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Extracellular vesicle microRNA-mediated transcriptional regulation may contribute to dementia with Lewy bodies molecular pathology

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Abstract

Objective: Dementia with Lewy bodies (DLB) is the second most common dementia. Advancing our limited understanding of its molecular pathogenesis is essential for identifying novel biomarkers and therapeutic targets for DLB. DLB is an α-synucleinopathy, and small extracellular vesicles (SEV) from people with DLB can transmit α-synuclein oligomerisation between cells. Post-mortem DLB brains and serum SEV from those with DLB share common miRNA signatures, and their functional implications are uncertain. Hence, we aimed to investigate potential targets of DLB-associated SEV miRNA and to analyse their functional implications. *Methods:* We identified potential targets of six previously reported differentially expressed miRNA genes in serum SEV of people with DLB (*MIR26A1*, *MIR320C2*, *MIR320D2*, *MIR548BA*, *MIR556*, and *MIR4722*) using *miRBase* and *miRDB* databases. We analysed functional implications of these targets using *EnrichR* gene set enrichment analysis and analysed their protein interactions using *Reactome* pathway analysis. *Results:* These SEV miRNA may regulate 4278 genes that were significantly enriched among the genes involved in neuronal development, cell-to-cell communication, vesicle-mediated transport, apoptosis, regulation of cell cycle, post-translational protein modifications, and autophagy lysosomal pathway, after Benjamini-Hochberg false discovery rate correction at 5%. The miRNA target genes and their protein interactions were significantly associated with several neuropsychiatric disorders and with multiple signal transduction, transcriptional regulation, and cytokine signalling pathways. *Conclusion:* Our findings provide *in-silico* evidence that potential targets of DLB-associated SEV miRNAs may contribute to Lewy pathology by transcriptional regulation. Experimental validation of these dysfunctional pathways is warranted and could lead to novel therapeutic avenues for DLB.

Significant outcomes

- Differentially expressed miRNA in serum SEV from people with DLB may contribute to the molecular pathogenesis of DLB by regulating expression of genes involved in neuronal development, cell-to-cell communication, vesicle-mediated transport, apoptosis, regulation of cell cycle, post-translational protein modifications, and autophagy lysosomal pathway.
- Potential target genes of the DLB-associated SEV miRNA were significantly enriched among the genes involved in axon guidance, focal adhesion, endocytosis, autophagy, mRNA surveillance, long-term potentiation, ubiquitin-mediated proteolysis, cholinergic and glutaminergic synapses, and neurotrophin, Wnt, mTOR, ErbB, and FoxO signalling pathways.
- Protein interactions of the potential target genes may contribute to DLB molecular pathogenesis by regulating cell cycle, protein phosphorylation, interleukin-6 signalling, transcriptional regulation, and post-transcriptional silencing by small RNA.

Limitations

- This study did not investigate the potential targets of other classes of SEV non-coding small RNA that were differentially expressed in people with DLB.
- miRNA target prediction is still an evolving scientific discipline.
- Further experimental validation of identified dysfunctional molecular processes and pathways is needed.

Introduction

Dementia with Lewy bodies (DLB) is the second most common neurodegenerative dementia (Rajkumar and Aarsland, 2020). Lewy bodies are intraneuronal eosinophilic cytoplasmic inclusion bodies. Post-mortem neuropathological examination of brains of people, who were clinically diagnosed to have DLB antemortem, often reveals Lewy bodies in brainstem, limbic system, and/or cerebral cortex (Garcia-Esparcia *et al.*, 2017). DLB is a primary α-synucleinopathy (Rajkumar and Aarsland, 2020), and α-synuclein aggregation is widely recognised as the key initial step in the formation of Lewy bodies (Beyer *et al.*, 2009). DLB causes earlier mortality (Oesterhus *et al.*, 2014), earlier nursing home admissions (Rongve *et al.*, 2014), poorer quality-of-life (Bostrom *et al.*, 2007), more frequent falls, higher care costs (Vossius *et al.*, 2014), and more caregivers' burden (Svendsboe *et al.*, 2016) than Alzheimer's disease (AD). Accurate distinction between DLB and AD is essential because they differ in their prognosis, neuropsychiatric symptoms, and pharmacological management (Watts *et al.*, 2023). Both AD and DLB are currently diagnosed by their clinical diagnostic criteria (McKeith *et al.*, 2017; WHO, 2018), and DLB is often misdiagnosed as AD or other dementia in clinical settings (Freer, 2017). Failing to diagnose DLB early, and treating visual hallucinations and challenging behaviours, which are more frequent in DLB than AD, with any antipsychotic medication risk potentially fatal adverse events like neuroleptic malignant syndrome. Reliable biomarkers from clinically collectable biological fluids (Ashton *et al.*, 2020) and safer effective treatments (Stinton *et al.*, 2015; Velayudhan *et al.*, 2017; Watts *et al.*, 2023) are urgently needed for DLB. Improving our current limited understanding of DLB molecular pathology is prerequisite for addressing this need and for improving clinical diagnosis and management of DLB (Zhang *et al.*, 2015).

Extracellular vesicles (EV) are cell-derived vesicles with lipid bilayer membrane that cannot replicate (Thery *et al.*, 2018). They are secreted into extracellular environment (Yanez-Mo *et al.*, 2015; Ibrahim and Khan, 2022), and they play an important role in communication between cells (Saint-Pol *et al.*, 2020). EV carry biologically active proteins, lipids and nucleic acids including messenger RNA (mRNA), microRNA (miRNA), other small non-coding RNA, mitochondrial DNA, and genomic DNA fragments from their cells of origin (Trotta *et al.*, 2018). Because of their potential for transferring biological information, several drug delivery systems using EV are being developed (Vader *et al.*, 2016; Mustajab *et al.*, 2022). EV are usually classified on the basis of their physical or biochemical characteristics. *The Minimal information for studies of extracellular vesicles* (MISEV2018) guidelines (Thery *et al.*, 2018) encourage using the term 'small extracellular vesicles' (SEV) for the EV that are smaller than 200 nm in size. SEV consist of heterogeneous population of EV including those called as *exosomes* (Kalluri and LeBleu, 2020).

SEV RNA cargo includes miRNA that can regulate expression of thousands of genes (Lu and Rothenberg, 2018). miRNA are approximately 22 nucleotides-long single-stranded non-coding RNA (Liu *et al.*, 2014). Several hundred nucleotides-long miRNA precursors, called pri-miRNA, are transcribed from miRNA genes usually by RNA polymerase-II. The pri-miRNA are processed first in nuclei and then in cytoplasm to generate small mature miRNA that regulate transcription of other genes (Vishnoi and Rani, 2023). miRNA exert important regulatory control over neuronal development and homeostasis (Liu *et al.*, 2014). Differentially expressed EV miRNA have been associated with neurodegenerative diseases such as Alzheimer's disease (Gamez-Valero *et al.*, 2019; Wang *et al.*, 2022), demyelinating diseases like multiple sclerosis (Ovchinnikova *et al.*, 2022), and psychiatric disorders such as schizophrenia and bipolar disorder (Du *et al.*, 2019; Gruzdev *et al.*, 2019).

SEV, separated from cerebrospinal fluid (CSF) of people with DLB, has been demonstrated to induce alpha-synuclein oligomerisation in H4 neuro-glioma cells (Stuendl *et al.*, 2016). Moreover, it has been shown that injection of brain-derived SEV from people with DLB into the brains of mice could induce α-synuclein oligomerisation (Ngolab *et al.*, 2017). As SEV may transmit disease-associated nucleic acids and proteins between cells (Candelario and Steindler, 2014), we hypothesise that differentially expressed SEV miRNA from people with DLB may contribute to DLB pathology by regulating expression of genes involved in the molecular pathways that are known to be associated with DLB. We have already reported an RNA sequencing (RNA-Seq) study that investigated differentially expressed genes (DEG) in post-mortem anterior cingulate and dorsolateral prefrontal cortices of people with DLB (Rajkumar *et al.*, 2020) and another RNA-Seq study that investigated serum SEV DEG in people living with DLB (Rajkumar *et al.*, 2021). Four miRNA genes, *MIR320C2*, *MIR320D2*, *MIR548BA* and *MIR4722*, were found significantly ($p < 0.05$) differentially expressed in both post-mortem DLB brains and serum SEV from people living with DLB (Rajkumar *et al.*, 2020; Rajkumar *et al.*, 2021). Differential expression levels of two more serum SEV miRNA DEG in DLB, *MIR26A1* and *MIR556*, could be replicated by high-throughput quantitative polymerase chain reaction (qPCR) (Rajkumar *et al.*, 2021). *MIR26A1* and *MIR556* were significantly upregulated, and other four miRNA genes were significantly downregulated in serum SEV from people living with DLB. In this study, we aimed to investigate the potential targets of the miRNA, transcribed by these six miRNA genes, and to analyse their downstream functional implications.

