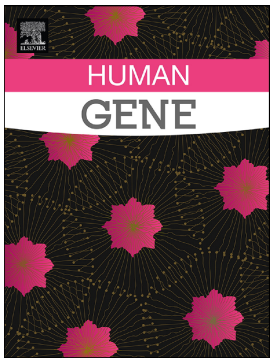

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Identification and Validation of Senescence-Related Genes in Parkinson's disease

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Identification and Validation of Senescence-Related Genes in Parkinson’s disease

Keywords: parkinson’s disease,senescence-raleted gene,machine learning,bioinformatics

Abstract
Parkinson's disease is a prevalent neurodegenerative disorder that is clinically identified by motor deficits. Its pathogenesis remains unclear and current treatments cannot reverse disease progression. Mounting evidence suggests that cellular senescence plays a crucial part in the development of Parkinson's disease. The analysis of genes related to aging in Parkinson's samples using bioinformatics has not been conducted so far. This study identified differentially expressed senescence genes using bioinformatics approaches and found genes RASL11B and PRRG1 to be highly correlated with Parkinson's, suggesting their potential as diagnostic and therapeutic targets. The miR-20 family of miRNAs may participate in Parkinson's pathogenesis by regulating these genes. Examining senescence genes within a senescence network framework, this study pioneers the investigation of their involvement in Parkinson's disease. It establishes the theoretical groundwork and identifies potential targets for the development of innovative diagnostic and therapeutic approaches focused on senescence. The present research reveals the important function of aging processes in the development of Parkinson's disease, enabling the advancement of novel diagnostic and therapeutic approaches for Parkinson's that focus on mechanisms related to aging.

1. Introduction

Parkinson's disease (PD) is a degenerative disorder of the nervous system that is identified by the degeneration of dopaminergic neurons in the substantia nigra region of the brain. This degeneration causes motor symptoms including tremors, stiffness, and slow movement. The condition affects approximately 2%-3% of individuals who are 65 years old and above(1), with an annual incidence rate ranging from 10-18 per 100,000 people(2). Moreover, the prevalence rate among individuals in my country who are 65 years old and above is recorded at 1.7%(3). The development of PD is intricate, encompassing interactions among hereditary and ecological elements(4-5). Nonetheless, recent studies suggest that the pathogenic mechanisms of PD could be associated with cellular senescence(6). Cellular senescence refers to a state of irreversible cell cycle arrest that is activated by different stressors, including telomere shortening(7-8), DNA damage(9-10), and oxidative stress(11). Senescent cells display various phenotypic alterations, such as modified gene expression, release of proinflammatory substances, and reduced sensitivity to growth factors(12). The buildup of aging cells has been linked to diseases associated with aging, including cancer, heart disease, and neurodegenerative disorders. Recent studies suggest that the buildup of aging cells might contribute to the development of PD. In the brains of individuals with PD, researchers have observed aging dopaminergic neurons, while aging astrocytes were discovered to release proinflammatory cytokines that contribute to neuroinflammation(13-14). Furthermore, it has been demonstrated that aging cells can hinder the functioning of mitochondria(15), which is believed to have a crucial impact on the development of PD. Hence, focusing on aged cells is seen as a hopeful path that may hinder or ease the beginning and advancement of PD by targeting the aged characteristic. The advancements in microarray technology and bioinformatics have significantly assisted the progress of biomedicine. At present, these bioinformatics examinations offer insightful hints to comprehend the origin of PD from various perspectives. Nevertheless, the precise molecular mechanisms connecting cellular senescence to PD pathology are still unknown. Consequently, our objective in this research is to examine PD-related GEO datasets from the standpoint of SAGs. The identification of SADEGs involved limma testing, WGCNA, and the intersection of 1302 genes related to senescence and senescence-associated genes. GO and GSEA were used to determine the possible biological roles and pathways of SADEGs. Next, the identification of hub genes linked to the progression of PD was carried out using SVM-RFE, LASSO logistic regression, and RF. Furthermore, we investigated the associations between the central genes and

Parkinson's disease. In the end, the validation set was used to confirm the expression levels of four central genes.

