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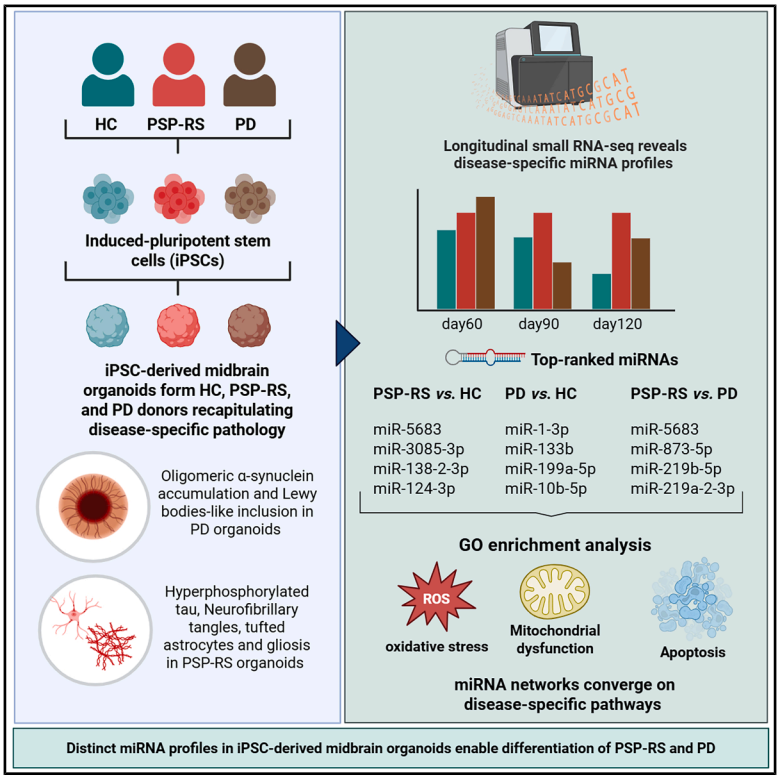
Distinct microRNA signatures define sporadic PSP-RS and PD in patient-derived midbrain organoids

Graphical abstract



Highlights

- iPSC midbrain organoids model PSP-RS and PD with disease-specific features
- Distinct miRNA signatures differentiate PSP-RS, PD, and healthy controls
- miR-5683, miR-873-5p, and miR-219 family differentiate PSP-RS from PD

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In brief

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Distinct microRNA signatures define sporadic PSP-RS and PD in patient-derived midbrain organoids

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SUMMARY

Progressive supranuclear Palsy–Richardson syndrome (PSP-RS) is a rare, rapidly progressive tauopathy often misdiagnosed as Parkinson's disease (PD) due to overlapping clinical features and the lack of reliable molecular biomarkers. To address this need, we generated human midbrain organoids from induced pluripotent stem cells (iPSCs) derived from individuals with sporadic PSP-RS, PD, and healthy controls (HCs), and performed longitudinal small RNA sequencing to profile microRNA (miRNA) signatures. These 3D organoids recapitulated disease-relevant pathologies, including tau hyperphosphorylation in PSP-RS and α-synuclein aggregation in PD. Transcriptomic analysis revealed dynamic, disease-specific miRNA signatures. Notably, miR-5683, miR-873-5p, miR-219b-5p, and miR-219a-2-3p were enriched in PSP-RS, while PD organoids showed increased expression of miR-1-3p and miR-133b. Differential expression analysis identified miR-5683, miR-3085-3p, and miR-124-3p as robust classifiers distinguishing PSP-RS from controls. Our findings support iPSC-derived midbrain organoids as a relevant platform for modeling atypical parkinsonian syndromes and uncovering candidate miRNA biomarkers for early and differential diagnosis.

INTRODUCTION

Neurodegenerative disorders such as Parkinson's disease (PD) and progressive supranuclear Palsy–Richardson syndrome (PSP-RS) impose a growing burden on aging populations worldwide. Although these diseases have distinct molecular etiologies—α-synuclein aggregation in PD and tau hyperphosphorylation and aggregation in PSP-RS—patients often exhibit overlapping early motor symptoms, leading to frequent misdiagnosis and delays in appropriate clinical management.[1,2] There is a critical unmet need for robust biomarkers capable of distinguishing these conditions at early, clinically ambiguous stages. PD is primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra and the pathological accumulation of α-synuclein, manifesting in bradykinesia, rigidity, and resting tremor.[3,4] In contrast, PSP-RS, the most prevalent clinical phenotype within the spectrum of progressive supranuclear palsy disorders, is marked by the widespread deposition of hyperphosphorylated tau in the midbrain, basal ganglia, and brainstem.[5] However, early-stage symptoms overlap between PD and PSP-RS and often mask these distinct molecular signatures,[1,6] complicating timely and accurate diagnosis. MicroRNAs (miRNAs), a class of small non-coding RNAs that modulate gene expression post-transcriptionally, have emerged as promising candidates for understanding the molecular underpinnings of neurodegenerative diseases. In the central nervous system (CNS), miRNAs play pivotal roles in cellular processes relevant to disease pathogenesis, including mitochondrial dysfunction, autophagy, protein aggregation, apoptosis, and neuroinflammation.[7–12] Their remarkable stability in biofluids and disease-specific expression patterns support their utility as both mechanistic effectors and potential biomarkers.[13–15] Nevertheless, direct sampling of CNS tissue is rarely feasible in living patients, and while peripheral sources such as blood and cerebrospinal fluid (CSF) offer more accessible options, they may not accurately reflect brain-specific miRNA dynamics, particularly in

early disease stages.[16] Previous studies have identified altered miRNA levels in blood from PD patients (e.g., miR-1-3p, miR-22-5p, and miR-29a-3p),[17] and in plasma from individuals with multiple system atrophy (MSA) (e.g., miR-24, miR-148b, miR-223, miR-324-3p, miR-339-5p).[18] However, these findings often lack disease-contextual modeling and require further validation in more representative systems. To address these limitations, we employed induced pluripotent stem cell (iPSC)-derived midbrain organoids as a human-relevant *in vitro* model to investigate miRNA dysregulation in PSP-RS and PD. These 3D organoids replicate key features of midbrain architecture, including the presence of dopaminergic neurons, glial cell populations, and hallmark disease-related proteinopathies.[19,20] Next, we performed small RNA sequencing on midbrain organoids generated from patients with sporadic PSP-RS, PD, and neurologically healthy controls (HCs) across multiple developmental time points to uncover miRNA signatures with diagnostic potential. Our findings reveal distinct and temporally consistent miRNA expression profiles that differentiate PSP-RS from both PD and healthy controls, as well as PD from controls, establishing a framework for the future development of miRNA-based biomarkers for early and accurate differential diagnosis of parkinsonian syndromes.

RESULTS

Midbrain organoids recapitulate key molecular and cellular features of the human midbrain

To model PD and PSP-RS in a physiologically relevant system, we generated iPSCs from four patients with sporadic PD, four with sporadic PSP-RS, and three healthy controls (HCs). A comprehensive list of single nucleotide variants (SNVs) associated with late-onset PD and PSP-RS is provided in Table S1. These variants were analyzed in our patient cohort using whole-genome sequencing data from our donor-derived lines. For PD, we specifically analyzed *SNCA*, *LRRK2*, *VPS35*, and *GBA*; for PSP, we analyzed *MAPT*. No causative or likely pathogenic variants were identified in any of the samples, supporting the notion that our patients are indeed sporadic. Demographic and clinical data for PSP-RS patients and healthy donors, previously reported,[21] are summarized in Table S2, together with corresponding information for PD patients. iPSCs from each group were pooled to generate representative midbrain organoids that capture disease-specific molecular phenotypes. This approach was adapted to minimize batch-specific technical variability and to enhance the detection of convergent, disease-associated miRNA signatures across multiple sporadic donors with PSP-RS and PD.[22] Prior characterization of these lines has been reported,[21,23] with additional data on PD-3 provided in Figure S1. Quantitative PCR (qPCR) confirmed strong upregulation of pluripotency markers (*OCT4*, *NANOG*, and *SOX2*) relative to parental lymphocytes (Figure S1A). Immunofluorescence confirmed NANOG, OCT4, SOX2, and TRA 1–60 expression in PD-3 iPSC colonies (Figure S1B). Trilineage differentiation potential of PD-3 iPSCs was assessed by qPCR for *GATA4* (endoderm), *HAND1* (mesoderm), and *TUBB3* (ectoderm) (Figure S1C), and immunofluorescence for BRAT (mesoderm), NESTIN (ectoderm), and SOX17 (endoderm) (Figure S1D), confirming successful trilineage differentiation capability. Organoid differentiation was tracked through integrated morphological, transcriptional, and immunocytochemical analyses. At days 4, 8, and 20, all lines formed spherical neuroepithelial structures (Figure 1A). Day 20 qPCR revealed robust induction of early midbrain markers, including *CNPY1A1*, *CORIN*, *EN1*, *NGN2*, *FOXA2*, *LMX1A/B*, *SHH*, *TH*, and *OTX1/2* (Figure 1B). Immunofluorescence confirmed spatial localization of midbrain identity markers: FOXA2 and LMX1A co-expression defined ventral midbrain progenitors (Figure 1C), and ZO-1 marked apical tight junctions surrounding $FOXA2^+$ rosettes (Figure 1D). By day 60, organoids expressed mature dopaminergic markers (*TH*, *NURR1* (*NR4A2*), *DDC*, *GIRK2*, *CALB1*, and *DAT*) (Figure 1E). Immunostaining confirmed TH-positive neurons co-expressing GIRK2 (a subtype-specific potassium channel), and CALB1 (calbindin) (Figure 1F), along with DDC (Dopa decarboxylase) and NFL (neurofilament light chain), indicating successful differentiation into mature midbrain dopaminergic neurons (Figure 1G).