Material and methods

Differentially expressed serum SEV miRNA in DLB

Our prior RNA-Seq study investigated serum SEV RNA profiles in people with DLB ($n = 10$) and age- and gender-matched comparisons ($n = 10$). That study obtained the 20 serum samples from the biobanks of three Norwegian cohorts that have obtained generic ethical approval for further studies using their serum samples (Selnes *et al.*, 2013; Rongve *et al.*, 2016; Fladby *et al.*, 2017). Serum samples from 10 people living with DLB and three gender- and age (±3 years)-matched comparisons without cognitive impairment or Parkinson's disease were obtained from the dementia study of western Norway (DemWest) (Rongve *et al.*, 2016). Another seven gender- and age-matched comparison serum samples were obtained from two cohorts at Akershus University Hospital dementia research centres (Selnes *et al.*, 2013; Fladby *et al.*, 2017). 70% ($n = 14$) of the samples were from men. The mean age of the people with DLB was 77.3 (SD = 3.7) years, and the mean age of the people in comparison group was 76.8 (SD = 4.1) years. Further details of the cohorts have been published elsewhere (Selnes *et al.*, 2013; Rongve *et al.*, 2016; Fladby *et al.*, 2017).

Our prior study separated SEV using an ultracentrifugation and OptiPrep™ (Sigma-Aldrich, UK) density gradient approach based on their buoyant density. Size distribution and concentration of separated EVs were verified using the Malvern NanoSight LM10

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nanoparticle analyser (Malvern Instruments Ltd., UK). Separation of SEVs was confirmed by Western blotting using antibodies against Flotillin-1 and CD63. Total RNA was isolated from SEV, and RNA samples were sequenced using the Illumina Hiseq-2500. We identified DEG using a previously experimentally validated *edgeR* algorithm (Anders *et al.*, 2013; Rajkumar *et al.*, 2015). Further details of this study have been published elsewhere (Rajkumar *et al.*, 2021). Those RNA-Seq raw data files are available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA530121.

MiRNA target discovery

We first identified the mature miRNA sequences, transcribed by the six differentially expressed miRNA genes (*MIR26A1*, *MIR320C2*, *MIR320D2*, *MIR548BA*, *MIR556*, and *MIR4722*) in DLB serum SEV, using the *miRbase* database (Liu *et al.*, 2014). '*MiRbase*' is a comprehensive database for miRNA sequences and annotations (Kozomara *et al.*, 2019). Expression of these six miRNA genes leads to nine mature miRNA sequences (hsa-miR-26a-5p, hsa-miR-26a-1-3p, hsa-miR-320c, hsa-miR-320d, hsa-mir-548ba, hsa-miR-556-5p, hsa-miR-556-3p, hsa-miR-4722-5p, and hsa-miR-4722-3p). We next identified potential targets of these miRNA using the *MiRDB* miRNA target prediction database (Liu and Wang, 2019). *MiRDB* analyses miRNA-target interactions using *MirTarget2* bioinformatics tool and data derived from high-throughput RNA sequencing experiments (Liu and Wang, 2019; Chen and Wang, 2020). *miRTarget2* can perform genome-wide miRNA target prediction using support vector machine framework (Benson *et al.*, 2007; Maglott *et al.*, 2007; Wang and El Naqa, 2008; Chen *et al.*, 2013). We combined all potential target genes of these miRNA and removed duplicates (Supplementary information-1).

Functional enrichment analyses

We employed the *EnrichR* functional enrichment analysis tool and identified the biological processes, molecular pathways, and disease processes that were significantly enriched among the potential target genes of these miRNA (Chen *et al.*, 2013; Kuleshov *et al.*, 2016). We repeated the *EnrichR* analyses for understanding the functional implications of the potential target genes of each miRNA gene of interest individually. *EnrichR* considers at least 204 gene set libraries that are constructed from prior published studies and major biological and biomedical online databases including *BioCarta*, the database of Genotypes and Phenotypes (dbGaP), *DisGeNET* (Pinero *et al.*, 2021), GeneSigDB (Culhane *et al.*, 2012), Online Mendelian Inheritance in Man (OMIM), and UK Biobank genome-wide association study *EnrichR* includes Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway information (Kanehisa and Goto, 2000; Kanehisa *et al.*, 2021). *EnrichR* calculates its p-values using Fisher's exact test and employs appropriate corrections for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) correction at 5% (q-values) (Benjamini and Hochberg, 1995).

Protein interactions of potential target genes

Interactome implies the total molecular interactions that occur within a cell (Yan *et al.*, 2018), and interactome analyses map these networks. Interactome analyses help improving our understanding of molecular pathways involved in neurodegenerative diseases (Haenig *et al.*, 2020). First, we identified significantly enriched molecular interaction pathways of the potential miRNA target genes using the *Reactome* pathway database (Fabregat *et al.*, 2017). We then expanded the *Reactome* enrichment analyses including all relevant protein-protein interactions from the *IntAct* protein interaction analysis database (Hermjakob *et al.*, 2004). Reactome calculates its p-values using over-representation analysis and hypergeometric distribution. It employs the Benjamini-Hochberg FDR correction at 5% (Benjamini and Hochberg, 1995).

Results

Biological processes

We identified 4278 potential target genes of these miRNA (Supplementary information-1). These potential target genes significantly (Fisher's exact test $p < 0.0001$) overlapped with the previously reported post-mortem DLB brain DEG (Rajkumar *et al.*, 2020) and with serum SEV DEG in people living with DLB (Rajkumar *et al.*, 2021). The potential target genes were significantly ($q < 0.05$) enriched among the genes associated with 62 biological processes after Benjamini-Hochberg FDR correction at 5% (Table 1; Supplementary information-2). The enriched biological processes clustered around communication between cells, post-translational protein modification, apoptosis, neuronal development, transcriptional regulation, regulation of cell cycle, autophagy lysosomal pathway, vesicle-mediated transport, plasma membrane organisation, and cellular macromolecule biosynthesis. The potential target genes were significantly enriched among the genes associated with several transcriptional regulation processes including regulation of transcription from RNA polymerase-II promoter ($q = 7.31 \times 10^{-8}$), negative regulation of gene expression ($q = 1.50 \times 10^{-6}$), regulation of DNA-templated transcription ($q = 1.55 \times 10^{-6}$), and regulation of gene expression ($q = 9.80 \times 10^{-5}$). Significantly enriched biological processes included apoptosis-associated regulation of apoptotic process ($q = 1.59 \times 10^{-5}$), negative regulation of programmed cell death ($q = 5.30 \times 10^{-4}$) and negative regulation of apoptotic process ($q = 0.0017$), as well as autophagy lysosomal pathway associated lysosomal transport ($q = 0.0196$) and negative regulation of autophagy ($q = 0.0065$) processes. Supplementary information-2 provides further details of all 62 significantly enriched biological processes with their gene ontology (GO) numbers, p-values, FDR-adjusted q-values, odds ratios, and overlapping genes.

Molecular functions

The potential target genes of these miRNA were significantly enriched among the genes associated with 35 molecular functions after Benjamini-Hochberg FDR correction at 5% (Supplementary information-2). These enriched molecular functions clustered around transcriptional regulation, protein phosphorylation, ubiquitin protease system (UPS), and histone acetylation. The potential target genes were significantly enriched among the genes associated with several molecular functions regulating transcription such as transcription coactivator activity ($q = 3.34 \times 10^{-5}$), transcription regulatory region DNA binding ($q = 0.0024$), RNA polymerase-II core promoter proximal region sequence-specific binding ($q = 0.0240$) transcription factor ($q = 0.0124$) and transcriptional activator ($q = 0.0139$) activities, activating transcription factor binding ($q = 0.0134$), RNA polymerase-II transcription cofactor activity ($q = 0.0329$), and transcription corepressor activity ($q = 0.0439$). The potential SEV miRNA target genes were significantly enriched among the genes involved in the UPS-related

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Table 1. Biological processes that were enriched among the potential target genes of differentially expressed small extracellular vesicle miRNA in people with dementia with Lewy bodies