2. Methodology and materials

2.1. Data download and processing

The data was sourced from the GEO database(16) available. **Table 1** provides a comprehensive list of the datasets analyzed in this study. For validation purposes, GSE20295 (17-18) was chosen as the testing dataset, whereas GSE49036(19) was utilized as an independent cohort of samples. We downloaded and processed the unprocessed information. The 'affy' R package(20) was utilized for preprocessing. The probe with the highest average expression was selected and labeled with the official gene symbol according to the platform annotation, for genes that were matched by multiple probes.

Table 1. Details of the selected datasets.

	GSE series	Platform	Sample size	Source	Mean age of PD(year)	Mean age of ND(year)
Training dataset	GSE20295	GPL96	78 PD[a)] and 18 ND[b)]	prefrontal cortex,putamen,substantia nigra	71.67	71.20
validation dataset	GSE49036	GPL570	20 PD and 9 ND	substantia nigra	69.75	71.00
	CellAge&MSigDB		1302			

[a)]PD,Parkinson's disease; [b)] ND,Non-disease groups

2.2. Genes associated with the process of aging and age-related changes

Genes associated with aging and the aging process were acquired from two different sources. The database for Genomic Resources of Human Aging(21) as well as the gene sets from MSigDB(22). A total of 1302 unique genes related to aging and cellular senescence were obtained by downloading genes with P values <0.05 from the following GO biological process gene sets: GOBP CELLULAR SENESCENCE (M11558), GOBP NEGATIVE REGULATION OF CELLULAR SENESCENCE (M14545), GOBP POSITIVE REGULATION OF CELLULAR SENESCENCE (M45293), GOBP REGULATION OF CELLULAR SENESCENCE (M45292), GOBP STRESS INDUCED PREMATURE SENESCENCE (M24710), and GOBP MULTICELLULAR ORGANISM AGING (M10108), and removing duplicates **(Table S1)**.

2.3. Analysis of differentially expressed genes

The normalizeBetweenArrays function from the limma R package was employed for the normalization of GSE20295 gene expression data and the identification of differential genes(DEGs). Simultaneously, the normalized data underwent principal component analysis using the factoextra R package to assess potential batch effects. Significantly differential expression genes were determined based on the criteria of $|log2FC| > 0.5$ and an adjusted p-value < 0.05. Subsequently, a volcano plot was generated using fold changes under log transfer and statistical significance criteria to visually represent the differentially expressed genes.

2.4. Identification of co-expressed genes

To identify modules of genes that are highly correlated and to associate these modules with clinical traits, we conducted a WGCNA using the 'WGCNA' package in R(23). To focus on the most informative genes, the top 25% most variant genes in GSE20295 were filtered prior to WGCNA. The co-expression network was constructed using the following key parameters: Soft thresholding power (β) = 16, which was chosen based on the scale-free topology criterion with an independent R^2 cutoff of 0.9 using the pickSoftThreshold function. The minimum module size was set to 30 genes, and the merge cut height was set to 0.25 in order to merge similar modules. Modules were associated with clinical traits by calculating Pearson's correlation coefficients between module eigengenes and trait data. Finally, hub genes in significantly correlated modules were functionally annotated using the clusterProfiler R package (v4.9.0.002) through Gene Ontology and KEGG pathway enrichment analysis(24).

2.5. Identification of senescence-associated differentially expressed genes

Using a heatmap, we identified SADEGs by overlapping DEGs, key modules (WGCNA), and senescence and senescence-associated genes.

2.6. Machine learning feature selection

The e1071 package provides the SVM-RFE(25-26) method for machine learning feature selection, which is efficient in inductive inference from a limited training set. Additionally, the glmnet package(27) offers Lasso regression with 10-fold cross-validation to optimize the model by reducing overfitting through coefficient shrinking(28). Moreover, the randomForest package(29) includes the Random Forest Recursive Feature Elimination (RFE) method, a supervised approach that ranks feature importance. Genes with a significance level greater than 0.25 were chosen. The performance of each model was assessed through leave-one-out cross-validation. Ultimately, the intersection of the top genes identified using these three

approaches yielded a strong signature consisting of four SADEGs linked to Parkinson's disease.

