and Therapeutic Approaches. *Int J Mol Sci* 18.
- Lettau M, Schmidt H, Kabelitz D and Janssen O. (2007) Secretory lysosomes and their cargo in T and NK cells. *Immunol Lett* 108, 10–19. [PubMed: 17097742]
- Li JY, Englund E, Holton JL et al. (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 14, 501–503. [PubMed: 18391963]
- Li Z, Liu F, He X, Yang X, Shan F and Feng J. (2019) Exosomes derived from mesenchymal stem cells attenuate inflammation and demyelination of the central nervous system in EAE rats by regulating the polarization of microglia. *Int Immunopharmacol* 67, 268–280. [PubMed: 30572251]
- Logan T, Bendor J, Toupin C, Thorn K and Edwards RH (2017) α -Synuclein promotes dilation of the exocytotic fusion pore. *Nat Neurosci* 20, 681–689. [PubMed: 28288128]
- Loria F, Vargas JY, Bousset L, Syan S, Salles A, Melki R and Zurzolo C. (2017) α -Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. *Acta Neuropathol* 134, 789–808. [PubMed: 28725967]
- Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ and Lee VM (2012) Pathological α -synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 338, 949–953. [PubMed: 23161999]
- Maheshwari R, Tekade M, Gondaliya P, Kalia K, D'Emanuele A and Tekade RK (2017) Recent advances in exosome-based nanovehicles as RNA interference therapeutic carriers. *Nanomedicine (Lond)* 12, 2653–2675. [PubMed: 28960165]
- Marras C, Beck JC, Bower JH et al. (2018) Prevalence of Parkinson's disease across North America. *NPJ Parkinsons Dis* 4, 21. [PubMed: 30003140]
- Masracchia C, Hnida M, Gerhardt E. et al. (2018) Membrane binding, internalization, and sorting of alpha-synuclein in the cell. *Acta Neuropathol Commun* 6, 79. [PubMed: 30107856]
- McMillan KJ, Murray TK, Bengoa-Vergniory N. et al. (2017) Loss of MicroRNA-7 Regulation Leads to α -Synuclein Accumulation and Dopaminergic Neuronal Loss In Vivo. *Mol Ther* 25, 2404–2414. [PubMed: 28927576]
- Medina DL, Fraldi A, Bouche V. et al. (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev Cell* 21, 421–430. [PubMed: 21889421]
- Mezey E, Dehejia A, Harta G, Papp MI, Polymeropoulos MH and Brownstein MJ (1998) Alpha synuclein in neurodegenerative disorders: murderer or accomplice? *Nat Med* 4, 755–757. [PubMed: 9662355]
- Mianehsaz E, Mirzaei HR, Mahjoubin-Tehran M, Rezaee A, Sahebhasagh R, Pourhanifeh MH, Mirzaei H and Hamblin MR (2019) Mesenchymal stem cell-derived exosomes: a new therapeutic approach to osteoarthritis? *Stem Cell Res Ther* 10, 340. [PubMed: 31753036]
- Minakaki G, Menges S, Kittel A. et al. (2018) Autophagy inhibition promotes SNCA/alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophagosome-exosome-like phenotype. *Autophagy* 14, 98–119. [PubMed: 29198173]