Biological processes	GO numbers of the enriched biological processes and their adjusted* p-values
Cell-to-cell communication	GO:0009967 (2.99×10^{-3}); GO:0007223 (4.77×10^{-3}); GO:0007265 (5.89×10^{-3}); GO:0046626 (1.94×10^{-2}); GO:0038127 (2.6×10^{-2}); GO:1902531 (4.03×10^{-2}); GO:0070102 (4.99×10^{-2})
Post-translational protein modification	GO:0006468 (6.28×10^{-8}); GO:0016310 (3.22×10^{-5}); GO:0046777 (2.28×10^{-3}); GO:0018105 (5.25×10^{-3}); GO:0018209 (8.56×10^{-3}); GO:0016579 (9.08×10^{-3}); GO:0006464 (9.08×10^{-3}); GO:0070646 (9.10×10^{-3}); GO:0033673 (2.95×10^{-2})
Apoptosis	GO:0042981 (1.59×10^{-5}); GO:0043069 (5.3×10^{-4}); GO:0043066 (1.67×10^{-3})
Neuronal development	GO:0007399 (4.7×10^{-6}); GO:0048666 (6.74×10^{-5}); GO:0031175 (3.37×10^{-7}); GO:0050773 (6.12×10^{-4}); GO:0048485 (1.37×10^{-3}); GO:0007411 (2.63×10^{-3}); GO:0021953 (2.88×10^{-3}); GO:0048812 (3.22×10^{-3}); GO:0048699 (4.77×10^{-3}); GO:0007409 (5.89×10^{-3}); GO:0001764 (6.44×10^{-3}); GO:0007416 (2.49×10^{-2}); GO:0030182 (2.07×10^{-2}); GO:1900006 (2.71×10^{-2}); GO:0061549 (3.95×10^{-2})
Regulation of Gene expression	GO:0006357 (7.31×10^{-8}); GO:0010629 (1.50×10^{-6}); GO:0045893 (1.55×10^{-6}); GO:0006355 (1.55×10^{-6}); GO:0045892 (2.42×10^{-6}); GO:0000122 (3.22×10^{-5}); GO:0045944 (3.28×10^{-5}); GO:1903506 (4.77×10^{-3}); GO:1903508 (9.48×10^{-5}); GO:0010468 (9.8×10^{-5}); GO:0010628 (1.27×10^{-4}); GO:1903507 (3.96×10^{-4}); GO:0006366 (3.27×10^{-3})
Regulation of cell cycle	GO:2000045 (6.74×10^{-5}); GO:2000134 (5.30×10^{-4}); GO:1902807 (3.27×10^{-3}); GO:0051726 (5.68×10^{-3})
Autophagy lysosomal pathway	GO:0010507 (6.49×10^{-3}); GO:0007041 (1.96×10^{-2})
Vesicle-mediated transport	GO:0016192 (4.77×10^{-3})
Plasma membrane organisation	GO:0120036 (1.87×10^{-3}); GO:0120039 (6.44×10^{-3}); GO:1903076 (1.25×10^{-2})
Cellular macromolecule biosynthesis	GO:2000113 (1.50×10^{-6}); GO:2000112 (6.12×10^{-4})

GO: Gene Ontology.
*Benjamini-Hochberg false discovery rate at 5%.

molecular functions, ubiquitin-protein transferase activity ($q = 0.0035$), and ubiquitin-protein ligase activity ($q = 0.0342$), that have been known to be associated with DLB pathology (Chowdhury and Rajkumar, 2020). Significantly enriched molecular functions included histone acetyltransferase activity ($q = 0.0188$) and several protein phosphorylation regulating functions such as protein serine/threonine kinase activity ($q = 8.65 \times 10^{-7}$), protein kinase activity ($q = 3.17 \times 10^{-6}$), protein kinase binding ($q = 4.30 \times 10^{-4}$), and protein kinase A regulatory subunit binding ($q = 0.0044$). Supplementary information-2 provides further details of all 35 significantly enriched molecular functions with their GO numbers, p-values, FDR-adjusted q-values, odds ratios, and overlapping genes.

Enriched molecular pathways

The potential target genes were significantly enriched for 59 KEGG pathways after Benjamini-Hochberg FDR correction at 5% (Table 2; Supplementary information-2). These enriched molecular pathways clustered around neuronal development, cell signalling, synaptic activity, programmed cell death, autophagy, UPS, and vesicle-mediated transport. They included axon guidance ($q = 1.69 \times 10^{-6}$), focal adhesion ($q = 5.08 \times 10^{-4}$), autophagy ($q = 8.78 \times 10^{-4}$), mRNA surveillance ($q = 0.0016$), long-term potentiation ($q = 0.0053$), ubiquitin-mediated proteolysis ($q = 0.0283$), cholinergic ($q = 0.0493$) and glutaminergic ($q = 0.0283$) synapses, ferroptosis ($q = 0.0350$), endocytosis ($q = 0.0493$), as well as Wnt ($q = 3.22 \times 10^{-4}$), mTOR ($q = 5.08 \times 10^{-4}$), ErbB ($q = 0.0018$), FoxO ($q = 0.0025$), and neurotrophin ($q = 0.0076$) signalling pathways. Further details of all 59 significantly enriched KEGG pathways with their pathway (hsa) numbers, p-values, FDR-adjusted q-values, odds ratios, and overlapping genes are presented in the supplementary information-2.

Disease processes

We assessed how the potential target genes were related to the genes that are known to be associated with specific disease processes in OMIM and *DisGeNET* (Pinero *et al.*, 2021) databases. The potential target genes were significantly enriched among the genes that are associated with 74 disease processes in the *DisGeNET* after Benjamini-Hochberg FDR correction at 5% (Supplementary information-2). These enriched disease processes included several neurodevelopmental disorders such as intellectual disability ($q = 7.58 \times 10^{-8}$), delayed speech and language development ($q = 1.47 \times 10^{-5}$), and autism ($q = 1.47 \times 10^{-5}$), as well as neuropsychiatric disorders such as schizophrenia ($q = 7.23 \times 10^{-4}$), speech impairment ($q = 6.37 \times 10^{-5}$), epilepsy ($q = 7.49 \times 10^{-4}$), cognitive disorders ($q = 0.0192$), bipolar disorder ($q = 0.0353$), and impaired cognition ($q = 0.0446$). Besides, the potential target genes were significantly ($p < 0.05$) enriched only among the genes associated with encephalopathy ($p = 0.0037$) in OMIM database, and this enrichment was not statistically significant after FDR correction (Supplementary information-2).

Protein interactions

We assessed the protein interaction pathways of the potential miRNA target genes using the *Reactome* pathway database (Fabregat *et al.*, 2017). The potential target genes were significantly ($p < 0.05$) overrepresented among 13 protein interaction pathways, and none of these associations were significant after FDR correction (Table 3; Supplementary information-2). These 13 protein interaction pathways indicated that the protein interactions of the potential target genes may contribute to DLB pathology by regulating transcription, cell cycle, signal transduction, and interleukin-6 signalling. Moreover, the potential target genes were significantly ($p < 0.05$) overrepresented among

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Table 2. Molecular pathways that were enriched among the potential target genes of differentially expressed small extracellular vesicle miRNA in people with dementia with Lewy bodies

KEGG Pathway	hsa number	Adjusted* *p*-value
Axon guidance	hsa04360	1.69×10^{-06}
Wnt signalling pathway	hsa04310	3.22×10^{-04}
mTOR signalling pathway	hsa04150	5.08×10^{-04}
Focal adhesion	hsa04510	5.08×10^{-04}
Autophagy	hsa04136	8.78×10^{-04}
Signalling pathways regulating pluripotency of stem cells	hsa04550	8.78×10^{-04}
mRNA surveillance pathway	hsa03015	1.58×10^{-3}
ErbB signalling pathway	hsa04012	1.78×10^{-3}
FoxO signalling pathway	hsa04068	2.49×10^{-3}
C-type lectin receptor signalling pathway	hsa04625	4.76×10^{-3}
Long-term potentiation	hsa04720	5.32×10^{-3}
AMPK signalling pathway	hsa04152	5.58×10^{-3}
MAPK signalling pathway	hsa04010	7.39×10^{-3}
Neurotrophin signalling pathway	hsa04722	7.63×10^{-3}
Rap1 signalling pathway	hsa04015	1.24×10^{-2}
T cell receptor signalling pathway	hsa04660	1.41×10^{-2}
cGMP-PKG signalling pathway	hsa04022	1.61×10^{-2}
Prolactin signalling pathway	hsa04917	1.64×10^{-2}
Phosphatidylinositol signalling system	hsa04070	1.67×10^{-2}
Glutamatergic synapse	hsa04724	2.82×10^{-2}
Ubiquitin-mediated proteolysis	hsa04120	2.82×10^{-2}
Oxytocin signalling pathway	hsa04921	2.97×10^{-2}
Protein processing in endoplasmic reticulum	hsa04141	3.02×10^{-2}
Sphingolipid signalling pathway	hsa04071	3.12×10^{-2}
Long-term depression	hsa04730	3.12×10^{-2}
Regulation of actin cytoskeleton	hsa04810	3.35×10^{-2}
PI3K-Akt signalling pathway	hsa04151	3.49×10^{-2}
Ferroptosis	hsa04216	3.49×10^{-2}
Phospholipase D signalling pathway	hsa04072	3.60×10^{-2}
Ras signalling pathway	hsa04014	3.60×10^{-2}
Cholinergic synapse	hsa04725	4.92×10^{-2}
Endocytosis	hsa04144	4.92×10^{-2}

KEGG: Kyoto Encyclopaedia of Genes and Genomes; hsa: *Homo sapiens*.
*Benjamini-Hochberg false discovery rate at 5%.

four protein interaction pathways after considering protein-protein interactions, and none of these associations were significant after FDR correction (Table 3; Supplementary information-2). These four protein interaction pathways indicated the possibility of the potential target genes influencing cell cycle, post-transcriptional silencing by small RNA, and phosphorylation of β-catenin that is one of the core molecules in the Wnt signalling pathway.