2.7. Identification of senescence-associated genes

In order to detect genes associated with senescence, we initially utilized the ggpubr package (30) to visualize and calculate the differential expression of the four SADEGs in PD and normal samples. Genes were classified as hub genes if they exhibited significant differential expression in both the training and validation datasets, with a p-value less than 0.05. A total of four hub genes were found to be significantly linked to senescence processes in PD.

2.8. Evaluation of senescence-associated DEGs by ssGSEA

To gain a deeper understanding of the functional roles of the identified SADEGs, we conducted ssGSEA and utilized the enrichplot tool(31) for visualization purposes. The clusterProfiler package(24) was used to apply ssGSEA on matrices of Pearson correlation between every SADEG and all remaining genes. The gene sets related to all metabolic pathways in KEGG were downloaded through the MSigDB database. The GSVA package (version 1.44.5) was used to calculate GSVA scores, which indicate the absolute enrichment of each gene set. We identified gene sets enriched for significant genes based on a significance threshold of $p<0.05$. Through this analysis, we were able to uncover the pathways and biological processes linked to the SADEGs in Parkinson's disease.

2.9. Development and verification of a nomogram diagnostic model

In order to assess the diagnostic capability of every SADEG contender, we contrasted their levels of expression in samples of PD and control, and formulated ROC curves. We exclusively chose genes with AUC > 0.7 in both the testing and validation datasets (GSE49036) to prevent overfitting. These selected genes were employed to create diagnostic models in the form of nomograms using the rms package(32). Subsequently, the nomograms were validated by calculating their AUC on the separate validation dataset.

2.10. Identifying miRNAs regulating hub genes

Construction of ARDEGs-miRNA networks. The miRNAs of central genes were predicted using TarBase v8 (33) using NetworkAnalyst 3.0 (34), a comprehensive network visualisation and analysis platform for gene expression analysis.The interaction network of ARDEGs-miRNAs was further visualised in Cytoscape software (v3.9.1) (35).

2.11. Assessment of immune invasion

To evaluate the infiltration of immune cells, we employed the CIBERSORT algorithm (36) to measure the ratios of 22 different types of immune cells using the combined expression profiles. Samples that had a CIBERSORT p-value less than 0.05 were selected for additional

analysis. We created bar graphs that show the proportion of the 22 different immune cells in samples from individuals with Parkinson's disease and control subjects. Additionally, we created a correlation matrix plot for the characteristic SADEGs using the ggcorrplot package.

2.12. Statistical analysis

Supporting packages were used to perform all analyses in R version 4.2.3 (64-bit). The statistical analysis employed the Student's t-test. Additionally, Pearson correlation was conducted for assessing the correlation between two genes and immune cell scores. Statistical significance was determined for all statistical tests with a p-value less than 0.05.

3. Results

3.1. Analysis of Principal Components and Identification of Differentially Expressed Genes

The process is described in **(Figure 1)**. Principal component analysis showed separation between PD patients and control ND subjects into two distinct groups **(Figure 2A)**. A total of 1523 genes were found to be differentially expressed (DEGs) between PD and controls, with 591 genes showing upregulation and 932 genes showing downregulation **(Figure 2B)**.