PD-derived organoids accumulate phosphorylated and oligomeric α-synuclein

To confirm the pathological relevance of the PD-derived organoids, we analyzed the α-synuclein phosphorylation and aggregation. Western blotting at day 90 revealed a significantly elevated phosphorylated α-synuclein at Ser129 (pS129) in PD organoids versus HC, with a prominent 14 kDa band (Figures 2A and 2B). High-molecular-weight oligomers (~238 kDa and ~55 kDa), indicative of α-synuclein multimers, were detected (Figure 2C), and densitometric analysis confirmed an increased oligomeric burden (Figure 2D). Intriguingly, Syn129 immunofluorescence revealed the presence of intracellular Lewy body-like inclusions in PD organoids, recapitulating a key pathological hallmark of Parkinson's disease (Figure 2E). Tyrosine hydroxylase (TH) levels were significantly reduced in PD compared to HC, reflecting dopaminergic neuron loss (Figure 2F). These findings validate the midbrain organoids as a physiologically relevant *in vitro* model for recapitulating α-synuclein pathology characteristic of Parkinson's disease. We previously demonstrated hallmark features of PSP-RS in this system, including neurofibrillary tangles, tufted astrocytes, and gliosis,[21] further highlighting its utility as a versatile and disease-relevant model for neurodegenerative disorders.

Time-resolved miRNA profiling reveals progressive, disease-specific expression signatures

To examine dynamic miRNA changes, we performed small RNA sequencing at days 60, 90, and 120. In PSP-RS vs. HC, 142 miRNAs were upregulated and 151 downregulated at day 60; increasing to 227 and 221, respectively, by day 120 (Figure 3A). PD vs. HC showed 231 upregulated and 195 downregulated miRNAs at day 60, rising to 262 and 234 by day 120 (Figure 3B). PSP-RS vs. PD comparisons revealed increasing divergence, with 120 upregulated and 158 downregulated miRNAs at day 60, and 163 and 230 at day 120 (Figure 3C). These patterns emphasize progressive, disease-specific miRNA dysregulation. We focused on persistently upregulated miRNA across all time points, prioritizing them for their therapeutic tractability. In PSP-RS vs. HC, 69 miRNAs (23%) were consistently elevated (Figure 3D); in PD vs. HC, 102 miRNAs (30.8%) met this criterion (Figure 3E). PSP-RS vs. PD revealed 34 miRNAs (15.7%) with sustained differential expression (Figure 3F). These stable

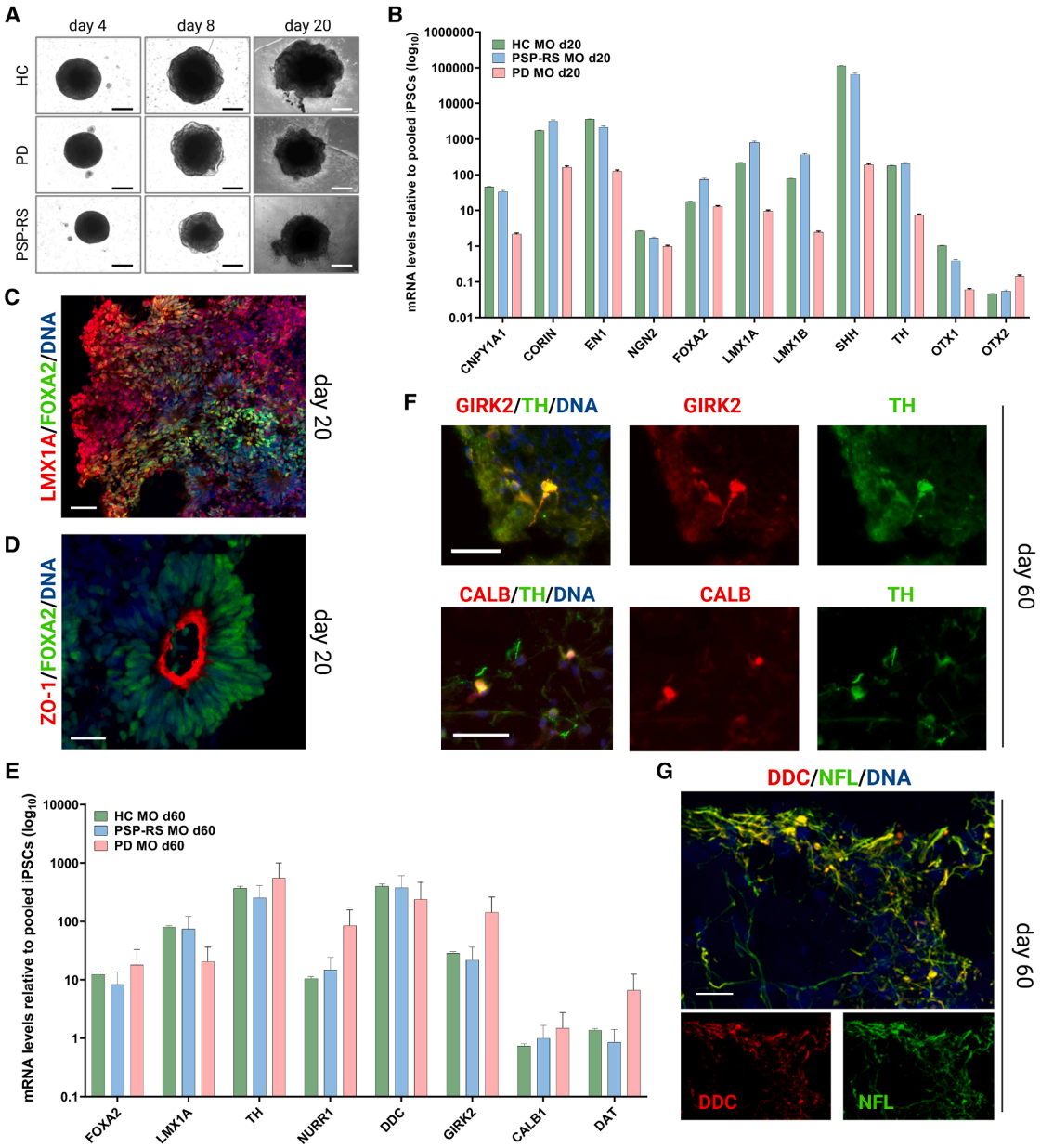

Figure 1. Generation of midbrain organoids (MOs)

(A) Representative brightfield images showing morphological development of midbrain organoids derived from healthy controls (HC), Parkinson's disease (PD), and progressive supranuclear Palsy-Richardson syndrome (PSP-RS) iPSCs at days 4, 8, and 20. Scale bars, 200 μm.

(B) Quantitative RT-PCR analysis of early midbrain progenitor markers at day 20. Data are expressed as fold change relative to pooled undifferentiated iPSCs (mean ± SEM, $n = 3$).

(C and D) Immunofluorescence staining at day 20 confirms midbrain regional identity and neuroepithelial organization, showing co-expression of FOXA2 with LMX1A (C) and ZO-1 (D). Nuclei are counterstained with DAPI (blue). Scale bars: 50 μm (C), 10 μm (D).

(E) RT-qPCR analysis of mature midbrain neuronal markers at day 60 demonstrates ongoing MO maturation across all conditions. Data are presented as fold change relative to undifferentiated iPSCs (mean ± SEM, $n = 3$).

(F) Immunofluorescence at day 60 confirms specification of dopaminergic neuronal subtypes, showing co-expression of TH with GIRK2 (A9 lineage, upper panels) and with CALB (A10 lineage, lower panels). Scale bars, 50 μm.

(G) Immunostaining for DDC (dopaminergic marker) and NFL (neuronal marker) at day 60 further validates midbrain neuronal identity. Scale bar, 50 μm. Nuclei are counterstained with DAPI (blue).

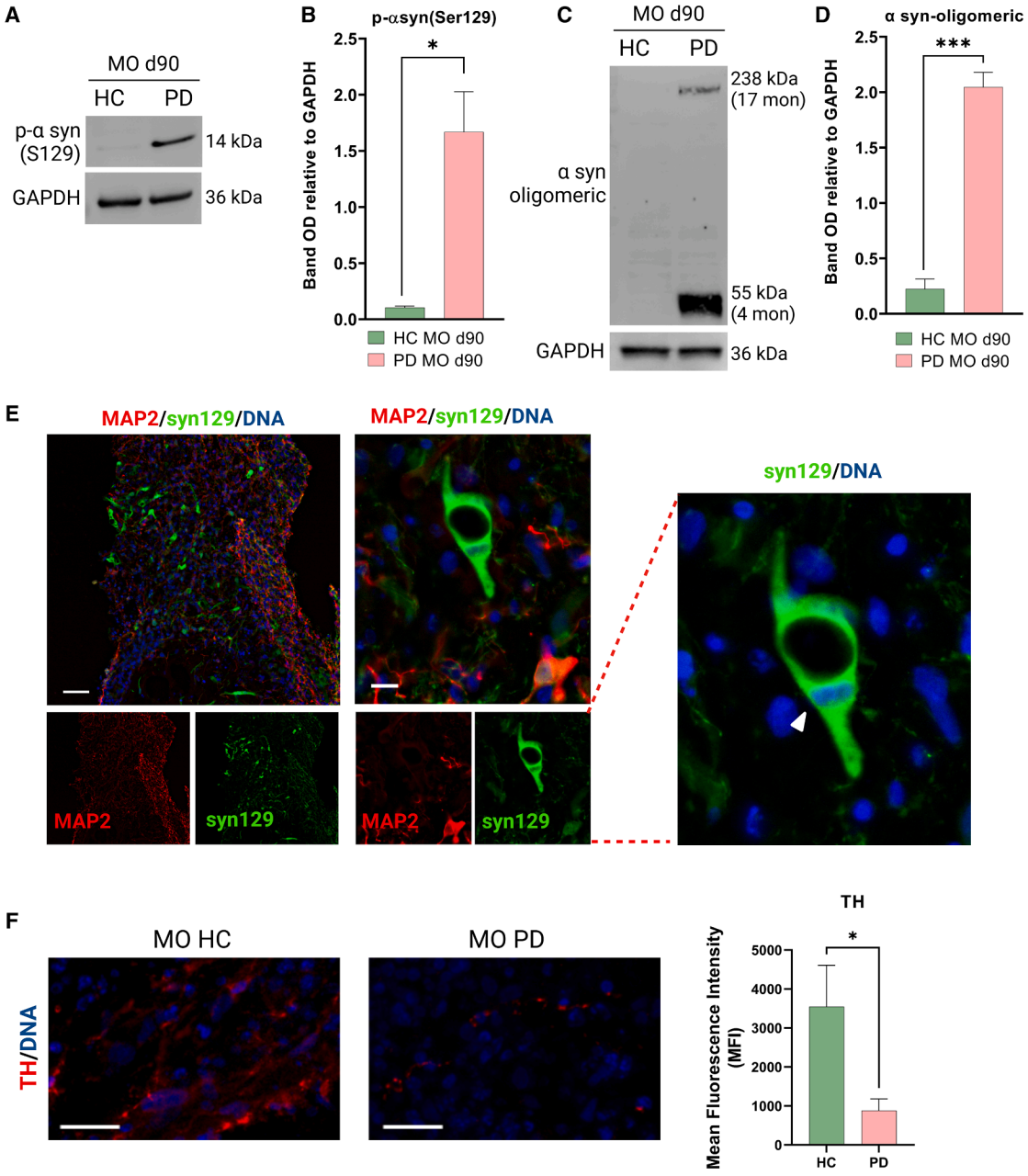

Figure 2. PD-derived MOs recapitulate hallmark pathological features

(A) Western blot analysis at day 90 reveals increased levels of phosphorylated α-synuclein at Ser129 (pS129) in PD midbrain organoids (MOs) compared to healthy controls (HC). GAPDH was used as a loading control.