Table 3. Protein interaction pathways* that were enriched among the potential target genes of differentially expressed small extracellular vesicle miRNA in people with dementia with Lewy bodies

Interaction pathway	*p*-value
MECP2 regulates neuronal receptors and channels	2.24×10^{-4}
MAPK1 (ERK2) activation	7.11×10^{-3}
Interleukin-6 signalling	7.53×10^{-3}
Aberrant regulation of mitotic G1/S transition due to RB1 defects	1.17×10^{-2}
Defective binding of RB1 mutants to E2F1,(E2F2, E2F3)	1.17×10^{-2}
L1CAM interactions	2.61×10^{-2}
Interleukin-6 family signalling	2.98×10^{-2}
FOXO-mediated transcription of cell cycle genes	3.58×10^{-2}
YAP1- and WWTR1 (TAZ)-stimulated gene expression	3.78×10^{-2}
RAF-independent MAPK1/3 activation	4.63×10^{-2}
Transcriptional regulation by MECP2	4.68×10^{-2}
TP53 regulates transcription of genes involved in cytochrome C release	4.81×10^{-2}
Diseases of mitotic cell cycle	4.89×10^{-2}
*After including protein-protein interactions**	
Aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects	3.44×10^{-3}
Defective binding of RB1 mutants to E2F1,(E2F2, E2F3)	3.44×10^{-3}
Beta-catenin phosphorylation cascade	4.24×10^{-2}
Post-transcriptional silencing by small RNAs	4.74×10^{-2}

*Derived from *Reactome* pathway database (https://reactome.org/).

Secondary analyses of target genes of individual miRNA

We repeated the functional enrichment and protein interaction analyses for the potential target genes of each of the six miRNA genes individually. These secondary analyses identified additional significantly ($q < 0.05$) enriched biological processes, molecular functions, and KEGG pathways, as well as nominally significant ($p < 0.05$) protein interaction pathways. Supplementary information-3 presents these additional findings. The potential target genes of hsa-miR-26a-5p and hsa-miR-26a-1-3p were significantly enriched among the genes associated with signal transduction, post-translational protein modifications, nucleotide binding, regulation of cellular catabolic process ($q = 0.0335$), thiol-dependent ubiquitinyl hydrolase activity ($q = 0.0085$), ubiquitin-like protein ligase activity ($q = 0.0461$), and RNA polymerase-II distal enhancer sequence-specific binding transcription factor activity ($q = 0.0461$). The potential target genes of hsa-miR-320d were significantly enriched among the genes involved in longevity regulating pathway ($q = 0.0019$) and transcriptional misregulation in cancer ($q = 0.0451$). They were significantly ($p < 0.05$) over-represented among nuclear receptor transcription ($p = 8.99 \times 10^{-5}$) and Cohesin loading onto chromatin ($p = 0.0464$) protein interaction pathways. Moreover, the potential target genes of hsa-mir-548ba were significantly enriched for ubiquitin-like protein ligase activity ($q = 0.019$) and protein carboxyl O-methyltransferase activity ($q = 0.0359$). Their protein interactions were significantly ($p < 0.05$) overrepresented among 19 protein interaction pathways including activation of G-protein gated potassium channels

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($p = 0.0034$), inhibition of voltage-gated Ca^{2+} channels via Gbeta/gamma subunits ($p = 0.0034$), GABA receptor activation ($p = 0.0132$), GABA-B receptor activation ($p = 0.0267$), RNA Polymerase-III transcription initiation from type-1 ($p = 0.0410$) and type-2 ($p = 0.0367$) promoters, and GRB2 events in ERBB2 signalling ($p = 0.0397$). Furthermore, functional enrichment and protein interaction analyses for the potential target genes of hsa-miR-556-5p and hsa-miR-556-3p did not reveal any additional significantly ($q < 0.05$) enriched biological processes, molecular functions, and KEGG pathways. Their protein interactions were significantly ($p < 0.05$) overrepresented among seven protein interaction pathways including FGFR2 ligand binding and activation ($p = 0.0068$) and voltage-gated potassium channels ($p = 0.0094$) (Supplementary information-3).

Discussion

This is the first study that systematically investigated the functional implications of differentially expressed miRNA in serum SEV from people with DLB. It adds evidence supporting the hypothesis that differentially expressed SEV miRNA may contribute to DLB molecular pathogenesis by widespread transcriptional regulation (Gamez-Valero *et al.*, 2019; Rajkumar *et al.*, 2021). The potential target genes of the differentially expressed SEV miRNA were significantly enriched among the genes involved in post-translational protein modifications (Manzanza *et al.*, 2021), transcriptional regulation (Chowdhury and Rajkumar, 2020; Feleke *et al.*, 2021), autophagy lysosomal pathway (Crews *et al.*, 2010; Arotcarena *et al.*, 2019), UPS (Bedford *et al.*, 2008; Zheng *et al.*, 2016), apoptosis (Mahul-Mellier *et al.*, 2020), regulation of cell cycle (Lee *et al.*, 2003), histone acetylation (Urbizu and Beyer, 2020), cell signalling (Beyer *et al.*, 2009; Garcia-Esparcia *et al.*, 2017), synaptic dysfunction (Overk and Masliah, 2014), and vesicle-mediated transport (Kurzawa-Akanbi *et al.*, 2021; Longobardi *et al.*, 2022) that have been associated with DLB pathology. Strengths of this study include employing appropriate FDR corrections, focusing on differentially expressed SEV miRNA that were either differentially expressed in both post-mortem DLB brains and serum SEV or replicated by high-throughput qPCR, and considering protein interactions. However, there were limitations including the methodological difficulties in miRNA target prediction (Riolo *et al.*, 2020), limited information regarding potential targets of other differentially expressed SEV small RNA in DLB, and investigating miRNA that were identified in total serum SEV population that was not enriched for neuronal origin (Rajkumar *et al.*, 2021).

Several lines of evidence indicate that post-mortem brain and CSF-derived EV carry pathogenic cargo that are capable of inducing alpha-synuclein oligomerisation and propagating Lewy pathology (Danzer *et al.*, 2012; Stuendl *et al.*, 2016; Ngolab *et al.*, 2017; Kurzawa-Akanbi *et al.*, 2021; Herman *et al.*, 2022). However, the molecular mechanisms by which the pathogenic EV propagate Lewy pathology remain uncertain (Choi *et al.*, 2021). SEV miRNA, derived from people with AD or Parkinson's disease (PD), have been shown to contribute towards neurodegeneration by transcriptional regulation of genes involved in neuroinflammation and autophagy (Vassileff *et al.*, 2020; Mavroeidi *et al.*, 2022). Current evidence supporting the uptake of SEV miRNA by cells and their transcriptional regulation in recipient cells (Hu *et al.*, 2012; Li *et al.*, 2021; Mustajab *et al.*, 2022) outweigh the evidence to the contrary (Albanese *et al.*, 2021). Moreover, several miRNA are known to regulate alpha-synuclein levels, functions and oligomerisation in people with PD (Zhao and Wang, 2019), and pertinent research involving people with DLB remains sparse. Such prior evidence and the findings of this study highlight the need for further experiments investigating the importance of SEV miRNA in the molecular pathogenesis of DLB.

MIR26A1, also known as *MIR26A*, is well known to regulate neuronal development and synaptic plasticity (Li and Sun, 2013). hsa-miR-26a-5p has been included in the blood-based 12 miRNA signature panel for AD (Leidinger *et al.*, 2013), and its expression level has been found to be significantly higher in people with frontotemporal dementia, when compared to people without dementia (Martinez and Peplow, 2022). The diagnostic biomarker potential of hsa-miR-26a-5p in DLB (Rajkumar *et al.*, 2021) warrants further investigation. Moreover, this study has identified *PTGS2* encoding cyclooxygenase-2 as one of the potential target genes of hsa-miR-26a-5p. Dual-luciferase and qPCR experiments have confirmed that hsa-miR-26a-5p directly targets and regulates transcription of *PTGS2* (Xie *et al.*, 2022). Transcriptional regulation of *PTGS2* by hsa-miR-26a-5p has been reported to lead to proliferation of AD pathology (Xie *et al.*, 2022), and further investigation investigating the contribution of this miRNA-mRNA pair in DLB pathology is needed.

Expression level of *MIR320C2* is reportedly significantly higher in plasma of people with AD (Nagaraj *et al.*, 2017), and it has been found to be significantly downregulated in post-mortem PD brains (Briggs *et al.*, 2015) as well as post-mortem DLB brains and serum SEV from people living with DLB (Rajkumar *et al.*, 2021). This supports the need for investigating the biomarker potential of hsa-miR-320c for differentiating DLB from AD in large cohorts. Similarly, *MIR320D2* has been found to be significantly upregulated in plasma of people with PD (Chen *et al.*, 2021), and it was significantly downregulated in post-mortem DLB brains and serum SEV from people with DLB (Rajkumar *et al.*, 2021). hsa-miR-320d may help differentiating DLB from PD dementia. Furthermore, hsa-miR-4722-5p is likely to regulate genes involved in signal transduction and autophagy (Liu *et al.*, 2022). It has been reported to be significantly higher in serum of people with AD and has been identified as a potential blood-based biomarker for AD (Soleimani Zakeri *et al.*, 2020; Liu *et al.*, 2022). *MIR4722* was significantly downregulated in post-mortem DLB brains (Rajkumar *et al.*, 2020) as well as serum SEV from people with DLB (Rajkumar *et al.*, 2021), and this may contribute to the impairment of autophagy lysosomal pathway in DLB (Crews *et al.*, 2010; Arotcarena *et al.*, 2019). Diagnostic biomarker and therapeutic potential of hsa-miR-4722-5p in DLB need further evaluation.