- Mollenhauer B, Trautmann E, Taylor P, Manninger P, Sixel-Döring F, Ebentheuer J, Trenkwalder C and Schlossmacher MG (2013) Total CSF α -synuclein is lower in de novo Parkinson patients than in healthy subjects. *Neurosci Lett* 532, 44–48. [PubMed: 23149132]
- Mor DE, Ugras SE, Daniels MJ and Ischiropoulos H. (2016) Dynamic structural flexibility of α -synuclein. *Neurobiol Dis* 88, 66–74. [PubMed: 26747212]
- Ngolab J, Trinh I, Rockenstein E. et al. (2017) Brain-derived exosomes from dementia with Lewy bodies propagate α -synuclein pathology. *Acta Neuropathol Commun* 5, 46. [PubMed: 28599681]
- Nonaka T, Watanabe ST, Iwatsubo T and Hasegawa M. (2010) Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases. *J Biol Chem* 285, 34885–34898. [PubMed: 20805224]
- Pan BT and Johnstone RM (1983) Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 33, 967–978. [PubMed: 6307529]
- Pardridge WM and Boado RJ (2012) Reengineering biopharmaceuticals for targeted delivery across the blood-brain barrier. *Methods Enzymol* 503, 269–292. [PubMed: 22230573]
- Parkkinen L, Pirttilä T and Alafuzoff I. (2008) Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance. *Acta Neuropathol* 115, 399–407. [PubMed: 18297293]
- Peng C, Trojanowski JQ and Lee VM (2020) Protein transmission in neurodegenerative disease. *Nat Rev Neurol* 16, 199–212. [PubMed: 32203399]
- Pérez M, Avila J and Hernández F. (2019) Propagation of Tau via Extracellular Vesicles. *Front Neurosci* 13, 698. [PubMed: 31312118]
- Poehler AM, Xiang W, Spitzer P. et al. (2014) Autophagy modulates SNCA/ α -synuclein release, thereby generating a hostile microenvironment. *Autophagy* 10, 2171–2192. [PubMed: 25484190]
- Polymeropoulos MH, Lavedan C, Leroy E. et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276, 2045–2047. [PubMed: 9197268]
- Postuma RB, Berg D, Stern M. et al. (2015) MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 30, 1591–1601. [PubMed: 26474316]
- Qu M, Lin Q, Huang L. et al. (2018) Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. *J Control Release* 287, 156–166. [PubMed: 30165139]
- Rani K, Mukherjee R, Singh E. et al. (2019) Neuronal exosomes in saliva of Parkinson's disease patients: A pilot study. *Parkinsonism Relat Disord* 67, 21–23. [PubMed: 31621600]
- Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ and Geuze HJ (1996) B lymphocytes secrete antigen-presenting vesicles. *J Exp Med* 183, 1161–1172. [PubMed: 8642258]
- Rappold PM and Tieu K. (2010) Astrocytes and therapeutics for Parkinson's disease. *Neurotherapeutics* 7, 413–423. [PubMed: 20880505]
- Rey NL, Steiner JA, Maroof N, Luk KC, Madaj Z, Trojanowski JQ, Lee VM and Brundin P. (2016) Widespread transneuronal propagation of α -synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. *J Exp Med* 213, 1759–1778. [PubMed: 27503075]
- Rodriguez L, Marano MM and Tandon A. (2018) Import and Export of Misfolded α -Synuclein. *Front Neurosci* 12, 344. [PubMed: 29875627]
- Rostami J, Holmqvist S, Lindström V, Sigvardson J, Westermarck GT, Ingelsson M, Bergström J, Roybon L and Erlandsson A. (2017) Human Astrocytes Transfer Aggregated Alpha-Synuclein via Tunneling Nanotubes. *J Neurosci* 37, 11835–11853. [PubMed: 29089438]
- Rustom A, Saffrich R, Markovic I, Walther P and Gerdes HH (2004) Nanotubular highways for intercellular organelle transport. *Science* 303, 1007–1010. [PubMed: 14963329]
- Sacino AN, Brooks M, Thomas MA et al. (2014) Intramuscular injection of α -synuclein induces CNS α -synuclein pathology and a rapid-onset motor phenotype in transgenic mice. *Proc Natl Acad Sci U S A* 111, 10732–10737. [PubMed: 25002524]
- Sargent D, Verchère J, Lazizzera C, Gaillard D, Lakhdar L, Streichenberger N, Morignat E, Bétemps D and Baron T. (2017) 'Prion-like' propagation of the synucleinopathy of M83 transgenic mice depends on the mouse genotype and type of inoculum. *J Neurochem* 143, 126–135. [PubMed: 28771723]