(B) Densitometric quantification of pS129-α-synuclein levels normalized to GAPDH. Data are presented as mean ± SEM ($n = 3$). $p < 0.05$, Welch's t test.

(C) Immunoblot showing elevated levels of oligomeric and tetrameric α-synuclein in PD MOs relative to controls. Molecular weights are indicated for α-synuclein multimers.

(D) Quantification of oligomeric α-synuclein signal intensity normalized to GAPDH. Data represent mean ± SEM ($n = 3$). $^{**}p < 0.001$, Welch's t test.

(E) Immunofluorescence analysis of PD MO cryosections stained for MAP2 and pS129-α-synuclein reveals intracellular inclusions resembling Lewy body-like structures (white arrowhead, enlarged in right panel). Nuclei were counterstained with DAPI. Scale bars, 50 μm; 10 μm higher magnification.

(F) Immunostaining for tyrosine hydroxylase (TH) and quantification of mean fluorescence intensity (MFI) show reduced dopaminergic marker expression in PD MOs compared to HC. Data are presented as mean ± SEM from at least 12 regions of interest (ROIs) across 3 biological replicates. $p < 0.05$, Welch's t test. Scale bars, 25 μm.

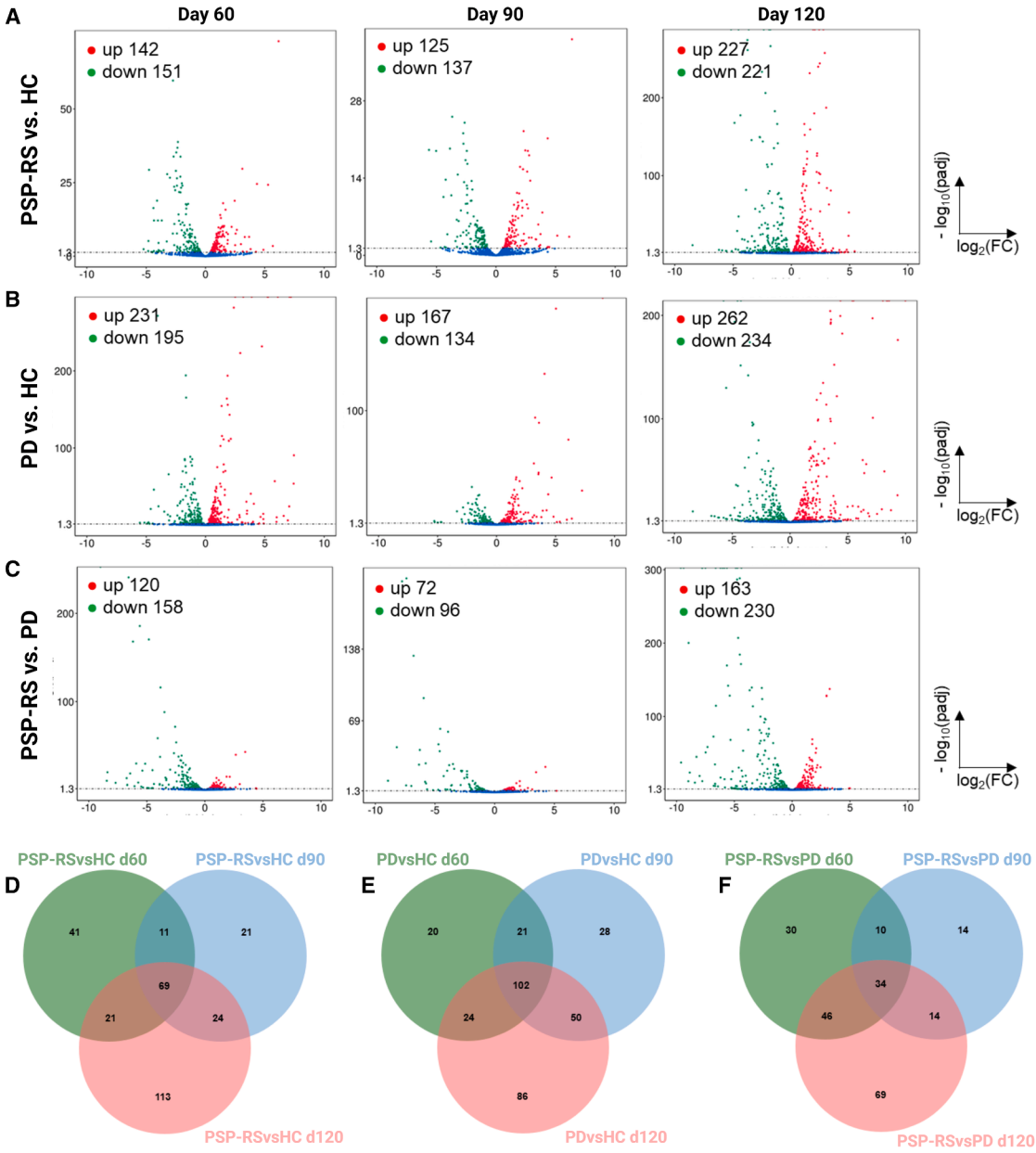

Figure 3. Differential expression of miRNAs in midbrain organoids across disease conditions and time points

(A–C) Volcano plots showing differentially expressed miRNAs at days 60, 90, and 120 for: (A) PSP-RS vs. healthy controls (HC), (B) PD vs. HC, and (C) PSP-RS vs. PD. Upregulated miRNAs (log_2 fold change >1, adjusted p value <0.05) are shown in red; downregulated miRNAs (log_2 fold change < −1, adjusted p value <0.05) are shown in green.

(D–F) Venn diagrams representing overlapping differentially expressed miRNAs across all three time points for: (D) PSP-RS vs. HC (n = 69), (E) PD vs. HC (n = 102), and (F) PSP-RS vs. PD (n = 34).

signatures were prioritized for target validation and pathways analysis. The complete list of common upregulated miRNAs is provided in Tables S3, S4, and S5.

Stable, disease-specific miRNA signatures distinguish PSP-RS and PD

To identify high-confidence candidate miRNAs, we filtered for those consistently upregulated across all three time points (day 60, 90, and 120) with average log_2 fold change >1.5. In the PSP-RS vs. HC comparison, nine miRNAs met this criterion: miR-5683, miR-3085-3p, miR-138-2-3p, miR-124-3p, miR-767-5p, miR-132-3p, miR-105-5p, miR-212-5p, and miR-129-5p (Table 1). miR-3085-3p showed the largest increase, from log_2 FC = 2.46 (day 60) to 4.75 (day 120). In PD vs. HC, 33 miRNAs were significantly upregulated, including miR-133b, miR-3120-3p, miR-214-5p, miR-199a-5p, miR-133a-5p, miR-10b-5p,

Table 1. Consistently upregulated miRNAs in PSP-RS compared to HC across all time points with log2 fold change >1.5

PSP-RS vs. HC	day 60		day 90		day 120		Average
miRNA	*log2FC*	*padj*	*log2FC*	*padj*	*log2FC*	*padj*	*log2 FC*
miR-5683	2.46	1.42e-19	2.78	7.47e-19	3.38	1.47e-81	2.88
miR-3085-3p	2.46	0.025	4.34	0.030	4.75	1.71e-05	3.85
miR-138-2-3p	1.67	0.005	2.23	2.26e-09	3.06	1.19e-84	2.32
miR-124-3p	1.59	2.06e-07	1.75	4.54e-09	2.30	1.51e-240	1.88
miR-767-5p	1.15	2.42e-08	1.41	3.50e-07	2.37	2.26e-91	1.65
miR-132-3p	1.09	1.66e-10	2.31	3.05e-23	2.39	0	1.93
miR-105-5p	1.07	2.80e-07	1.76	4.68e-12	2.36	4.07e-104	1.73
miR-212-5p	0.85	5.56e-05	1.95	6.02e-12	2.47	1.57e-102	1.76
miR-129-5p	0.63	0.005	2.45	9.50e-20	2.75	0	1.95

miR-3120-5p, miR-1-3p, miR-1245b-3p, miR-199a-3p, miR-133a-3p, miR-10b-3p, miR-143-3p, miR-145-3p, miR-143-5p, miR-193a-5p, miR-483-3p, miR-196b-5p, miR-199b-5p, miR-542-3p, miR-1299, miR-140-3p, miR-483-5p, miR-140-5p, miR-486-5p, miR-486-3p, miR-455-3p, miR-450a-5p, miR-1247-5p, miR-450b-5p, miR-455-5p, miR-212-5p, and miR-574-5p (Table 2). Comparative analysis between PSP-RS and PD identified four miRNAs selectively enriched in PSP-RS: miR-5683, miR-873-5p, miR-219a-2-3p, and miR-219b-5p (Table 3), which were subsequently prioritized for downstream target validation.