The potential target genes of the investigated SEV miRNA in DLB include *UBE3A*, *UBE3B*, and 11 ubiquitin-conjugating enzyme genes. The potential target genes were significantly enriched among the genes involved in ubiquitin-protein transferase activity, ubiquitin-protein ligase activity, ubiquitin-mediated proteolysis, lysosomal transport, and negative regulation of autophagy. Dysfunction of autophagy lysosomal pathway and/or UPS leads to accumulation of pathologically misfolded proteins and progression of Lewy pathology (Zheng *et al.*, 2016). Recent transcriptomic studies (Pietrzak *et al.*, 2016; Nelson *et al.*, 2018; Santpere *et al.*, 2018; Rajkumar *et al.*, 2020) and a systematic review (Chowdhury and Rajkumar, 2020) have reported statistically significant downregulation of several autophagy lysosomal pathway and UPS-associated genes in post-mortem DLB brains. Statistically significant downregulation of UPS-associated genes, *UBE3A*, *USP47*, and *PSMD4*, and of the protein ubiquitination

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pathway in serum SEV from people with DLB have been reported (Rajkumar *et al.*, 2021). The UPS closely interacts with the autophagy lysosomal pathway, and their dysfunction was shown to be sufficient for causing Lewy body-like inclusions in mice models (Zheng *et al.*, 2009). Their dysfunction leads to cytoplasmic accumulation of alpha-synuclein and other misfolded proteins that can set off a vicious cycle by inhibiting neuronal lysosomal activity further (Mazzulli *et al.*, 2011). Moreover, our findings showed that the potential target genes were likely to regulate transcription of genes involved in protein phosphorylation and post-translational protein modifications. Phosphorylation and other post-translational modifications of alpha-synuclein contribute substantially towards molecular pathogenesis of DLB (Outeiro *et al.*, 2019; Manzanza *et al.*, 2021). Additionally, the potential target genes may play a role in DLB pathogenesis by impacting Wnt signalling pathway and associated β-catenin phosphorylation cascade that regulate microglia-mediated neuroinflammation and synaptic plasticity (Rajkumar *et al.*, 2020; Yang and Zhang, 2020).

Our findings support the biological plausibility of the differentially expressed SEV miRNA contributing towards DLB pathogenesis by transcriptional regulation of other genes. Therapeutic potential of these differentially expressed SEV miRNA and their target genes are warranted. Further experiments are required for verifying predicted miRNA targets as well as identified dysfunctional molecular processes and pathways. The potential regulation of post-translational modifications by SEV miRNA-mediated transcription, which directly act upon alpha-synuclein and other proteins and their functions, provides additional layers of regulatory cross-talk that could be targeted for therapeutic benefit. Moreover, SEV miRNA have opened a novel avenue for identifying blood-based diagnostic biomarkers for DLB. There is an urgent need for an adequately powered blood-based SEV RNA sequencing study that is not biased by post-transcriptional RNA modifications (Shi *et al.*, 2021). Discovered potential diagnostic biomarker miRNA and other small RNA should be evaluated in large replication cohorts. As gene expression changes often differ with disease progression, measuring the expression levels of potential diagnostic biomarkers at various clinical stages of DLB is preferred. Besides, investigating SEV that are enriched for neuronal (Mustapic *et al.*, 2017) and/or microglial (Winston *et al.*, 2021) origin by immunoprecipitation may facilitate the discovery of novel diagnostic biomarkers and therapeutic targets for DLB.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/neu.2023.27.

Authors' contribution. APR conceived this study, and all authors were involved in designing the study. APR identified the differentially expressed serum SEV miRNA in DLB. FBI performed all bioinformatic analyses and wrote the initial draft. All authors were involved in interpretation of the results and further critical revisions of the manuscript. All authors have approved the final version of the manuscript.

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References

Albanese M, Chen YA, Huls C, Gartner K, Tagawa T, Mejias-Perez E, Keppler OT, Gobel C, Zeidler R, Shein M, Schutz AK and Hammerschmidt W (2021) MicroRNAs are minor constituents of extracellular vesicles that are rarely delivered to target cells. *PLOS Genetics* **17**, e1009951.

Anders S, Mccarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W and Robinson MD (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. *Nature Protocols* **8**, 1765–1786.

Arotcarena ML, Teil M and Dehay B (2019) Autophagy in synucleinopathy: the overwhelmed and defective machinery. *Cells* **8**, 565.

Ashton NJ, Hye A, Rajkumar AP, Leuzy A, Snowden S, Suarez-Calvet M, Karikari TK, Scholl M, La Joie R, Rabinovici GD, Hoglund K, Ballard C, Hortobagyi T, Svenningsson P, Blennow K, Zetterberg H and Aarsland D (2020) An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders. *Nature reviews. Neurology* **16**, 265–284.

Bedford L, Hay D, Paine S, Rezvani N, Mee M, Lowe J and Mayer RJ (2008) Is malfunction of the ubiquitin proteasome system the primary cause of alpha-synucleinopathies and other chronic human neurodegenerative disease? *Biochimica et Biophysica Acta* **1782**, 683–690.

Benjamini Y and Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Statistical Methodology* **57**, 289–300.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J and Wheeler DL (2007) GenBank. *Nucleic Acids Research* **35**, D21–D25.

Beyer K, Domingo-Sabat M and Ariza A (2009) Molecular pathology of Lewy body diseases. *International Journal of Molecular Sciences* **10**, 724–745.

Bostrom F, Jonsson L, Minthon L and Londos E (2007) Patients with dementia with lewy bodies have more impaired quality of life than patients with Alzheimer disease. *Alzheimer Disease & Associated Disorders* **21**, 150–154.

Briggs CE, Wang Y, Kong B, Woo TU, Iyer LK and Sonntag KC (2015) Midbrain dopamine neurons in Parkinson's disease exhibit a dysregulated miRNA and target-gene network. *Brain Research* **1618**, 111–121.

Candelario KM and Steindler DA (2014) The role of extracellular vesicles in the progression of neurodegenerative disease and cancer. *Trends in Molecular Medicine* **20**, 368–374.

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR and Ma'ayan A (2013) Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14**(1), 128.

Chen Q, Deng N, Lu K, Liao Q, Long X, Gou D, Bi F and Zhou J (2021) Elevated plasma miR-133b and miR-221-3p as biomarkers for early Parkinson's disease. *Scientific Reports* **11**, 15268.

Chen Y and Wang X (2020) miRDB: an online database for prediction of functional microRNA targets. *Nucleic acids research* **48**, D127–D131.

Choi YR, Park SJ and Park SM (2021) Molecular events underlying the cell-to-cell transmission of alpha-synuclein. *The FEBS Journal* **288**, 6593–6602.

Chowdhury A and Rajkumar AP (2020) Systematic review of gene expression studies in people with Lewy body dementia. *Acta Neuropsychiatrica* **32**, 281–292.

Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, Hansen L, Adame A, Galasko D, Masliah E (2010) Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One* **5**, e9313.

Culhane AC, Schroder MS, Sultana R, Picard SC, Martinelli EN, Kelly C, Haibe-Kains B, Kapushesky M, St Pierre AA, Flahive W, Picard KC, Gusenleitner D, Papenhausen G, O'connor N, Correll M and Quackenbush J (2012) GeneSigDB: a manually curated database and resource for analysis of gene expression signatures. *Nucleic Acids Research* **40**, D1060–D1066.

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. *Molecular Neurodegeneration* **7**, 42.

Du Y, Yu Y, Hu Y, Li XW, Wei ZX, Pan RY, Li XS, Zheng GE, Qin XY, Liu QS, Cheng Y (2019) Genome-wide, integrative analysis implicates exosome-derived microRNA dysregulation in schizophrenia. *Schizophrenia Bulletin* **45**, 1257–1266.

Fabregat A, Sidiropoulos K, Viteri G, Forner O, Marin-Garcia P, Arnau V, D'eustachio P, Stein L and Hermjakob H (2017) Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics* **18**(1), 142.

Feleke R, Reynolds RH, Smith AM, Tilley B, SaG Taliun, Hardy J, Matthews PM, Gentleman S, Owen DR, Johnson MR, Srivastava PK and Ryten M (2021) Cross-platform transcriptional profiling identifies common and distinct molecular pathologies in Lewy body diseases. *Acta Neuropathologica* **142**, 449–474.

Fladby T, Palhaugen L, Selnes P, Waterloo K, Brathen G, Hessen E, Almdahl IS, Arntzen KA, Auning E, Eliassen CF, Espenes R, Grambaite R, Grontvedt GR, Johansen KK, Johnsen SH, Kalheim LF, Kirsebom BE, Muller KI, Nakling AE, Rongve A, Sando SB, Siafarikas N, Stav AL, Tecelao S, Timon S, Bekkelund SI and Aarsland D (2017) Detecting at-risk Alzheimer's disease cases. *Journal of Alzheimers Disease* **60**, 97–105.