- Sarkar S, Dammer EB, Malovic E. et al. (2020) Molecular Signatures of Neuroinflammation Induced by α -Synuclein Aggregates in Microglial Cells. *Front Immunol* 11, 33. [PubMed: 32082315]
- Sarko DK and McKinney CE (2017) Exosomes: Origins and Therapeutic Potential for Neurodegenerative Disease. *Front Neurosci* 11, 82. [PubMed: 28289371]
- Schrag A, Zhelev SS, Hotham S, Merritt RD, Khan K and Graham L. (2019) Heterogeneity in progression of prodromal features in Parkinson's disease. *Parkinsonism Relat Disord*.
- Shi M, Kovac A, Korff A. et al. (2016) CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. *Alzheimers Dement* 12, 1125–1131. [PubMed: 27234211]
- Shi M, Liu C, Cook TJ et al. (2014) Plasma exosomal α -synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta Neuropathol* 128, 639–650. [PubMed: 24997849]
- Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R and Goedert M. (1997) α -Synuclein in Lewy bodies. *Nature* 388, 839–840. [PubMed: 9278044]
- Stuendl A, Kunadt M, Kruse N, Bartels C, Moebius W, Danzer KM, Mollenhauer B and Schneider A. (2016) Induction of α -synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain* 139, 481–494. [PubMed: 26647156]
- Surmeier DJ, Obeso JA and Halliday GM (2017) Parkinson's Disease Is Not Simply a Prion Disorder. *J Neurosci* 37, 9799–9807. [PubMed: 29021297]
- Tanik SA, Schultheiss CE, Volpicelli-Daley LA, Brunden KR and Lee VM (2013) Lewy body-like α -synuclein aggregates resist degradation and impair macroautophagy. *J Biol Chem* 288, 15194–15210. [PubMed: 23532841]
- Théry C, Witwer KW, Aikawa E. et al. (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 7, 1535750.
- Tsunemi T, Hamada K and Krainc D. (2014) ATP13A2/PARK9 regulates secretion of exosomes and α -synuclein. *J Neurosci* 34, 15281–15287. [PubMed: 25392495]
- Tsunemi T, Perez-Rosello T, Ishiguro Y. et al. (2019) Increased Lysosomal Exocytosis Induced by Lysosomal Ca. *J Neurosci* 39, 5760–5772. [PubMed: 31097622]
- Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ and Lötvalld JO (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 9, 654–659. [PubMed: 17486113]
- Vella LJ, Hill AF and Cheng L. (2016) Focus on Extracellular Vesicles: Exosomes and Their Role in Protein Trafficking and Biomarker Potential in Alzheimer's and Parkinson's Disease. *Int J Mol Sci* 17, 173. [PubMed: 26861304]
- Vella LJ, Sharples RA, Lawson VA, Masters CL, Cappai R and Hill AF (2007) Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *J Pathol* 211, 582–590. [PubMed: 17334982]
- Vella LJ, Sharples RA, Nisbet RM, Cappai R and Hill AF (2008) The role of exosomes in the processing of proteins associated with neurodegenerative diseases. *Eur Biophys J* 37, 323–332. [PubMed: 18064447]
- Vilaça-Faria H, Salgado AJ and Teixeira FG (2019) Mesenchymal Stem Cells-derived Exosomes: A New Possible Therapeutic Strategy for Parkinson's Disease? *Cells* 8.
- Wakabayashi K, Takahashi H, Ohama E and Ikuta F. (1990) Parkinson's disease: an immunohistochemical study of Lewy body-containing neurons in the enteric nervous system. *Acta Neuropathol* 79, 581–583. [PubMed: 1972853]
- Wang H, Ye Y, Zhu Z. et al. (2016) MiR-124 Regulates Apoptosis and Autophagy Process in MPTP Model of Parkinson's Disease by Targeting to Bim. *Brain Pathol* 26, 167–176. [PubMed: 25976060]
- Wang Q, Yu J, Kadungure T, Beyene J, Zhang H and Lu Q. (2018) ARMMs as a versatile platform for intracellular delivery of macromolecules. *Nat Commun* 9, 960. [PubMed: 29511190]
- Wollert T and Hurley JH (2010) Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* 464, 864–869. [PubMed: 20305637]
- Xu J, Camfield R and Gorski SM (2018) The interplay between exosomes and autophagy - partners in crime. *J Cell Sci* 131.

- Yáñez-Mó M, Siljander PR, Andreu Z. et al. (2015) Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 4, 27066. [PubMed: 25979354]
- Zhao ZH, Chen ZT, Zhou RL, Zhang X, Ye QY and Wang YZ (2018) Increased DJ-1 and α -Synuclein in Plasma Neural-Derived Exosomes as Potential Markers for Parkinson's Disease. *Front Aging Neurosci* 10, 438. [PubMed: 30692923]
- Zhuang X, Xiang X, Grizzle W. et al. (2011) Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther* 19, 1769–1779. [PubMed: 21915101]
- Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G and Amigorena S. (1998) Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 4, 594–600. [PubMed: 9585234]