Top-ranked miRNAs and target networks define disease-specific regulatory programs

To refine disease-specific miRNA signatures, we selected the top upregulated miRNAs with the lowest adjusted *p* values from each pairwise comparison. A heatmap of TPM-normalized expression values (Figure 4A) revealed distinct sample clustering by disease group, underscoring the discriminatory capacity of the selected miRNAs. In the PSP-RS group, the most prominently upregulated miRNAs included miR-5683, miR-3085-3p, miR-138-2-3p, and miR-124-3p (Figure 4B). In contrast PD organoids exhibited increased expression of miR-1-3p, miR-133b, miR-199a-5p, and miR-10b-5p (Figure 4C); the direct comparison between PSP-RS and PD revealed selective enrichment of miR-5683, miR-873-5p, miR-219a-2-3p, and miR-219b-5p (Figure 4D). In PSP-RS organoids, miRNA target analysis revealed significant repression of *CCNB1*, *POLQ*, and *SLC7A11* (targets of miR-5683); *QPCTL* (miR-3085-3p); *REST*, *RPL7L1*, and *VPS13A* (miR-138-2-3p); and *VIM*, *SOX9*, and *CDK6* (miR-124-3p), compared to HC (Figure 4E). In PD organoids, validated repressed targets included *GJA1*, *ZNF215*, and *ZZZ3* (miR-1-3p); *HIF1A*, *ATF6*, and *GSK3B* (miR-199a-5p); *SESN3*, *CXCR4*, and *RB1CC1* (miR-133b); as well as *RPS8*, *RPS15A*, and *BRCA1* (miR-10b-5p) (Figure 4F). Direct comparison between PSP-RS and PD further highlighted confirmed selective repression of *POLQ*, *USP1*, and *HNRNPU* (miR-5683); *ZNF480*, *SPB1*, and *CANX* (miR-219b-5p); *CDK3*, *EN2*, and *ADAR* (miR-873-5p); and *CCNE2*, *SESN3*, and *CDK6* (miR-219a-2-3p) (Figure 4G). To validate the robustness of these miRNA signatures identified in pooled midbrain organoids, we examined their expression patterns in individual organoids derived from single iPSCs lines (PSP-RS-1, PD-1, and HC-1). Consistent with pooled data, miR-5683, miR-3085-3p, miR-138-2-3p, and miR-124-3p were significantly upregulated in PSP-RS-1 organoids relative to HC-1 (Figure S2A). Similarly, miR-1-3p and miR-10b-5p were elevated in PD-1 compared to HC-1 (Figure S2B). The PSP-RS-1 vs. PD-1 comparisons confirmed significantly higher expression of miR-5683, miR-219b-5p, miR-873-5p, and miR-219a-2-3p in PSP-RS organoids (Figure S2C). We next assessed expression of selected miRNA target genes, as identified via integrative target prediction and pathway enrichment. In PSP-RS-1 organoids, target genes including *CCNB1* and *POLQ* (miR-5683), *QPCTL* (miR-3085-3P), *RPL7L1* and *VPS13A* (miR-138-2-3p), *SOX9* and *CDK6* (miR-124-3p) were significantly downregulated relative to HC-1 (Figure S2D). In PD-1, targets such as *GJA1* and *ZNF215* (miR-1-3p), *RPS15A*, and *BRCA1* (miR-10b-5p) showed reduced expression compared to HC-1 (Figure S2E). Comparison of PSP-RS-1 and PD-1 revealed selective downregulation of *POLQ* and *USP1* (miR-5683), *EN2* and *ADAR* (miR-873-5p), *ZNF480*, and *SPB1* (miR-219b-5p), *CDK6* and *SESN3* (miR-219a-2-3p) in PSP-RS-1 (Figure S2F).

Gene ontology analysis of selected miRNA target genes

To investigate the biological relevance of candidate miRNAs, we performed gene ontology (GO) enrichment analysis on their predicted target genes to identify associated pathways and functional categories. Enrichment profiles were generated for each comparison PSP-RS vs. HC (Figure S3A), PD vs. HC (Figure S3B), and PSP-RS vs. PD (Figure S3C) to identify distinct functional pathways associated with disease-specific miRNA targets. To further refine mechanistic insights, we performed individual GO enrichment analyses for each candidate miRNA, enabling the identification of miRNA-specific functional signatures. In PSP-RS, miR-5683 and miR-124-3p targets were enriched in pathways related to synaptic organization, cytoskeletal remodeling, organelle fission, spindle microtubule attachment to kinetochores, and chromosome segregation (Figure 5A). While some of these functions are associated with mitosis, they may reflect disruptions in glial or progenitor dynamics relevant to PSP pathology. In PD, targets of miR-133b, miR-199a-5p, miR-10b-5p, and miR-1-3p were enriched in pathways linked to synaptic function, mitochondrial homeostasis, and oxidative stress response, hallmarks feature of α-synuclein pathology

Table 2. Consistently upregulated miRNAs in PD compared to HC across all time points with log2 fold change >1.5

PD vs. HC	day 60		day 90		day 120		Average
miRNA	*log2FC*	*padj*	*log2FC*	*padj*	*log2FC*	*padj*	*log2FC*
miR-133b	7.25	1.40e-30	7.43	1.95e-54	9.33	5.85e-177	12.00
miR-3120-3p	4.38	2.52e-27	7.06	7.72e-25	8.17	7.76e-50	6.54
miR-214-5p	4.38	2.52e-27	7.05	7.83e-25	8.17	7.76e-50	6.54
miR-199a-5p	4.06	4.72e-133	7.08	0	8.36	0	6.50
miR-133a-5p	4.83	1.04e-07	4.87	3.48e-15	9.30	9.01e-27	6.34
miR-10b-5p	3.16	2.06e-54	7.19	0	8.57	0	6.31
miR-3120-5p	3.39	1.14e-07	6.59	1.56e-12	8.74	2.65e-12	6.24
miR-1-3p	5.03	3.15e-190	5.22	0	8.42	0	6.22
miR-1245b-3p	5.36	0.003	5.98	8.62e-05	6.86	1.90e-06	6.07
miR-199a-3p	3.60	4.39e-90	6.10	0	8.03	0	5.91
miR-133a-3p	0.67	0.036	4.75	1.54e-232	7.14	6.45e-198	4.17
miR-10b-3p	2.47	0.001	6.00	1.03e-10	7.94	3.22e-13	5.47
miR-143-3p	3.54	6.34e-45	5.28	0	6.42	3.43e-48	5.08
miR-145-3p	2.81	0.0003	4.87	2.49e-08	6.76	1.93e-09	4.82
miR-143-5p	3.19	6.14e-09	2.37	2.91e-11	5.42	2.32e-16	3.66
miR-193a-5p	2.06	2.55e-05	4.07	2.75e-28	6.60	6.88e-57	4.25
miR-483-3p	2.30	6.66e-10	3.44	6.11e-31	6.29	8.34e-61	4.01
miR-196b-5p	2.97	0.0025	3.89	0.0074	3.90	0.0014	3.59
miR-199b-5p	2.18	1.19e-32	3.18	0	5.39	0	3.58
miR-542-3p	1.64	1.94e-40	2.50	0	5.50	0	3.21
miR-1299	2.67	2.86e-11	2.88	7.76e-11	3.89	1.59e-20	3.15
miR-140-3p	1.08	4.22e-20	3.90	0	4.36	0	3.11
miR-483-5p	2.10	1.07e-05	2.68	8.04e-08	3.95	1.69e-18	2.91
miR-140-5p	0.88	1.12e-06	2.93	6.73e-224	4.52	4.18e-183	2.78
miR-486-5p	1.86	7.95e-18	1.97	7.79e-110	3.54	1.15e-196	2.46
miR-486-3p	1.87	1.79e-17	1.96	4.10e-110	3.53	6.35e-194	2.45
miR-455-3p	0.86	0.0001	2.13	1.60e-112	3.48	6.42e-205	2.16
miR-450a-5p	1.13	8.59e-07	1.35	2.32e-17	3.52	2.86e-98	2.00
miR-1247-5p	1.14	0.010	1.92	0.0003	2.60	4.96e-05	1.89
miR-450b-5p	0.80	3.23e-09	1.23	1.95e-54	3.51	1.32e-192	1.85
miR-455-5p	0.94	6.11e-10	1.80	1.08e-164	2.64	2.39e-125	1.79
miR-212-5p	0.49	0.037	1.63	1.40e-46	2.78	7.93e-60	1.63
miR-574-5p	0.44	0.027	0.96	3.06e-18	3.14	1.93e-114	1.51

(Figure 5B). In the PSP-RS vs. PD comparison, miR-219a-2-3p and miR-219b-5p targets were associated with mRNA transport, p53 signaling, and glycosyltransferase activity (Figure 5C). miR-5683 emerged as a consistently enriched and discriminatory miRNA across all comparisons, reinforcing its biomarker potential. miRNAs without validated targets or significant enrichment (*e.g.*, miR-3085-3p, miR-138-2-3p, and miR-873-5p) were excluded from GO analysis. To further investigate the relevance of disease-associated miRNAs, we performed KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis on their predicted targets (Figure S4). In PSP-RS organoids, miR-124-3p was linked to pathways involved in intracellular signaling and cytoskeletal dynamics, including PI3K-Akt, MAPK, Ras, and Rap1 signaling (Figure S4A). In PD organoids, miR-1-3p and miR-133b enriched similar signaling pathways, with additional involvement in dopaminergic synapse and JAK-STAT signaling (Figures S4B and S4C). miR-199a-5p and miR-10b-5p were associated with apoptosis, cellular senescence, and p53 signaling (Figures S4D and S4E), underscoring stress-related mechanisms in PD. Notably, miR-219a-2-3p, differentially expressed in PSP-RS versus PD, selectively enriched the p53 pathway (Figure S4F), suggesting a disease-specific regulatory role. These analyses reveal both shared and distinct miRNA-driven pathway alterations in PSP-RS and PD.

Dysregulation of oxidative stress, mitochondrial dynamics, and apoptosis in PSP-RS and PD organoids

To link miRNA profiles to functional outcomes, we examined expression of genes and proteins involved in oxidative stress, mitochondrial dynamics, and apoptosis in midbrain organoids.