Freer J (2017) UK lags far behind Europe on diagnosis of dementia with Lewy bodies. *BMJ* **358**, j3319.

Gamez-Valero A, Campdelacreu J, Vilas D, Ispierto L, Rene R, Alvarez R, Armengol MP, Borras FE and Beyer K (2019) Exploratory study on microRNA profiles from plasma-derived extracellular vesicles in Alzheimer's disease and dementia with Lewy bodies. *Translational Neurodegeneration* **8**, 31.

Garcia-Esparcia P, Lopez-Gonzalez I, Grau-Rivera O, Garcia-Garrido MF, Konetti A, Llorens F, Zafar S, Carmona M, Del Rio JA, Zerr I, Gelpi E and Ferrer I (2017) Dementia with Lewy bodies: molecular pathology in the frontal cortex in typical and rapidly progressive forms. *Frontiers in Neurology* **8**, 89.

Gruzdev SK, Yakovlev AA, Druzhkova TA, Guekht AB and Gulyaeva NV (2019) The missing link: how exosomes and miRNAs can help in bridging psychiatry and molecular biology in the context of depression, bipolar disorder and schizophrenia. *Cellular and Molecular Neurobiology* **39**, 729–750.

Haenig C, Atias N, Taylor AK, Mazza A, Schaefer MH, Russ J, Riechers SP, Jain S, Coughlin M, Fontaine JF, Freibaum BD, Brusendorf L, Zenkner M, Porras P, Stroedicke M, Schnoegl S, Arnsburg K, Boeddrich A, Pigazzini L, Heutink P, Taylor JP, Kirstein J, Andrade-Navarro MA, Sharan R and Wanker EE (2020) Interactome mapping provides a network of neurodegenerative disease proteins and uncovers widespread protein aggregation in affected brains. *Cell Reports* **32**, 108050.

Herman S, Djaldetti R, Mollenhauer B and Offen D (2022) CSF-derived extracellular vesicles from patients with Parkinson's disease induce symptoms and pathology. *Brain* **146**, 209–224.

Hermjakob H, Montecchi-Palazzi L, Lewington C, Mudali S, Kerrien S, Orchard S, Vingron M, Roechert B, Roepstorff P, Valencia A, Margalit H, Armstrong J, Bairoch A, Cesareni G, Sherman D and Apweiler R (2004) IntAct: an open source molecular interaction database. *Nucleic Acids Research* **32**, D452–D455.

Hu G, Drescher KM and Chen XM (2012) Exosomal miRNAs: biological properties and therapeutic potential. *Frontiers in Genetics* **3**, 56.

Ibrahim SA and Khan YS (2022) Histology, Extracellular Vesicles. Treasure Island, FL: StatPearls.

Kalluri R and Lebleu VS (2020) The biology, function, and biomedical applications of exosomes. *Science* **367**, eaau6977.

Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M and Tanabe M (2021) KEGG: integrating viruses and cellular organisms. *Nucleic Acids Research* **49**, D545–D551.

Kanehisa M and Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **28**, 27–30.

Kozomara A, Birgaoanu M and Griffiths-Jones S (2019) miRBase: from microRNA sequences to function. *Nucleic Acids Research* **47**, D155–D162.

Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, Mcdermott MG, Monteiro CD, Gundersen GW and Ma'ayan A (2016) Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research* **44**, W90–W97.

Kurzawa-Akanbi M, Tammireddy S, Fabrik I, Gliaudelyte L, Doherty MK, Heap R, Matecko-Burmann I, Burmann BM, Trost M, Lucocq JM, Gherman AV, Fairfoul G, Singh P, Burte F, Green A, Mckeith IG, Hartlova A, Whitfield PD and Morris CM (2021) Altered ceramide metabolism is a feature in the extracellular vesicle-mediated spread of alpha-synuclein in Lewy body disorders. *Acta Neuropathologica* **142**, 961–984.

Lee SS, Kim YM, Junn E, Lee G, Park KH, Tanaka M, Ronchetti RD, Quezado MM and Mouradian MM (2003) Cell cycle aberrations by alpha-synuclein over-expression and cyclin B immunoreactivity in Lewy bodies. *Neurobiology of Aging* **24**(5), 687–696.

Leidinger P, Backes C, Deutscher S, Schmitt K, Mueller SC, Frese K, Haas J, Ruprecht K, Paul F, Stahler C, Lang CJ, Meder B, Bartfai T, Meese E and Keller A (2013) A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biology* **14**, R78.

Li B, Cao Y, Sun M and Feng H (2021) Expression, regulation, and function of exosome-derived miRNAs in cancer progression and therapy. *The FASEB Journal* **35**, e21916.

Li B and Sun H (2013) MiR-26a promotes neurite outgrowth by repressing PTEN expression. *Molecular Medicine Reports* **8**, 676–680.

Liu B, Li J and Cairns MJ (2014) Identifying miRNAs, targets and functions. *Briefings in Bioinformatics* **15**, 1–19.

Liu W and Wang X (2019) Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. *Genome Biology* **20**, 18.

Liu Y, Xu Y and Yu M (2022) MicroRNA-4722-5p and microRNA-615-3p serve as potential biomarkers for Alzheimer's disease. *Experimental and Therapeutic Medicine* **23**, 241.

Longobardi A, Nicsanu R, Bellini S, Squitti R, Catania M, Tiraboschi P, Saraceno C, Ferrari C, Zanardini R, Binetti G, Di Fede G, Benussi L and Ghidoni R (2022) Cerebrospinal fluid EV concentration and size are altered in Alzheimer's disease and dementia with Lewy bodies. *Cells* **11**, 462.

Lu TX and Rothenberg ME (2018) MicroRNA. *Journal of Allergy and Clinical Immunology* **141**, 1202–1207.

Maglott D, Ostell J, Pruitt KD and Tatusova T (2007) Entrez Gene: gene-centered information at NCBI. *Nucleic Acids Research* **35**, D26–D31.

Mahul-Mellier AL, Burtscher J, Maharjan N, Weerens L, Croisier M, Kuttler F, Leleu M, Knott GW and Lashuel HA (2020) The process of Lewy body formation, rather than simply alpha-synuclein fibrillization, is one of the major drivers of neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America* **117**, 4971–4982.

Manzanza NO, Sedlackova L and Kalaria RN (2021) Alpha-synuclein post-translational modifications: implications for pathogenesis of Lewy body disorders. *Frontiers in Aging Neuroscience* **13**, 690293.

Martinez B and Peplow PV (2022) MicroRNA biomarkers in frontotemporal dementia and to distinguish from Alzheimer's disease and amyotrophic lateral sclerosis. *Neural Regeneration Research* **17**, 1412–1422.

Mavroeidi P, Vetsi M, Dionysopoulou D and Xilouri M (2022) Exosomes in alpha-synucleinopathies: propagators of pathology or potential candidates for nanotherapeutics? *Biomolecules* **12**, 957.

Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA and Krainc D (2011) Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* **146**, 37–52.

Mckeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, Aarsland D, Galvin J, Attems J, Ballard CG, Bayston A, Beach TG, Blanc F, Bohnen N, Bonanni L, Bras J, Brundin P, Burn D, Chen-Plotkin A, Duda JE, El-Agnaf O, Feldman H, Ferman TJ, Ffytche D, Fujishiro H, Galasko D, Goldman JG, Gomperts SN, Graff-Radford NR, Honig LS, Iranzo A, Kantarci K, Kaufer D, Kukull W, Lee VMY, Leverenz JB, Lewis S, Lippa C, Lunde A, Masellis M, Masliah E, Mclean P, Mollenhauer B, Montine TJ, Moreno E, Mori E, Murray M, O'brien JT, Orimo S, Postuma RB, Ramaswamy S, Ross OA, Salmon DP, Singleton A, Taylor A, Thomas A, Tiraboschi P, Toledo JB, Trojanowski JQ, Tsuang D, Walker Z, Yamada M and Kosaka K (2017) Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology* **89**, 88–100.

Mustajab T, Kwamboka MS, Choi DA, Kang DW, Kim J, Han KR, Han Y, Lee S, Song D, Chwae YJ (2022) Update on extracellular vesicle-based vaccines and therapeutics to combat COVID-19. *International Journal of Molecular Sciences* **23**, 11247.

Mustapic M, Eitan E, Werner JK Jr., Berkowitz ST, Lazaropoulos MP, Tran J, Goetzl EJ and Kapogiannis D (2017) Plasma extracellular vesicles enriched

https://doi.org/10.1017/neu.2023.27 Published online by Cambridge University Press

for neuronal origin: a potential window into brain pathologic processes. *Frontiers in Neuroscience* **11**, 278.

Nagaraj S, Laskowska-Kaszub K, Debski KJ, Wojsiat J, Dabrowski M, Gabryelewicz T, Kuznicki J and Wojda U (2017) Profile of 6 microRNA in blood plasma distinguish early stage Alzheimer's disease patients from non-demented subjects. *Oncotarget* **8**, 16122–16143.