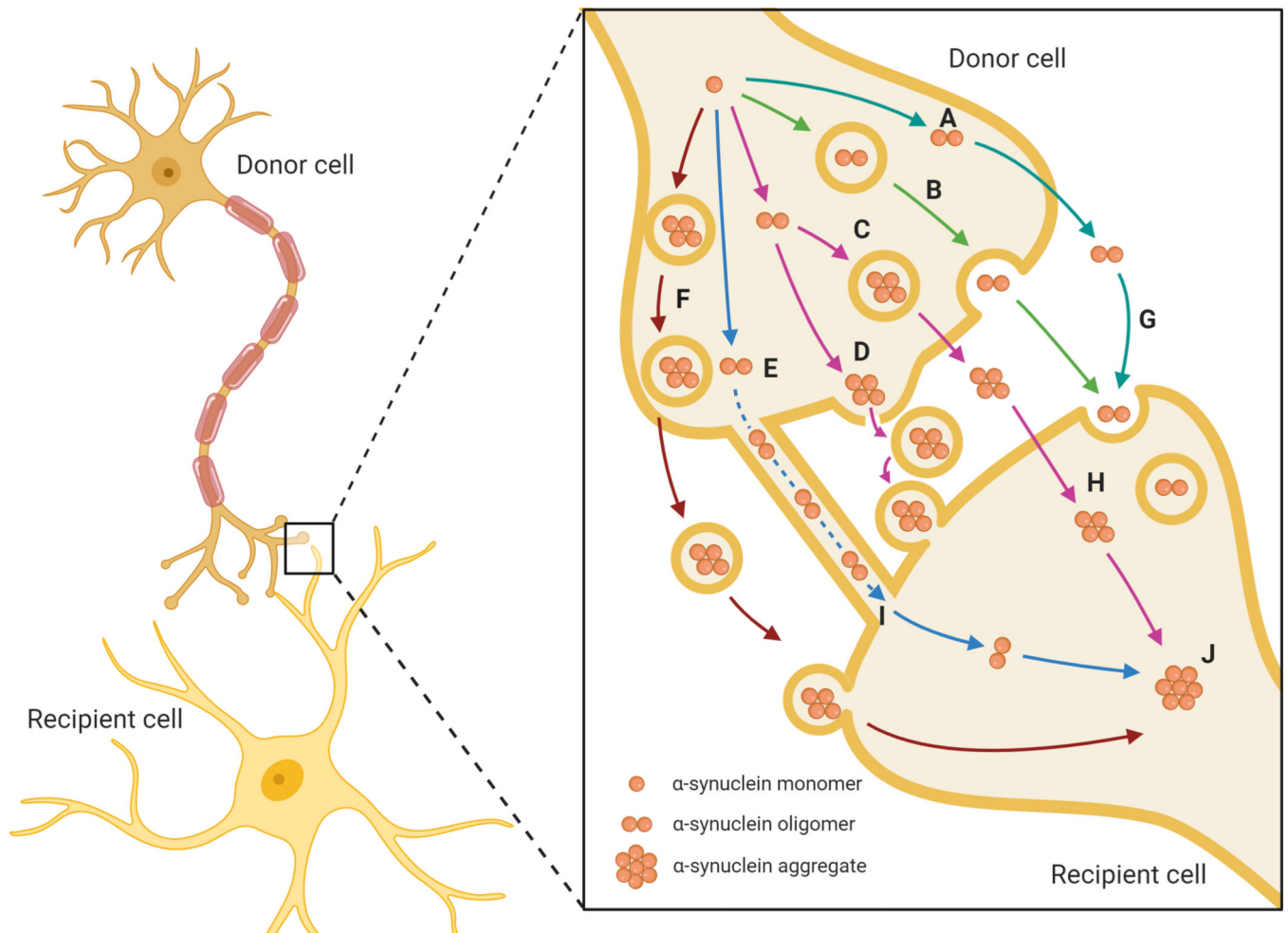


Figure 1. Proposed mechanisms of intercellular α -synuclein transmission.

Cellular mechanisms of α -synuclein transmission between cells facilitate the propagation of protein pathology in PD, whereby protein ‘seeds’ are transferred from donor cells into the extracellular space, as naked protein or in vesicles such as exosomes. These monomers and oligomers then act as seeds for protein aggregation in the recipient cells and can activate neuroinflammatory responses from glial cells. Mechanisms of transmission include the non-vesicular release of free α -synuclein (A), exocytosis (B), secretory lysosome release (C), microvesicular shedding (D), transport via tunnelling nanotubes (E) and exosomal transport (F). Uptake mechanisms include endocytosis (G), membrane perforation or pore formation (H) and exosome uptake (I), leading to α -synuclein aggregation formation (J).