Table 3. Consistently upregulated miRNAs in PSP-RS compared to PD across all time points with log2 fold change >1.5

PSP-RS vs. PD	day 60		day 90		day 120		Average
miRNA	*log2FC*	*padj*	*log2FC*	*padj*	*log2FC*	*padj*	*log2FC*
miR-5683	1.87	1.14e-15	2.10	2.00e-11	2.45	1.43e-31	2.14
miR-873-5p	1.31	1.09e-07	1.82	1.94e-09	2.03	3.42e-51	1.72
miR-219b-5p	0.76	1.93e-08	1.24	4.74e-05	2.97	1.64e-128	1.66
miR-219a-2-3p	0.76	1.91e-08	1.24	4.74e-05	2.98	3.38e-129	1.66

qPCR revealed disease-specific alterations in antioxidant gene expression. In PSP-RS organoids, *SOD2* and *GPX1* were downregulated, while *NRF2* expression remained unchanged. In contrast, PD organoids exhibited significant downregulation of *SOD2*, *GPX1*, and *NRF2*, suggesting a more extensive disruption of the antioxidant defense pathway (Figure 6A). Oxidative stress was further evaluated at the protein level, with particular focus on NRF2, a master regulator of the antioxidant response. NRF2 expression was consistently reduced in both PD and PSP-RS organoids compared to HC (Figures 6B and S5A). Mitochondrial dynamics were also disrupted. PSP-RS organoids displayed reduced expression of MFN2, FIS1, OPA1, and DRP1, indicative of impaired mitochondrial fusion and fission processes. In contrast, PD organoids showed increased levels of MFN1, FIS1, and DRP1 alongside decreased OPA1, consistent with a shift toward enhanced mitochondrial fragmentation (Figure 6C). To further confirm mitochondrial dysfunction, we assessed the expression of PGC-1α and NDUFS1, key regulators of mitochondrial biogenesis and electron transport chain activity, respectively. Both proteins were significantly downregulated in PD and PSP-RS compared to HC; conversely, PARKIN, an E3 ubiquitin ligase essential for mitochondrial quality control through mitophagy,[24] was significantly upregulated in both PD and PSP-RS compared to HC (Figures 6D and S5B). In parallel, mitochondrial morphology was quantitatively assessed by immunostaining for TOM20, followed by image-based analysis using validated protocol for mitochondrial morphometric profiling.[25] This analysis revealed a marked increase in mitochondrial puncta and a concomitant reduction in elongated, interconnected networks in both disease groups, indicative of disrupted mitochondrial dynamics (Figure 6E). To investigate whether these alterations were associated with apoptotic signaling, we examined the expression of pro-apoptotic markers. *BAX* and *FAS*, along with stress-responsive genes *CELF2* and *PEG3*, were significantly upregulated in PD organoids (Figure S5C), suggesting activation of apoptotic and cellular stress pathways. Similar results were previously shown in PSP-RS organoids.[21] Apoptotic dysregulation was further confirmed at the protein level, with elevated expression of the pro-apoptotic marker BAX and reduced levels of the anti-apoptotic protein BCL-XL in both PSP-RS and PD organoids relative to HC (Figures 6F and S5D). These findings were corroborated by TUNEL assay, which revealed a significant increase in apoptotic cells in both disease groups, with PD displaying the highest proportion of TUNEL-positive cells ($p < 0.01$) (Figure 6G). Together, these results suggest that miRNA-mediated regulatory networks converge on key cellular oxidative pathways, namely oxidative stress, mitochondrial dysfunction, and apoptosis, in a disease-specific manner.

DISCUSSION

Although PSP-RS and PD share overlapping early clinical presentations, they are biologically distinct neurodegenerative disorders. PD is primarily associated with α-synuclein aggregation and dopaminergic neurodegeneration, while PSP-RS is marked by widespread deposition of hyperphosphorylated tau in the brainstem and basal ganglia regions.[5] This symptomatic overlap often delays accurate diagnosis and hampers early therapeutic intervention. Consequently, the identification of molecular biomarkers capable of distinguishing PD and PSP-RS at prodromal stages remains a critical unmet clinical need. Consequently, the identification of molecular biomarkers that can reliably distinguish between PD and PSP-RS, particularly at prodromal stages, remains a critical unmet clinical need. MicroRNAs (miRNAs), which regulate gene expression post-transcriptionally, have emerged as promising candidates for biomarker discovery in neurodegeneration.[26] Dysregulated miRNAs are known to influence neuroinflammation, synaptic function, mitochondrial dynamics, and apoptosis.[27–29] However, most miRNA studies to date rely on peripheral fluids, which may not accurately reflect the central nervous system (CNS) molecular landscape, particularly in early disease.[16] To address this gap, we employed iPSC-derived midbrain organoids as a physiologically relevant 3D model of PD and PSP-RS. These organoids recapitulate key aspects of midbrain architecture and neuropathology, including the presence of dopaminergic neurons, glial cell populations, and hallmark proteinopathies such as α-synuclein inclusions and tau tangles.[19,30,31] Importantly, the use of iPSC-derived midbrain organoids provides a human, three-dimensional neural model that enables the investigation of miRNA signatures within a disease-intrinsic and CNS-relevant context. This system supports longitudinal miRNA profiling under controlled experimental conditions, allowing the capture of molecular alterations directly associated with neuronal pathology while minimizing confounding influences from systemic factors, such as peripheral inflammation, age-related comorbidities, or medication exposure. To enhance the robustness of miRNA signal detection and mitigate batch-specific technical variability, we employed a donor-pooling strategy whereby iPSC lines from multiple donors within each group (PSP-RS, PD, and healthy controls) were combined prior to organoids differentiation. While this approach may obscure individual-specific variability, it facilitates the identification of convergent, disease-associated miRNA signatures that are reproducibly observed across genetically diverse backgrounds. This strategy is consistent with recent organoid-based transcriptomic frameworks, such as the study by Bolaños et al.,[22] which

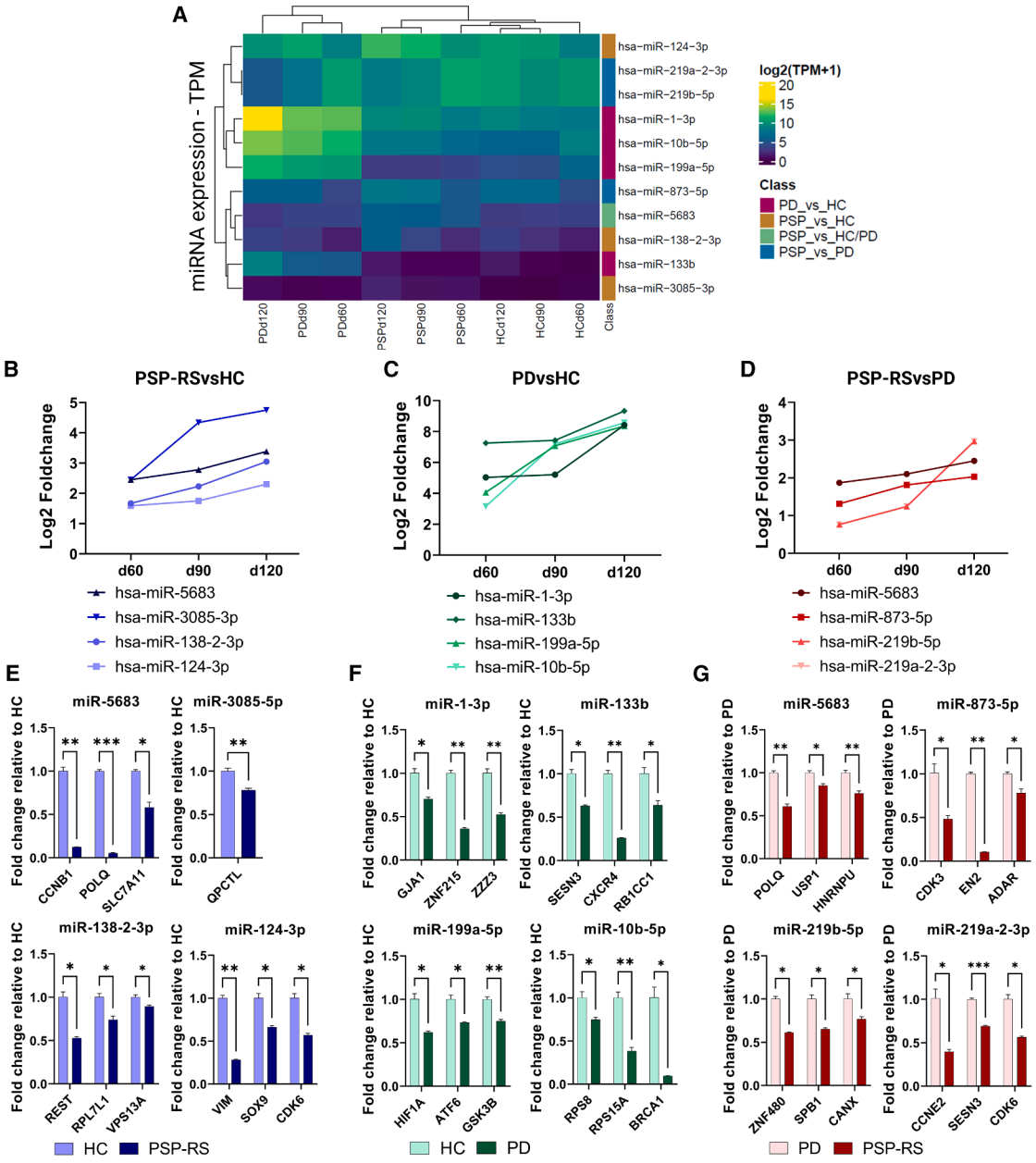

Figure 4. Identification and validation of differentially expressed miRNAs and their predicted target genes

(A) Heatmap displaying the expression profiles (TPM: transcripts per million) of selected differentially expressed miRNAs across healthy control (HC), Parkinson's disease (PD), and PSP-Richardson syndrome (PSP-RS) midbrain organoids at different time points. Unsupervised clustering reveals disease- and condition-specific miRNA expression patterns.

(B–D) Temporal expression trends of representative miRNAs in: (B) PSP-RS vs. HC, (C) PD vs. HC, (D) PSP-RS vs. PD. Expression values are shown as $\log_2$ fold change at days 60, 90, and 120.