Nelson PT, Wang WX, Janse SA and Thompson KL (2018) MicroRNA expression patterns in human anterior cingulate and motor cortex: a study of dementia with Lewy bodies cases and controls. *Brain Research* **1678**, 374–383.

Ngolab J, Trinh I, Rockenstein E, Mante M, Florio J, Trejo M, Masliah D, Adame A, Masliah E and Rissman RA (2017) Brain-derived exosomes from dementia with Lewy bodies propagate alpha-synuclein pathology. *Acta Neuropathologica Communications* **5**, 46.

Oesterhus R, Soennesyn H, Rongve A, Ballard C, Aarsland D and Vossius C (2014) Long-term mortality in a cohort of home-dwelling elderly with mild Alzheimer's disease and Lewy body dementia. *Dementia and Geriatric Cognitive Disorders* **38**, 161–169.

Outeiro TF, Koss DJ, Erskine D, Walker L, Kurzawa-Akanbi M, Burn D, Donaghy P, Morris C, Taylor JP, Thomas A, Attems J and Mckeith I (2019) Dementia with Lewy bodies: an update and outlook. *Molecular Neurodegeneration* **14**, 5.

Ovchinnikova LA, Zalevsky AO and Lomakin YA (2022) Extracellular vesicles in chronic demyelinating diseases: prospects in treatment and diagnosis of autoimmune neurological disorders. *Life (Basel)* **12**, 1943.

Overk CR and Masliah E (2014) Pathogenesis of synaptic degeneration in Alzheimer's disease and Lewy body disease. *Biochemical Pharmacology* **88**, 508–516.

Pietrzak M, Papp A, Curtis A, Handelman SK, Kataki M, Scharre DW, Rempala G and Sadee W (2016) Gene expression profiling of brain samples from patients with Lewy body dementia. *Biochemical and Biophysical Research Communications* **479**, 875–880.

Pinero J, Sauch J, Sanz F and Furlong LI (2021) The DisGeNET cytoscape app: exploring and visualizing disease genomics data. *Computational and Structural Biotechnology Journal* **19**, 2960–2967.

Rajkumar AP and Aarsland D (2020) Dementia with Lewy bodies. In Geddes JR, Andreasen NC and Goodwin GM (ed), New Oxford Textbook of Psychiatry, 3rd edn. Oxford: Oxford University press.

Rajkumar AP, Bidkhori G, Shoaie S, Clarke E, Morrin H, Hye A, Williams G, Ballard C, Francis P, Aarsland D (2020) Postmortem cortical transcriptomics of Lewy body dementia reveal mitochondrial dysfunction and lack of neuroinflammation. *The American Journal of Geriatric Psychiatry* **28**, 75–86.

Rajkumar AP, Hye A, Lange J, Manesh YR, Ballard C, Fladby T and Aarsland D (2021) Next-generation RNA-sequencing of serum small extracellular vesicles discovers potential diagnostic biomarkers for dementia with Lewy bodies. *The American Journal of Geriatric Psychiatry* **29**, 573–584.

Rajkumar AP, Qvist P, Lazarus R, Lescai F, Ju J, Nyegaard M, Mors O, Borglum AD, Li Q and Christensen JH (2015) Experimental validation of methods for differential gene expression analysis and sample pooling in RNA-seq. *BMC Genomics* **16**, 548.

Riolo G, Cantara S, Marzocchi C and Ricci C (2020) miRNA targets: from prediction tools to experimental validation. *Methods and Protocols* **4**, 1.

Rongve A, Soennesyn H, Skogseth R, Oesterhus R, Hortobagyi T, Ballard C, Auestad BH and Aarsland D (2016) Cognitive decline in dementia with Lewy bodies: a 5-year prospective cohort study. *BMJ Open* **6**, e010357.

Rongve A, Vossius C, Nore S, Testad I and Aarsland D (2014) Time until nursing home admission in people with mild dementia: comparison of dementia with Lewy bodies and Alzheimer's dementia. *International Journal of Geriatric Psychiatry* **29**, 392–398.

Saint-Pol J, Gosselet F, Duban-Deweer S, Pottiez G and Karamanos Y (2020) Targeting and crossing the blood-brain barrier with extracellular vesicles. *Cells* **9**, 851–851.

Santpere G, Garcia-Esparcia P, Andres-Benito P, Lorente-Galdos B, Navarro A and Ferrer I (2018) Transcriptional network analysis in frontal cortex in Lewy body diseases with focus on dementia with Lewy bodies. *Brain Pathology* **28**(3), 315–333.

Selnes P, Aarsland D, Bjornerud A, Gjerstad L, Wallin A, Hessen E, Reinvang I, Grambaite R, Auning E, Kjaervik VK, Due-Tonnessen P, Stenset V and Fladby T (2013) Diffusion tensor imaging surpasses cerebrospinal fluid as predictor of cognitive decline and medial temporal lobe atrophy in subjective cognitive impairment and mild cognitive impairment. *Journal of Alzheimers Disease* **33**, 723–736.

Shi J, Zhang Y, Tan D, Zhang X, Yan M, Zhang Y, Franklin R, Shahbazi M, Mackinlay K, Liu S, Kuhle B, James ER, Zhang L, Qu Y, Zhai Q, Zhao W, Zhao L, Zhou C, Gu W, Murn J, Guo J, Carrell DT, Wang Y, Chen X, Cairns BR, Yang XL, Schimmel P, Zernicka-Goetz M, Cheloufi S, Zhang Y, Zhou T and Chen Q (2021) PANDORA-seq expands the repertoire of regulatory small RNAs by overcoming RNA modifications. *Nature Cell Biology* **23**(4), 424–436.

Soleimani Zakeri NS, Pashazadeh S and Motieghader H (2020) Gene biomarker discovery at different stages of Alzheimer using gene co-expression network approach. *Scientific Reports* **10**, 12210.

Stinton C, Mckeith I, Taylor JP, Lafortune L, Mioshi E, Mak E, Cambridge V, Mason J, Thomas A and O'brien JT (2015) Pharmacological management of Lewy body dementia: a systematic review and meta-analysis. *American Journal of Psychiatry* **172**, 731–742.

Stuendl A, Kunadt M, Kruse N, Bartels C, Moebius W, Danzer KM, Mollenhauer B and Schneider A (2016) Induction of alpha-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain* **139**, 481–494.

Svendsboe E, Terum T, Testad I, Aarsland D, Ulstein I, Corbett A and Rongve A (2016) Caregiver burden in family carers of people with dementia with Lewy bodies and Alzheimer's disease. *International Journal of Geriatric Psychiatry* **31**, 1075–1083.

Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre D C, Bach J-M, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, Beckham C, Bedina Zavec A, Benmoussa A, Berardi AC, Bergese P, Bielska E, Blenkiron C, Bobis-Wozowicz S, Boilard E, Boireau W, Bongiovanni A, Borràs FE, Bosch S, Boulanger CM, Breakefield X, Breglio AM, Brennan MÁ, Brigstock DR, Brisson A, Broekman MLD, Bromberg JF, Bryl-Górecka P, Buch S, Buck AH, Burger D, Busatto S, Buschmann D, Bussolati B, Buzás EI, Byrd JB, Camussi G, Carter DRF, Caruso S, Chamley LW, Chang Y-T, Chen C, Chen S, Cheng L, Chin AR, Clayton A, Clerici SP, Cocks A, Cocucci E, Coffey RJ, Cordeiro-da-Silva A, Couch Y, Coumans FAW, Coyle B, Crescitelli R, Criado MF, D'Souza-Schorey C, Das S, Datta Chaudhuri A, de Candia P, EF De Santana Junior, De Wever O, del Portillo HA, Demaret T, Deville S, Devitt A, Dhondt B, Di Vizio D, Dieterich LC, Dolo V, Dominguez Rubio AP, Dominici M, Dourado MR, Driedonks TAP, Duarte FV, Duncan HM, Eichenberger RM, Ekström K, EL Andaloussi S, Elie-Caille C, Erdbrügger U, Falcón-Pérez JM, Fatima F, Fish JE, Flores-Bellver M, Försönits Aás, Frelet-Barrand A, Fricke F, Fuhrmann G, Gabrielsson S, Gámez-Valero A, Gardiner C, Gärtner K, Gaudin R, Gho YS, Giebel B, Gilbert C, Gimona M, Giusti I, Goberdhan DCI, Görgens Aé, Gorski SM, Greening DW, Gross JC, Gualerzi A, Gupta GN, Gustafson D, Handberg A, Haraszti RA, Harrison P, Hegyesi H, Hendrix A, Hill AF, Hochberg FH, Hoffmann KF, Holder B, Holthofer H, Hosseinkhani B, Hu G, Huang Y, Huber V, Hunt S, Ibrahim AG-E, Ikezu T, Inal JM, Isin M, Ivanova A, Jackson HK, Jacobsen S, Jay SM, Jayachandran M, Jenster G, Jiang L, Johnson SM, Jones JC, Jong A, Jovanovic-Talisman T, Jung S, Kalluri R, Kano S-I, Kaur S, Kawamura Y, Keller ET, Khamari D, Khomyakova E, Khvorova A, Kierulf P, Kim KP, Kislinger T, Klingeborn M, DJ Klinke II, Kornek M, Kosanović MM, Kovács Árpád F, Krämer-Albers E-M, Krasemann S, Krause M, Kurochkin IV, Kusuma GD, Kuypers Sören, Laitinen S, Langevin SM, Languino LR, Lannigan J, Lässer C, Laurent LC, Lavieu G, Lázaro-Ibáñez E, Le Lay S, Lee M-S, Lee YXF, Lemos DS, Lenassi M, Leszczynska A, Li ITS, Liao K, Libregts SF, Ligeti E, Lim R, Lim SK, Linē A, Linnemannstöns K, Llorente A, Lombard CA, Lorenowicz MJ, Lörincz Ákos M, Lötvall J, Lovett J, Lowry MC, Loyer X, Lu Q, Lukomska B, Lunavat TR, Maas SLN, Malhi H, Marcilla A, Mariani J, Mariscal J, Martens-Uzunova ES, Martin-Jaular L, Martinez M C,

https://doi.org/10.1017/neu.2023.27 Published online by Cambridge University Press