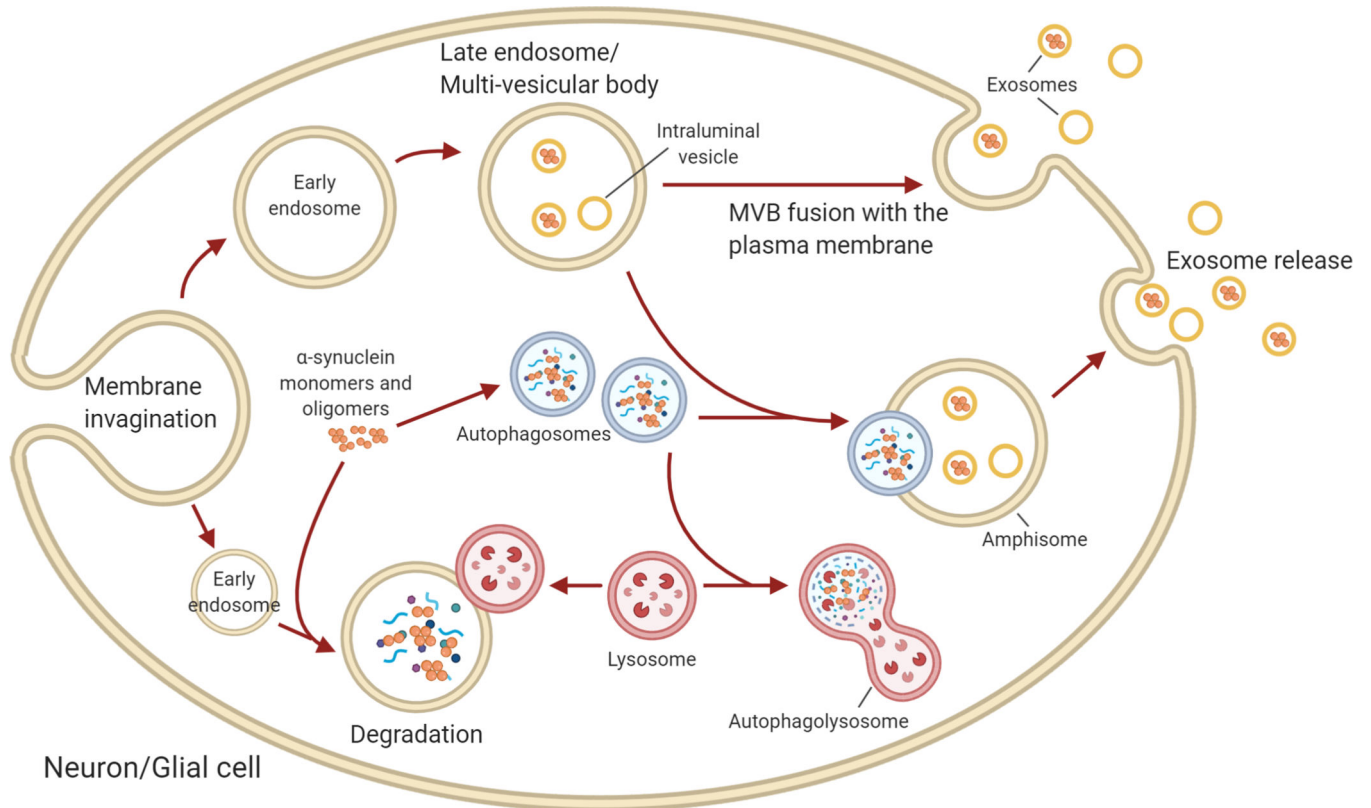


Figure 2. Intracellular vesicle trafficking and autophagy.

Intracellular α -syn can be sorted into endosomes/multi-vesicular bodies or autophagosomes. Internalisation of portions of the cellular plasma membrane leads to formation of early endosomes, in which further membrane invagination produces intraluminal vesicles (ILVs) which accumulate inside of the late endosome/multivesicular body (MVB). These late endosomes and their constituent ILVs can contain a mixture of cellular components, including genetic material and protein, such as α -syn. MVBs can fuse with the plasma membrane to release the ILVs into the extracellular space as exosomes, or they can fuse with autophagosomes to form amphisomes. Amphisomes are intermediates in autophagic processing, which are also capable of fusing with the cellular membrane to mediate exosome release. Alternatively, α -syn may be internalised to endosomes which fuse with lysosomes for degradation, or encompassed into autophagosomes and degraded by the traditional autophagic pathway following fusion with lysosomes to produce the autophagolysosome. Amphisomes can also be degraded by the lysosome (not shown). Impaired autophagy flux such as due to lysosomal dysfunction enhances exosome release from amphisomes and MVBs in attempts to reduce intracellular α -syn levels.