(E–G) RT-qPCR validation of predicted target genes of selected differentially expressed miRNAs: (E) PSP-RS vs. HC, (F) PD vs. HC, (G) PSP-RS vs. PD. Bar graphs represent fold changes in gene expression relative to controls. Data are shown as mean ± SEM (n = 3). Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; Welch's t-test.

demonstrated that pooled differentiations yield reproducibility and cell-type specific molecular profiles. Our results uncovered temporally stable, disease-specific miRNA signatures that robustly differentiate PSP-RS from PD and from healthy controls. Notably, PSP-RS organoids exhibited consistent upregulation of miR-5683, miR-3085-3p, miR-138-2-3p, and miR-124-3p compared to control, and miR-5683, miR-873-5p, miR-219a-2-3p, and miR-219b-5p, compared to PD organoids. In

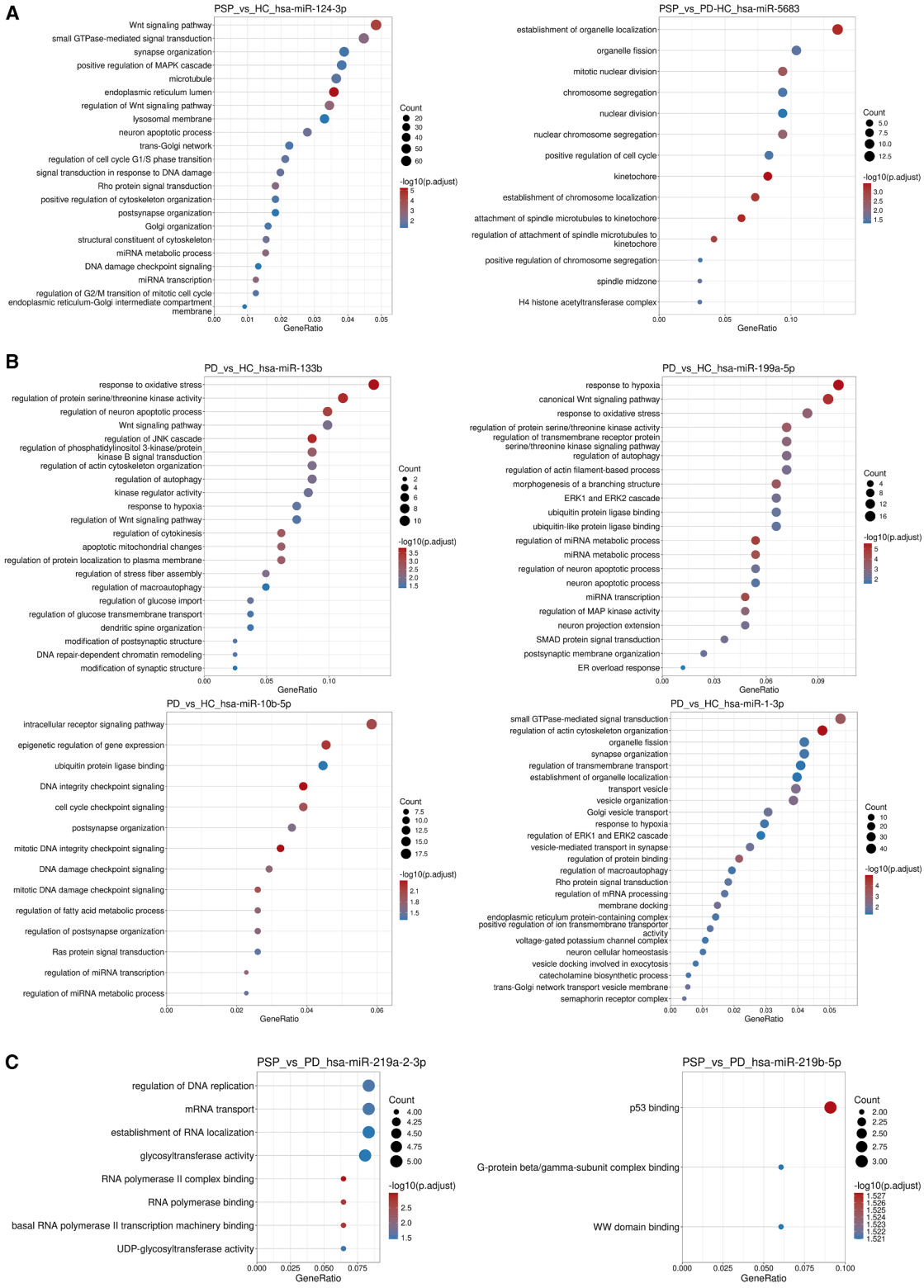

Figure 5. Gene ontology (GO) enrichment analysis of predicted target genes of differentially expressed miRNAs

(A) GO enrichment analysis of target genes for miR-124-3p (left) and miR-5683 (right), showing significantly enriched biological processes in PSP-RS vs. HC and PSP-RS vs. PD comparisons, respectively.

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contrast, PD organoids showed increased expression of miR-1-3p, miR-133b, miR-10b-5p, and miR-199a-5p. These distinct miRNA profiles align with the molecular pathologies of their respective diseases. Gene ontology (GO) enrichment analysis of validated targets revealed divergent regulatory networks. In PSP-RS, dysregulated miRNAs were associated with cytoskeletal remodeling, mitotic spindle organization, and chromosome segregation—hallmarks of tau-induced microtubule instability and aberrant activation of cell cycle pathways in postmitotic neurons. Specifically, miR-124-3p and miR-5683 regulated targets involved in mitotic control and organelle dynamics,[32] supporting their functional relevance in PSP-RS. In contrast, PD-associated miRNAs targeted genes linked to synaptic signaling, mitochondrial homeostasis, and oxidative stress—core processes affected by α-synuclein toxicity. For example, miR-133b, miR-10b-5p, and miR-199a-5p modulated pathways involving ERK/JNK signaling, glucose metabolism, and proteostasis, all implicated in PD pathogenesis. These findings are consistent with prior reports linking these miRNAs to dopaminergic dysfunction and neuronal stress responses. Among the differentially expressed miRNAs, the miR-219 family (miR-219a-2-3p and miR-219b-5p) emerged as robust PSP-RS-specific markers. These miRNAs regulate genes involved in mRNA transport, glycosylation, and p53 signaling,[33,34] suggesting roles in transcriptional homeostasis and neuronal integrity. Their selective upregulation in PSP-RS organoids and repression of targets, such as *ZNF480*, *CANX*, and *SPB1* implicate them in disease-specific regulatory programs.[5] Several of the top miRNA signatures identified in midbrain organoids align with previously reported clinical data from plasma or cerebrospinal fluid (CSF) studies, underscoring their potential translational relevance. For example, elevated plasma levels of miR-133b have been reported in early-stage PD patients.[35] Notably, miR-133b is enriched in midbrain dopaminergic neurons and plays a critical role in their differentiation and maintenance.[36] Its upregulation in PD organoids may therefore reflect pre-dendritic, compensatory changes aimed at preserving neuronal identity and function. Similarly, miR-1-3p, which has been reported as downregulated in whole blood of *de novo* PD patients but upregulated in early-onset PD,[37] showed increased expression in our PD organoids. This apparent dual behavior may reflect stage-specific regulation, with our model capturing early, preclinical disease states. In the context of PSP-RS, miR-873-3p has been found significantly elevated in the CSF of PSP patients from early to advanced disease stage.[38] Correspondingly, our PSP-RS organoids showed upregulation of the complementary strand, miR-873-5p, suggesting that dysregulation of the miR-873 duplex may represent an early, intrinsic molecular hallmark of tauopathy. Beyond the top four miRNAs highlighted in each comparison, we also identified several additional miRNAs that were consistently upregulated across days 60, 90, and 120 of differentiation and are supported by independent clinical evidence (Tables 1, 2, and 3). For instance, miR-214, significantly upregulated in serum of individuals with prodromal PD compared to both healthy controls and patients with advanced disease,[39] was consistently elevated in our PD organoids, suggesting a role in early pathophysiological responses preceding neurodegeneration. Similarly, miR-145-3p, previously found elevated in the saliva of PD patients,[40] was also upregulated in PD organoids, reinforcing its potential as non-invasive biomarker detectable both centrally and peripherally. In PSP-RS organoids, miR-138, implicated in tau pathology (phosphorylation and aggregation) and previously shown to be upregulated in Alzheimer's disease brain tissues,[41] was significantly elevated, suggesting conserved pathogenic roles across tauopathies. Collectively, these convergences between organoids derived and circulating miRNA signatures support the translational relevance of our findings and suggest that select miRNAs may serve as stable, disease-relevant biomarkers that originate from CNS pathology yet are accessible through peripheral biofluids. Although the primary aim of this study was not a comprehensive molecular characterization, we performed targeted validation to begin linking disease-specific miRNA signatures to functional outcomes. We investigated key stress-response pathways in midbrain organoids, focusing on mitochondrial homeostasis, redox balance, and programmed cell death. PSP-RS organoids exhibited clear signs of mitochondrial instability and impaired oxidative resilience, while PD organoids showed broader dysregulation, characterized by heightened oxidative stress and increased apoptotic susceptibility. These phenotypes were corroborated by TUNEL assays, which revealed elevated apoptosis in both disease models, with a more pronounced burden in PD. Together, these findings suggest that distinct miRNA regulatory networks drive disease-specific molecular cascades converging on mitochondrial dysfunction and cell death. Our results nominate select miRNAs as promising candidates for further validation in patient cohorts and underscore the utility of iPSC-derived midbrain organoids as a physiologically relevant platform for dissecting neurodegenerative mechanisms and discovering CNS-specific biomarkers.

Limitations of the study

To establish the clinical relevance of our findings, validation in larger, independent patient cohorts—including both peripheral biofluids and CNS-derived specimens such as cerebrospinal fluid and postmortem brain tissue—is essential. Furthermore, current midbrain organoid systems lack key cellular components, such as microglia and vasculature, and incompletely model mature neuronal circuitry. Future iterations incorporating vascularization and co-culture systems will enhance physiological fidelity. Finally, functional interrogation of candidate miRNAs

(B) GO analysis of predicted targets for miR-133b, miR-199a-5p, miR-10b-5p, and miR-1-3p, differentially expressed in PD vs. HC comparisons, highlighting key pathways related to oxidative stress, neuronal apoptosis, autophagy, and synaptic organization.
(C) GO terms enriched among predicted targets of miR-219a-2-3p and miR-219b-5p, which were differentially expressed in PSP-RS vs. PD. Enriched terms include RNA processing, transcription regulation, and protein binding functions. Each dot represents a significantly enriched GO term, with dot size indicating the number of genes (Count) associated with the term and color representing the adjusted *p* value ($\log_{10}$ *padj*). GeneRatio refers to the proportion of target genes associated with a given GO term.