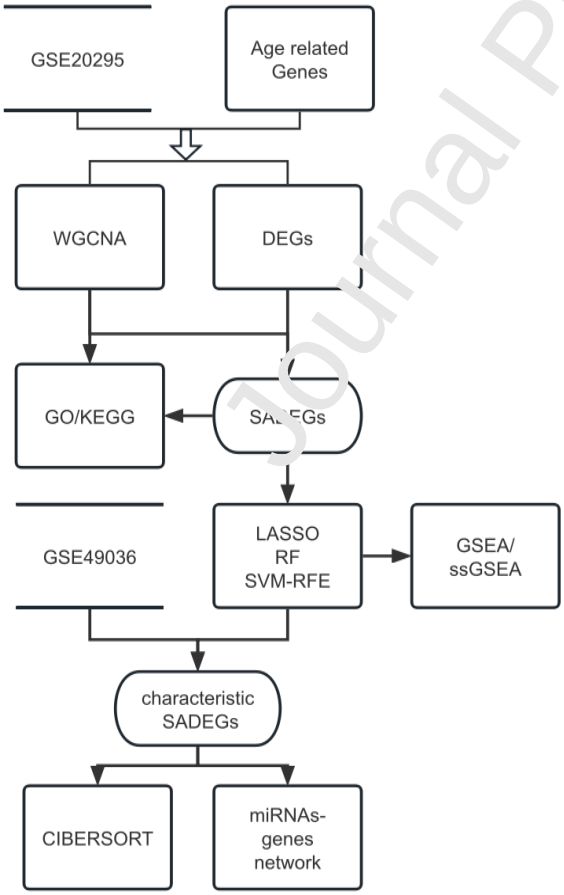

Figure 1. Diagram showing study flow.

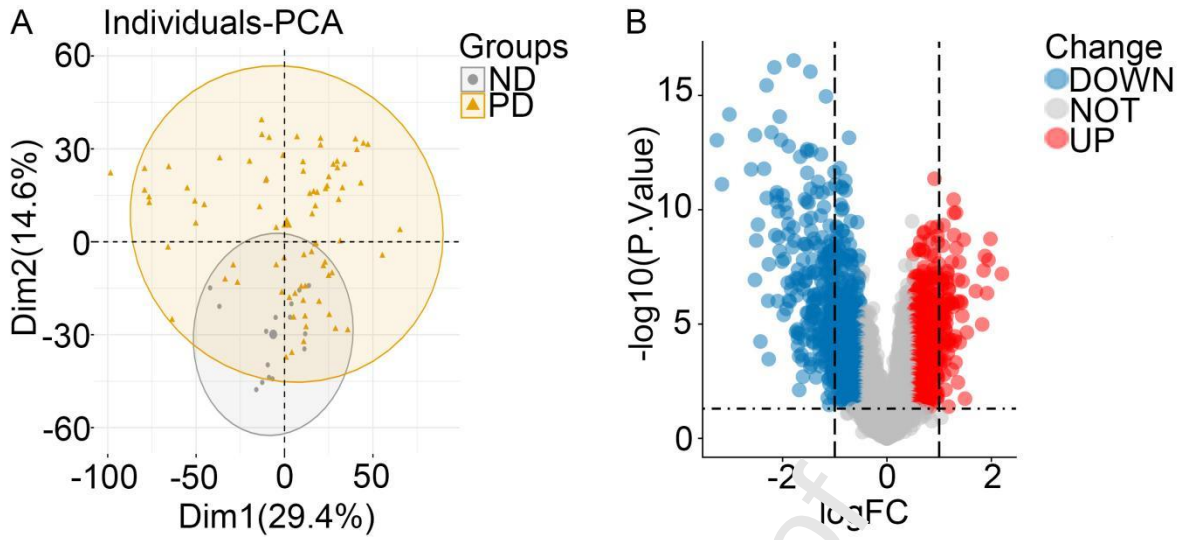

Figure 2. Identifying DEGs. (A)Principal component analysis(PCA). (B)A volcano plot comparing DEGs between Parkinson' s disease and non-disease groups.

3.2. Analysis of gene co-expression networks using weighted methods

By utilizing a gentle threshold β=9, we established a network with a topology that closely resembles a scale-free structure **(Figure 3A)**. **Figure 3B** illustrates the clustering of 10 modules containing the top 25% variance genes. The analysis of Pearson correlation indicated that the black module, consisting of 86 genes, and the pink module, consisting of 74 genes, showed the strongest association with the group (PD vs ND) **(Figure 3C)**. Functional enrichment analysis of the black and pink modules showed enrichment for biological processes including cell junction assembly, cellular components like glutamatergic synapses, and molecular functions such as actin binding **(Figures 3D-E)**. The most enriched KEGG pathway was calcium signaling **(Figure 3E)**.