Martins VR, Mathieu M, Mathivanan S, Maugeri M, McGinnis LK, McVey MJ, DG Meckes Jr, Meehan KL, Mertens I, Minciacchi VR, Möller A, Møller Jørgensen M, Morales-Kastresana A, Morhayim J, Mullier Fçois, Muraca M, Musante L, Mussack V, Muth DC, Myburgh KH, Najrana T, Nawaz M, Nazarenko I, Nejsum P, Neri C, Neri T, Nieuwland R, Nimrichter L, Nolan JP, Nolte-'t Hoen ENM, Noren Hooten N, O'Driscoll L, O'Grady T, O'Loghlen A, Ochiya T, Olivier M, Ortiz A, Ortiz LA, Osteikoetxea X, Østergaard O, Ostrowski M, Park J, Pegtel DM, Peinado H, Perut F, Pfaffl MW, Phinney DG, Pieters BCH, Pink RC, Pisetsky DS, Pogge von Strandmann E, Polakovicova I, Poon IKH, Powell BH, Prada I, Pulliam L, Quesenberry P, Radeghieri A, Raffai RL, Raimondo S, Rak J, Ramirez MI, Raposo Gça, Rayyan MS, Regev-Rudzki N, Ricklefs FL, Robbins PD, Roberts DD, Rodrigues SC, Rohde E, Rome S, Rouschop KMA, Rughetti A, Russell AE, Saá P, Sahoo S, Salas-Huenuleo E, Sánchez C, Saugstad JA, Saul MJ, Schiffelers RM, Schneider R, Schøyen TH, Scott A, Shahaj E, Sharma S, Shatnyeva O, Shekari F, Shelke GV, Shetty AK, Shiba K, Siljander PR-M, Silva AM, Skowronek A, OL Snyder II, Soares RP, Sódar BW, Soekmadji C, Sotillo J, Stahl PD, Stoorvogel W, Stott SL, Strasser EF, Swift S, Tahara H, Tewari M, Timms K, Tiwari S, Tixeira R, Tkach M, Toh WS, Tomasini R, Torrecilhas AC, Tosar JP, Toxavidis V, Urbanelli L, Vader P, van Balkom BWM, van der Grein SG, Van Deun J, van Herwijnen MJC, Van Keuren-Jensen K, van Niel G, van Royen ME, van Wijnen AJ, Vasconcelos M H, IJ Vechetti Jr, Veit TD, Vella LJ, Velot Émilie, Verweij FJ, Vestad B, Viñas JL, Visnovitz Tás, Vukman KV, Wahlgren J, Watson DC, Wauben MHM, Weaver A, Webber JP, Weber V, Wehman AM, Weiss DJ, Welsh JA, Wendt S, Wheelock AM, Wiener Zán, Witte L, Wolfram J, Xagorari A, Xander P, Xu J, Yan X, Yáñez-Mó Mía, Yin H, Yuana Y, Zappulli V, Zarubova J, Žėkas V, Zhang J-Y, Zhao Z, Zheng L, Zheutlin AR, Zickler AM, Zimmermann P, Zivkovic AM, Zocco D and Zuba-Surma EK (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. *Journal of Extracellular Vesicles* **7**, 1535750.

Trotta T, Panaro MA, Cianciulli A, Mori G, Di Benedetto A and Porro C (2018) Microglia-derived extracellular vesicles in Alzheimer's Disease: a double-edged sword. *Biochemical Pharmacology* **148**, 184–192.

Urbizu A and Beyer K (2020) Epigenetics in Lewy body diseases: impact on gene expression, utility as a biomarker, and possibilities for therapy. *International Journal of Molecular Sciences* **21**, 4718.

Vader P, Mol EA, Pasterkamp G and Schiffelers RM (2016) Extracellular vesicles for drug delivery. *Advanced Drug Delivery Reviews* **106**, 148–156.

Vassileff N, Cheng L and Hill AF (2020) Extracellular vesicles - propagators of neuropathology and sources of potential biomarkers and therapeutics for neurodegenerative diseases. *Journal of Cell Science* **133**, jcs243139.

Velayudhan L, Ffytche D, Ballard C and Aarsland D (2017) New therapeutic strategies for Lewy body dementias. *Current Neurology and Neuroscience Reports* **17**, 68.

Vishnoi A and Rani S (2023) miRNA biogenesis and regulation of diseases: an updated overview. *Methods in Molecular Biology* **2595**, 1–12.

Vossius C, Rongve A, Testad I, Wimo A and Aarsland D (2014) The use and costs of formal care in newly diagnosed dementia: a three-year prospective follow-up study. *The American Journal of Geriatric Psychiatry* **22**, 381–388.

WHO (2018) International Classification of Diseases for Mortality and Morbidity Statistics (11th Revision) [Online]. World Health Organization. Available at https://icd.who.int/browse11/l-m/en (accessed 02 December 2022).

Wang X and El Naqa IM (2008) Prediction of both conserved and nonconserved microRNA targets in animals. *Bioinformatics* **24**, 325–332.

Wang Y, Yuan P, Ding L, Zhu J, Qi X, Zhang Y, Li Y, Xia X and Zheng JC (2022) Circulating extracellular vesicle-containing microRNAs reveal potential pathogenesis of Alzheimer's disease. *Frontiers in Cellular Neuroscience* **16**, 955511.

Watts KE, Storr NJ, Barr PG and Rajkumar AP (2023) Systematic review of pharmacological interventions for people with Lewy body dementia. *Aging Ment Health* **27**, 203–216.

Winston CN, Sarsoza F, Spencer B and Rissman RA (2021) Characterizing blood-based, microglial derived exosomes (MDEs) as biomarkers for Alzheimer's disease. *Alzheimer's & Dementia* **17**, e055371.

Xie T, Pei Y, Shan P, Xiao Q, Zhou F, Huang L and Wang S (2022) Identification of miRNA-mRNA pairs in the Alzheimer's disease expression profile and explore the effect of miR-26a-5p/PTGS2 on amyloid-beta induced neurotoxicity in Alzheimer's disease cell model. *Frontiers in Aging Neuroscience* **14**, 909222.

Yan S, Nagle DG, Zhou Y and Zhang W (2018) Application of systems biology in the research of TCM formulae. In Zhang W-D (ed), Systems Biology and Its Application in TCM Formulas Research. London: Academic Press.

Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borras FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colas E, Cordeiro-Da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Kramer-Albers EM, Laitinen S, Lasser C, Lener T, Ligeti E, Line A, Lipps G, Llorente A, Lotvall J, Mancek-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'T Hoen EN, Nyman TA, O'driscoll L, Olivan M, Oliveira C, Pallinger E, Del Portillo HA, Reventos J, Rigau M, Rohde E, Sammar M, Sanchez-Madrid F, Santarem N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van Der Grein SG, Vasconcelos MH, Wauben MH and De Wever O (2015) Biological properties of extracellular vesicles and their physiological functions. *Journal of Extracellular Vesicles* **4**, 27066.

Yang Y and Zhang Z (2020) Microglia and Wnt pathways: prospects for inflammation in Alzheimer's disease. *Frontiers in Aging Neuroscience* **12**, 110.

Zhang Q, Kim YC and Narayanan NS (2015) Disease-modifying therapeutic directions for Lewy-Body dementias. *Frontiers in Neuroscience* **9**, 293.

Zhao L and Wang Z (2019) MicroRNAs: game changers in the regulation of alpha-synuclein in Parkinson's disease. *Parkinson's Disease* **2019**, 1–10.

Zheng Q, Huang T, Zhang L, Zhou Y, Luo H, Xu H and Wang X (2016) Dysregulation of ubiquitin-proteasome system in neurodegenerative diseases. *Frontiers in Aging Neuroscience* **8**, 303.

Zheng Q, Li J and Wang X (2009) Interplay between the ubiquitin-proteasome system and autophagy in proteinopathies. *International Journal of Physiology, Pathophysiology and Pharmacology* **1**, 127–142.

https://doi.org/10.1017/neu.2023.27 Published online by Cambridge University Press