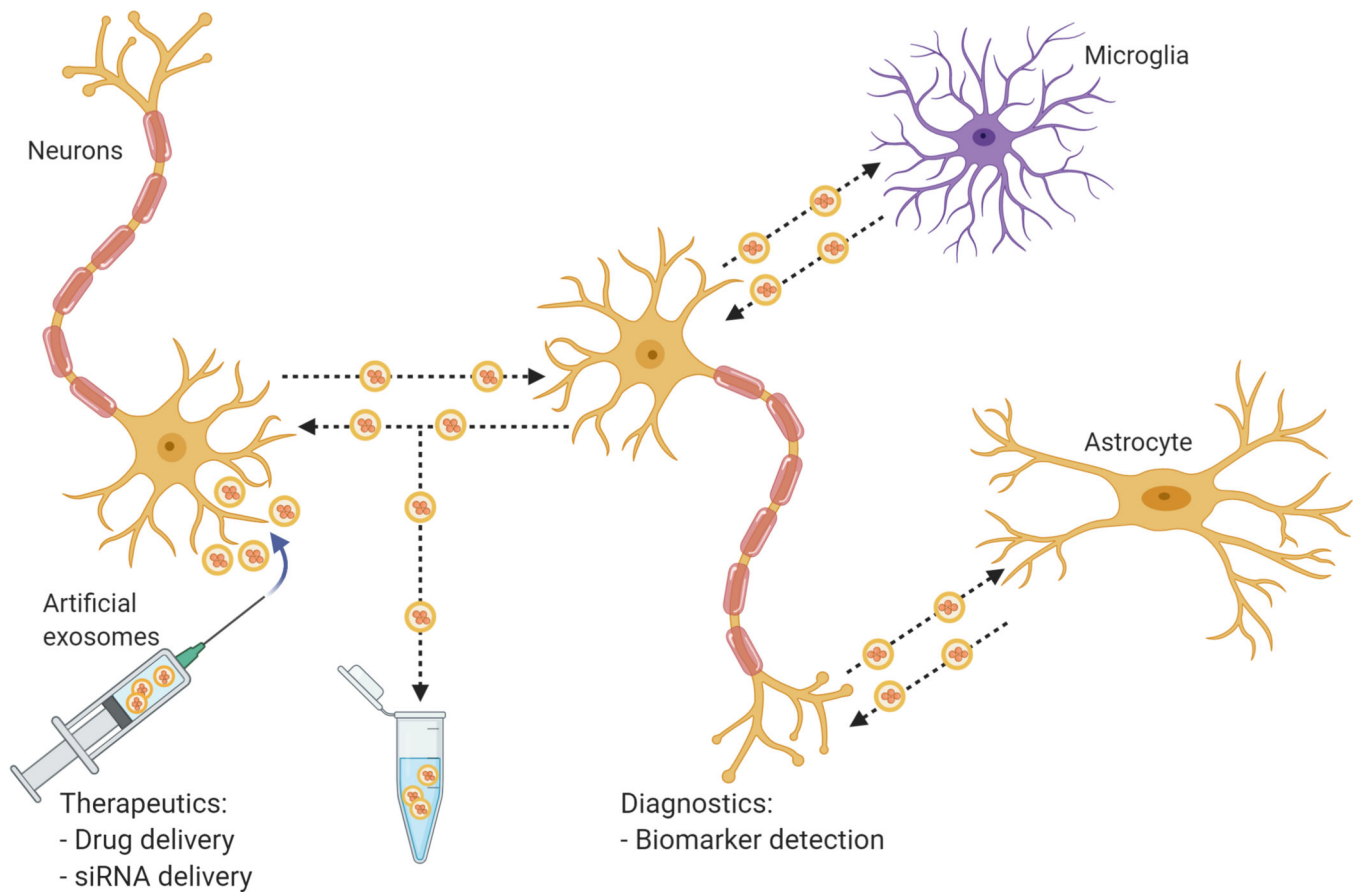


Figure 3. Potential roles of exosomes in neuropathology and clinical applications.

Exosomes can bi-directionally transfer proteins and genetic material between neurons and glial cells such as microglia and astrocytes. Such cell-cell interactions contribute to pathological progression and spread in neurodegenerative diseases such as PD. On the other hand, exosomes and other exosome-like extracellular vesicles have also been investigated as potential tools for the delivery of therapeutics and biomarkers.