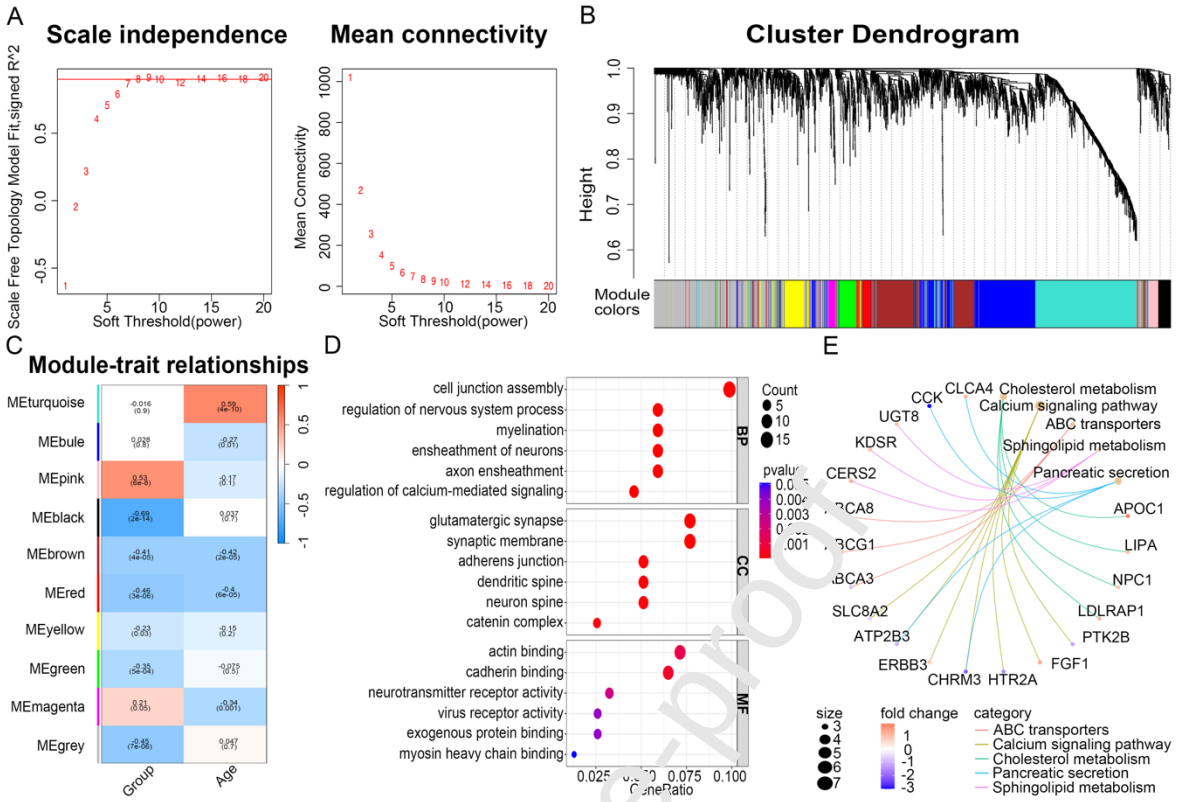

Figure 3. Co-expression modules. (A) Analysis of soft threshold power from 1 to 20 as a function of scale-free fitting indexes . (B) Clustering dendrograms. According to dynamic tree cutting, genes were clustered into different modules through hierarchical clustering analysis with the threshold of 0.25 was used. Each color represents each module. (C) The correlation heatmap between eigengenes of modules and clincal features. (D) GO of biological processes (BP), cellular component (CC), and molecular functions (MF) for black module and pink module. (E) KEGG black module and pink module.

3.3. Identification of key genes by integrating

In **Figure 4A**, we identified 16 overlapping genes (SADEGs) by overlapping the DEGs, WGCNA module genes (black and pink), and genes related to aging. **Figure 4B** displays a heat map illustrating the expression patterns of these SADEGs in PD patients compared to controls. The analysis of GO and KEGG pathways showed that the SADEGs were enriched in various biological processes, including the metabolism of phosphatidylcholine, regulation of phospholipid metabolism, and catabolism of glycerophospholipids **(Figure S1)**.