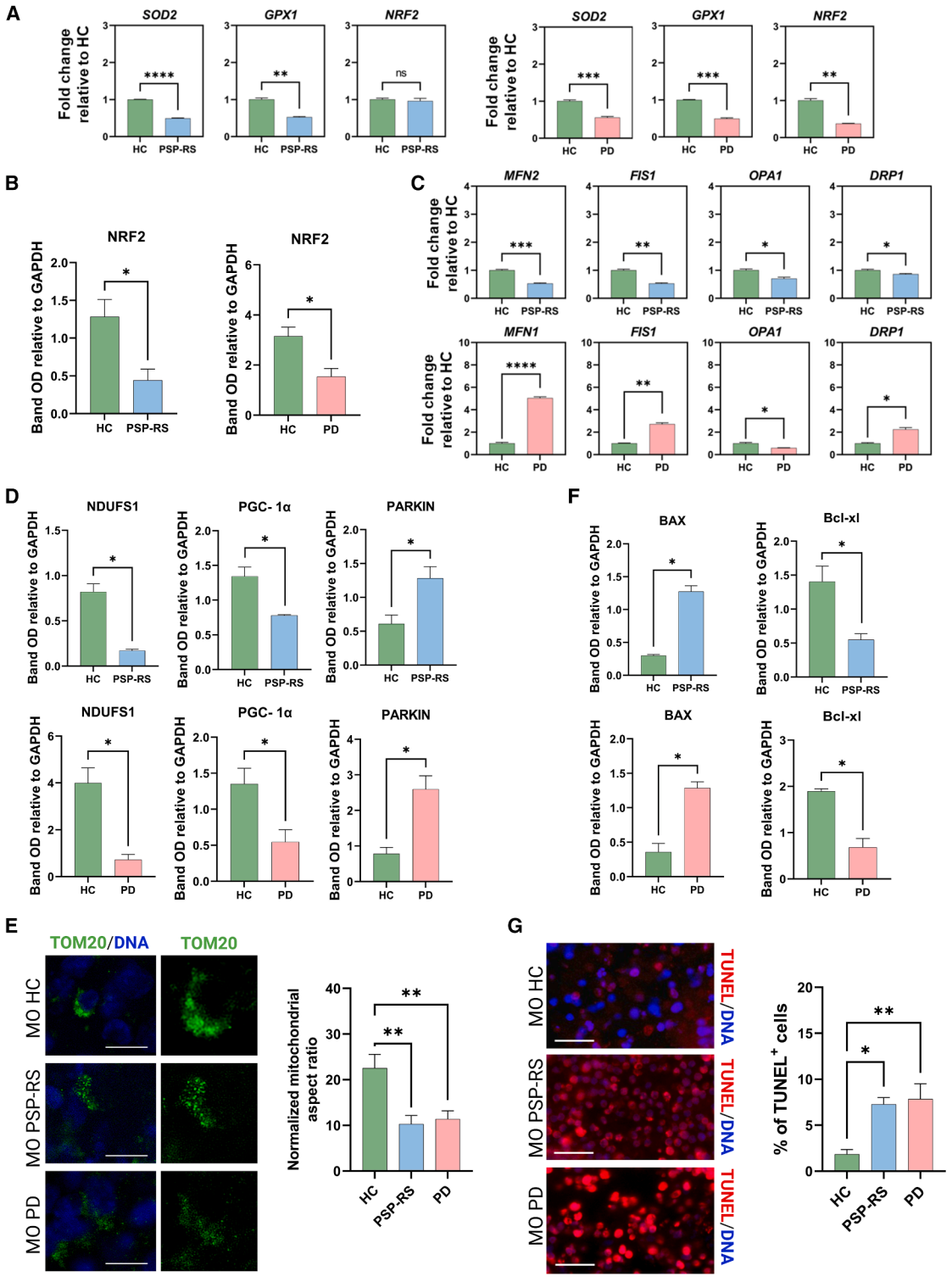

Figure 6. Mitochondrial dysfunction, oxidative stress, and DNA damage response impairments in PD and PSP-RS midbrain organoids

(A) RT-qPCR analysis of oxidative stress response genes (*SOD2*, *GPX1*, and *NRF2*) in PSP-RS and PD organoids compared to healthy controls (HC).

(B) Western blot quantification of NRF2 expression in PD and PSP-RS patient-derived samples relative to healthy controls.

(C) Expression of genes involved in mitochondrial dynamics (MFN2, FIS1, OPA1, and DRP1) in PSP-RS and PD organoids vs. HC.

(D) Western blot quantification of NDUFS1, PGC-1α, and PARKIN expression in PD and PSP-RS patient-derived samples relative to healthy controls.

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using tools, such as antagomirs or CRISPR-based knockout strategies will be critical to delineate their causal roles in PSP-RS and PD pathogenesis.

RESOURCE AVAILABILITY

Lead contact

Additional information and data are available from the lead contact, Dr. Elvira Immacolata Parrotta (parrotta@unicz.it) upon reasonable request.

Materials availability

Requests for materials and reagents should also be directed to the lead contact, Dr. Elvira Immacolata Parrotta (parrotta@unicz.it).

Data and code availability

- **Data**: the sRNA-seq data discussed in this paper have been deposited in NCBI's Gene Expression Omnibus[42] and are accessible through GEO Series accession number GSE294029 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE294029), as reported in the key resources table.
- **Code**: this paper does not report original code.
- **Additional information**: further details needed to replicate the analyses presented in this study can be obtained from the lead contact Dr. Elvira Immacolata Parrotta (parrotta@unicz.it) upon request.

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AUTHOR CONTRIBUTIONS

E.I.P. and G.C. conceived and designed the study. D.V., C.Z., S.S., V.L., G.L. B., M.G.T., and I.L. performed the experiments. D.V., C.Z., and S.S. conducted data analysis. D.V., C.Z., S.S., and D.B. generated the figures; D.B., F.C., E.F., and G.P. carried out the bioinformatics analyses. Andrea Quattrone and Aldo Quattrone were responsible for patient recruitment and provided clinical samples and data. E.I.P. and G.C. wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used ChatGPT in order to assist with English language editing. After using this tool or service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS
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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Owolabi, L. (2013). Progressive supranuclear palsy misdiagnosed as Parkinson's disease: a case report and review of literature. Ann. Med. Health Sci. Res. *3*, S44–S47. https://doi.org/10.4103/2141-9248.121221.
2. Stein, Z.L.-G. (1985). Parkinson's Disease and Progressive Supranuclear Palsy. Arch. Intern. Med. *145*, 952. https://doi.org/10.1001/archinte.1985.00360050222047.
3. Obeso, J.A., Stamelou, M., Goetz, C.G., Poewe, W., Lang, A.E., Weintraub, D., Burn, D., Halliday, G.M., Bezard, E., Przedborski, S., et al. (2017). Past, Present, and Future of Parkinson's Disease: A Special Essay on the 200th Anniversary of the Shaking Palsy. Mov. Disord. *32*, 1264–1310. https://doi.org/10.1002/mds.27115.
4. Grazia, S.M., and Goedert, M. (2018). Neurodegeneration and the ordered assemblymof α-synuclein. Cell Tissue Res. *373*, 137–148. https://doi.org/10.1007/s00441-017-2706-9.
5. Stamelou, M., de Silva, R., Arias-Carrión, O., Boura, E., Höllerhage, M., Oertel, W.H., Ulrich, M., and Höglinger, G.U. (2010). Rational therapeutic approaches to progressive supranuclear palsy. Brain *133*, 1578–1590. https://doi.org/10.1093/brain/awq115.
6. Dickson, D.W. (2012). Parkinson's disease and parkinsonism: neuropathology. Cold Spring Harb. Perspect. Med. *2*, a009258. https://doi.org/10.1101/cshperspect.a009258.
7. Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory funcions. Cell *136*, 215–233. https://doi.org/10.1016/j.cell.2009.01.002.

(E) Representative images of TOM20 immunofluorescence and quantification of mitochondrial morphology, showing mitochondrial puncta and interconnected networks expressed as normalized mitochondrial aspect ratio. At least 10 ROI were analyzed for each condition. Scale bar 10 μm (F) Elevated expression of the pro-apoptotic protein BAX and reduced levels of the anti-apoptotic protein BCL-XL in patient-derived samples compared to HC.
(G) Representative TUNEL staining images and quantification showing increased apoptosis in PD and PSP-RS organoids vs. HC. Nuclei counterstained with DAPI (blue). Scale bar, 25 μm. Quantification based on manual counting of at least 10,000 cells per condition. All data are presented as mean ± SEM from three biological replicates. Statistical significance: ns = not significant; $p < 0.05$, $p < 0.01$, $^{*}p < 0.001$, $^{**}p < 0.0001$. Welch's t test was used unless otherwise specified; Tukey's one-way ANOVA test was applied for (E) and (G).

8. Ha, M., and Kim, V.N. (2014). Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. *15*, 509–524. https://doi.org/10.1038/nrm3838.

9. Lau, P., and de Strooper, B. (2010). Dysregulated microRNAs in neurodegenerative disorders. Semin. Cell Dev. Biol. *21*, 768–773. https://doi.org/10.1016/j.semcdb.2010.01.009.

10. Lusardi, T.A., Phillips, J.I., Wiedrick, J.T., Harrington, C.A., Lind, B., Lapidus, J.A., Quinn, J.F., and Saugstad, J.A. (2017). MicroRNAs in Humand Cerebrospinal Fluid as Biomarkers for Alzheimer's Disease. J. Alzheimers Dis. *55*, 1223–1233. https://doi.org/10.3233/JAD-160835.

11. Roser, A.-E., Caldi Gomes, L., Schünemann, J., Maass, F., and Lingor, P. (2018). Circulating miRNAs as Diagnostic Biomarkers for Parkinson's Disease. Front. Neurosci. *12*, 625. https://doi.org/10.3389/fnins.2018.00625.

12. Burgos, K., Malenica, I., Metpally, R., Courtright, A., Rakela, B., Beach, T., Shill, H., Adler, C., Sabbagh, M., Villa, S., et al. (2014). Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. PLoS One *9*, e94839. https://doi.org/10.1371/journal.pone.0106174.

13. Ćwiklińska, A., Procyk, G., Koziorowski, D., and Szlufik, S. (2024). The role of MicroRNAs in Progressive Supranuclear Palsy-A Systematic Review. Int. J. Mol. Sci. *25*, 8243. https://doi.org/10.3390/ijms25158243.

14. Pavelka, L., Rauschenberger, A., Hemedan, A., Ostaszewski, M., Glaab, E., and Krüger, R.; NCER-PD Consortium (2024). Converging peripheral blood microRNA profiles in Parkinson's disease and progressive supranuclear palsy. Brain Commun. *6*, fcae187. https://doi.org/10.1093/braincomms/fcae187.

15. Schulz, J., Takousis, P., Wohlers, I., Itua, I.O.G., Dobricic, V., Rücker, G., Binder, H., Middleton, L., Ioannidis, J.P.A., Perneczky, R., et al. (2019). Meta-analyses identify differentially expressed microRNAs in Parkinson's disease. Ann. Neurol. *85*, 835–851. https://doi.org/10.1002/ana.25490.