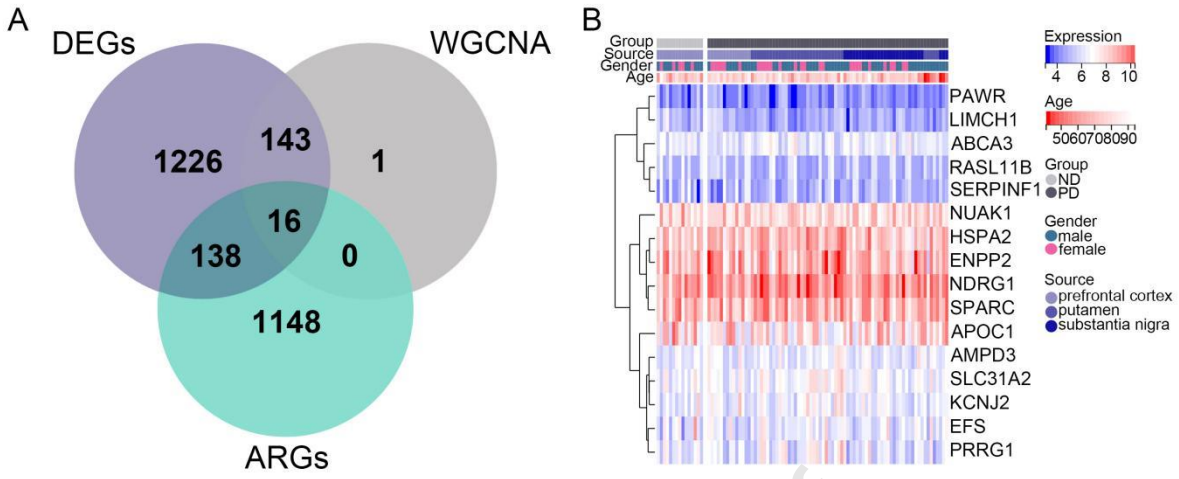

Figure 4. Identification of senescence-associated DEGs (SADEGs). (A) Identification of SADEGs with a venn diagram. (B) A heat map showing the expression of SADEGs.

3.4. Screening candidate diagnostic genes using machine learning

To further identify crucial diagnostic genes, three different machine learning algorithms were utilized. In **Figure 5A-B**, the diagnostic markers chosen by LASSO regression analysis were 7 genes (RASL11B, NDRG1, NUAK1, PAWR, SPARC, PRRG1, and AMPD3). Using the random forest algorithm, the top 10 genes by importance score were RASL11B, SERPINF1, NDRG1, ABCA3, PAWR, APOC1, ENPP2, PRRG1, AMPD3 and SPARC **(Figure 5C-D)**. SVM-RFE algorithm minimzed error with 5 features(RASL11B, AMPD3, PRRG1, HSPA2 and SPARC)**(Figure 5E-F)**. The ultimate AUC values for the three models SVM-RFE, LASSO, and RF were 0.86, 0.99, and 1, respectively. Notably, the RF model demonstrated the most superior performance**(Figure 5G)**. By integrating these results, 4 SADEGs were obtained(RASL11B, AMPD3, PRRG1 and SPARC)**(Figure 5H)**.