16. Todorov, H., Weiβbach, S., Schlichtholz, L., Mueller, H., Hartwich, D., Gerber, S., and Winter, J. (2024). Stage-specific expression patterns and co-targeting relationships among miRNAs in the developing mouse cerebral cortex. Commun. Biol. *7*, 1366. https://doi.org/10.1038/s42003-024-07092-7.

17. Margis, R., Margis, R., and Rieder, C.R.-M. (2011). Identification of blood microRNAs associated with Parkinson's disease. J. Biotechnol. *152*, 96–101. https://doi.org/10.1016/j.jbiotec.2011.01.023.

18. Vallelunga, A., Ragusa, M., Di Mauro, S., Iannitti, T., Pilleri, M., Biundo, R., Weis, L., Di Pietro, C., De Iuliis, A., Nicoletti, A., et al. (2014). Identification of circulating microRNAs for the differential diagnosis of Parkinson's disease and Multiple System Atrophy. Front. Cell. Neurosci. *8*, 156. https://doi.org/10.3389/fncel.2014.00156.

19. Qian, X., Song, H., and Ming, G.-L. (2019). Brain organoids: advances, applications and challenges. Development *146*, dev166074. https://doi.org/10.1242/dev.166074.

20. Louise Nickels, S., Modamio, J., Mendes-Pinheiro, B., Monzel, A.S., Betsou, F., and Schwamborn, J.C. (2020). Reproducible generation of human midbrain organoids for *in vitro* modeling of Parkinson's disease. Stem Cell Res. *46*, 10187. https://doi.org/10.1016/j.scr.2020.101870.

21. Parrotta, E.I., Lucchino, V., Zannino, C., Valente, D., Scalise, S., Bressan, D., Benedetto, G.L., Iazzetta, M.R., Talarico, M., Gagliardi, M., et al. (2025). Modeling Sporadic Progressive Supranuclear Palsy in 3D Midbrain Organoids: Recapitulating Disease Features for In Vitro Diagnosis and Drug Discovery. Ann. Neurol. *97*, 845–859. https://doi.org/10.1002/ana.27172.

22. Antón-Bolaños, N., Faravelli, I., Tyler, F., Andreadis, S., Kastli, R., Trattato, S., Adiconis, X., Wei, A., Kumar, A.S., Di Bella, D.J., et al. (2024). Brain Chimeroids reveal individual susceptibility to neurotoxic triggers. Nature *631*, 1–8. https://doi.org/10.1038/s41586-024-07578-8.

23. Benedetto, G., Zannino, C., Valente, D., Covello, R., Scalise, S., Lucchino, V., Quattrone, A., Parrotta, E.I., Quattrone, A., and Cuda, G. (2025). Generation of hiPSCs lines from three sporadic Parkinson's disease patients. Stem Cell Res. *82*, 103632. https://doi.org/10.1016/j.scr.2024.103632.

24. Harper, J.W., Ordureau, A., and Heo, J.-M. (2018). Building and decoding ubiquitin chains for mitophagy. Nat. Rev. Mol. Cell Biol. *19*, 93–108. https://doi.org/10.1038/nrm.2017.129.

25. Nag, S., Szederkenyi, K., Yip, C.M., and McQuibban, G.A. (2023). Protocol for evaluating mitochondrial morphology changes in response to CCCP-induced stress through oper-source image processing software. STAR Protoc. *4*, 102745. https://doi.org/10.1016/j.xpro.2023.102745.

26. Krauskopf, J., Verheijen, M., Kleinjans, J.C., de Kok, T.M., and Caiment, F. (2015). Development and Regulatory Application of Microrna Biomarkers. Biomark. Med. *9*, 1137–1151. https://doi.org/10.2217/bmm.15.50.

27. Meza-Sosa, K.F., Valle-García, D., Pedraza-Alva, G., and Pérez-Martínez, L. (2012). Role of microRNAs in central nervous system development and pathology. J. Neurosci. Res. *90*, 1–12. https://doi.org/10.1002/jnr.22701.

28. Sharma, S., and Lu, H.-C. (2018). microRNAs in Neurodegeneration: Current Findings and Potential Impacts. J. Alzheimers Dis. Parkinsonism *8*, 420. https://doi.org/10.4172/2161-0460.1000420.

29. Juźwik, C.A., S Drake, S., Zhang, Y., Paradis-Isler, N., Sylvester, A., Amar-Zifkin, A., Douglas, C., Morquette, B., Moore, C.S., and Fournier, A.E. (2019). microRNA dysregulation in neurodegenerative diseases: A systematic review. Prog. Neurobiol. *182*, 101664. https://doi.org/10.1016/j.pneurobio.2019.101664.

30. Jusop, A.S., Thanaskody, K., Tye, G.J., Dass, S.A., Wan Kamarul Zaman, W.S., and Nordin, F. (2023). Development of brain organoid technology derived from iPSC for the neurodegenerative disease modelling: a glance through. Front. Mol. Neurosci. *16*, 1173433. https://doi.org/10.3389/fnmol.2023.1173433.

31. Becerra-Calixto, A., Mukherjee, A., Ramirez, S., Sepulveda, S., Sinha, T., Al-Lahham, R., De Gregorio, N., Gherardelli, C., and Soto, C. (2023). Lewy Body-like Pathology and Loss of Dopaminergic Neurons in Midbrain Organoids Derived from Familial Parkinson's Disease Patient. Cells *12*, 625. https://doi.org/10.3390/cells12040625.

32. Gao, J., Wang, L., Liu, J., Xie, F., Su, B., and Wang, X. (2017). Abnormalities of Mitochondrial Dynamics in Neurodegenerative Diseases. Antioxidants *6*, 25. https://doi.org/10.3390/antiox6020025.

33. Wong, T.-S., Li, G., Li, S., Gao, W., Chen, G., Gan, S., Zhang, M., Li, H., Wu, S., and Du, Y. (2023). G protein-coupled receptors in neurodegenerative diseases and psychiatric disorders. Signal Transduct. Target. Ther. *8*, 177. https://doi.org/10.1038/s41392-023-01427-2.

34. Sushma, A.C.M., and Mondal, A.C. (2019). Role of GPCR signaling and calcium dysregulation in Alzheimer's disease. Mol. Cell. Neurosci. *101*, 103414. https://doi.org/10.1016/j.mcn.2019.103414.

35. 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. Sci. Rep. *11*, 15268. https://doi.org/10.1038/s41598-021-94734-z.

36. Kim, J., Inoue, K., Ishii, J., Vanti, W.B., Voronov, S.V., Murchison, E., Hannon, G., and Abeliovich, A. (2007). A MicroRNA feedback circuit in midbrain dopamine neurons. Science *317*, 1220–1224. https://doi.org/10.1126/science.1140481.

37. Margis, R., Margis, R., and Carlos, R.M.R. (2011). Identification of blood microRNAs associated to Parkinson's disease. J. Biotechnol. *152*, 96–101. https://doi.org/10.1016/j.jbiotec.2011.01.023.

38. Nonaka, W., Takata, T., Iwama, H., Komatsubara, S., Kobara, H., Kamada, M., Deguchi, K., Touge, T., Miyamoto, O., Nakamura, T., et al. (2022). A cerebrospinal fluid microRNA analysis: Progressive supranuclear palsy. Mol. Med. Rep. *25*, 88. https://doi.org/10.3892/mmr.2022.12604.

39. Li, L., Ren, J., Pan, C., Li, Y., Xu, J., Dong, H., Chen, Y., and Liu, W. (2021). Serum miR-214 Serves as a Biomarker for Prodromal Parkinson's Disease (2021). Front. Aging Neurosci. *13*, 700959. https://doi.org/10.3389/fnagi.2021.700959.

40. Salaramoli, S., Hamid, R.J., and Hashemy, S.I. (2023). Salivary Biomarkers: Noninvasive Ways for Diagnosis of Parkinson's Disease. Neurol. Res. Int. *2023*, 3555418. https://doi.org/10.1155/2023/3555418.

41. Boscher, E., Goupil, C., Petry, S., Keraudren, R., Loiselle, A., Planel, E., and Hébert, S.S. (2021). MicroRNA-138 Overexpression Alters Aβ42 Levels and Behavior in Wildtype Mice (2021). Front. Neurosci. *14*, 591138. https://doi.org/10.3389/fnins.2020.591138.

42. Edgar, R., Domrachev, M., and Lash, A.E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository (2002). Nucleic Acids Res. *30*, 207–210. https://doi.org/10.1093/nar/30.1.207.

43. Parrotta, E.I., Scalise, S., Taverna, D., De Angelis, M.T., Sarro, G., Gaspari, M., Santamaria, G., and Cuda, G. (2019). Comprehensive proteogenomic analysis of human embryonic and induced pluripotent stem cells. J. Cell Mol. Med. *23*, 5440–5453. https://doi.org/10.1111/jcmm.14426.

44. Sozzi, E., Nilsson, F., Kajtez, J., Parmar, M., and Fiorenzano, A. (2022). Generation of Human Ventral Midbrain Organoids Derived from Pluripotent Stem Cells. Curr. Protoc. *2*, e555. https://doi.org/10.1002/cpz1.555.

45. Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. *10*, R25. https://doi.org/10.1186/gb-2009-10-3-r25.

46. Friedländer, M.R., Mackowiak, S.D., Li, N., Chen, W., and Rajewsky, N. (2012). miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res. *40*, 37–52. https://doi.org/10.1093/nar/gkr688.

47. Wen, M., Shen, Y., Shi, S., and Tang, T. (2012). miREvo: An Integrative microRNA Evolutionary Analysis Platform for next-generation sequencing experiments. BMC Bioinf. *13*, 140. https://doi.org/10.1186/1471-2105-13-140.

48. Ru, Y., Kechris, K.J., Tabakoff, B., Hoffman, P., Radcliffe, R.A., Bowler, R., Mahaffey, S., Rossi, S., Calin, G.A., Bemis, L., and Theodorescu, D. (2014). The multiMiR R package and database: integration of microRNA-target interactions along with their disease and drug association. Nucleic Acids Res. *42*, e133. https://doi.org/10.1093/nar/gku631.

49. Cui, S., Yu, S., Huang, H.-Y., Lin, Y.-C.-D., Huang, Y., Zhang, B., Xiao, J., Zuo, H., Wang, J., Li, Z., et al. (2025). miRTarBase 2025: updates to the collection of experimentally validated microRNA-target interactions. Nucleic Acids Res. *53*, D147–D156. https://doi.org/10.1093/nar/gkae1072.

50. Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., et al. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation *2*, 100141. https://doi.org/10.1016/j.xinn.2021.100141.