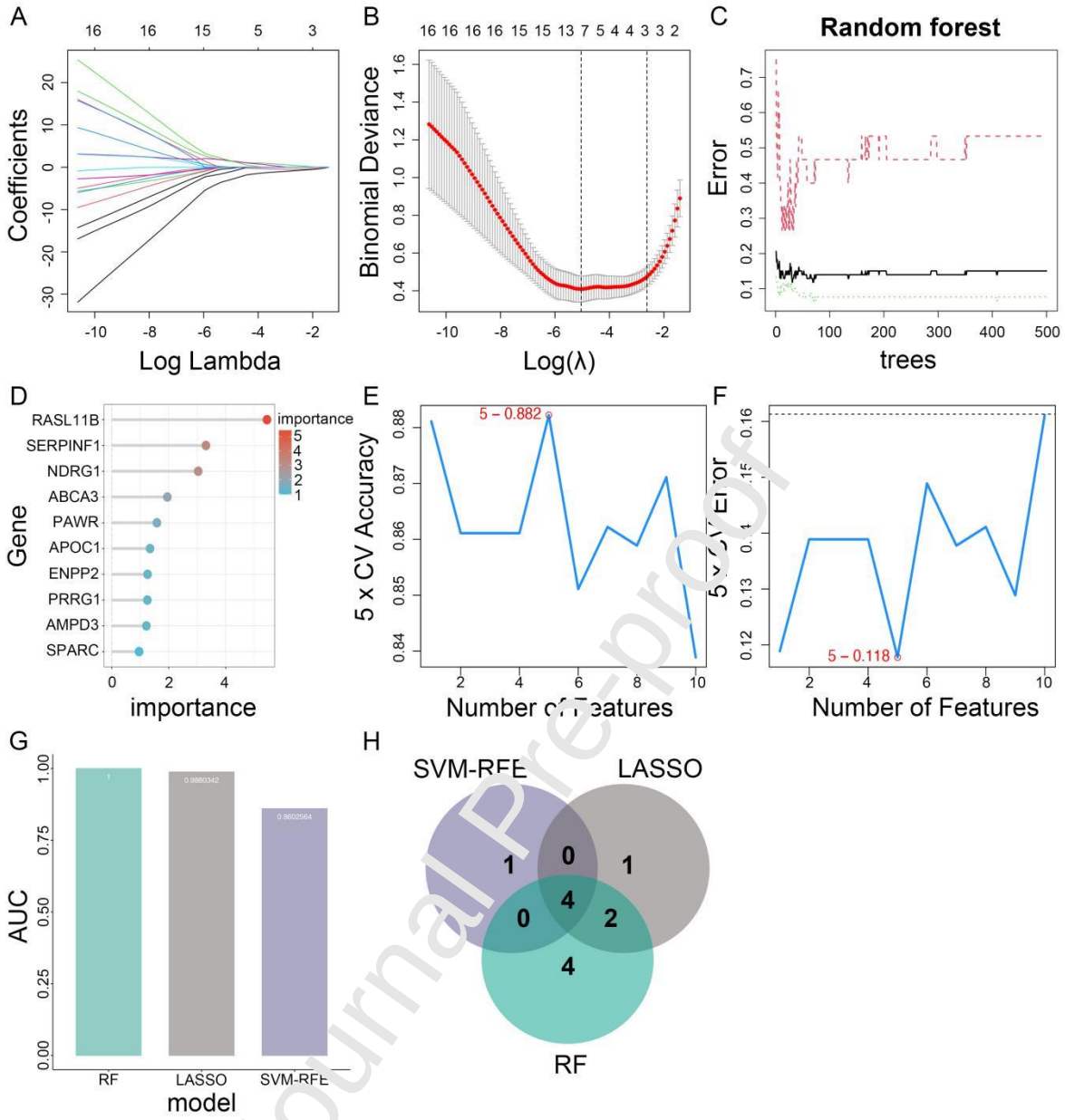

Figure 5. Selection of signature genes among genes associated with Parkinson's onset and SADEGs. (A) Ten cross-validations of adjusted parameter selection in the LASSO model. Each curve corresponds to one gene. (B) LASSO coefficient analysis. Vertical dashed lines are plotted at the best lambda. (C) Relationship between the number of random forest trees and error rates. (D) Ranking of the relative importance of genes. (E-F) SVM-RFE algorithm for feature gene selection. (G) SVM-RFE,LASSO, and RF performance in dataset. (H) Venn diagram showing the feature genes shared by SVM-RFE,LASSO, and RF.

3.5. Diagnostic performance of identified genes

In the GSE20295 dataset, the mRNA levels of the 4 potential SADEGs indicated that RASL11B exhibited a significant decrease in PD compared to controls, whereas AMPD3, PRRG1, and SPARC showed a significant increase in PD **(Figure S2)**. The four genes

successfully differentiated PD patients from controls ($p<0.05$), suggesting their potential as diagnostic biomarkers for PD.

3.6. Gene Set Enrichment Analysis (GSEA) for single genes

The four SADEGs were assessed using GSEA to determine the signaling pathways linked to them**(Figure 6A-D)**. The gene RASL11B was linked to pathways related to asthma and the metabolism of cholesterol.The gene AMPD3 was linked to resistance against antifolate drugs and pathways related to endocrine function and calcium reabsorption. PRRG1 was linked to sphingolipid and fatty acid metabolism pathways. The association of SPARC was linked to the metabolism of glycerolipid and fatty acid. Strong correlations were observed in KEGG gene sets **(Figure 6E)**.

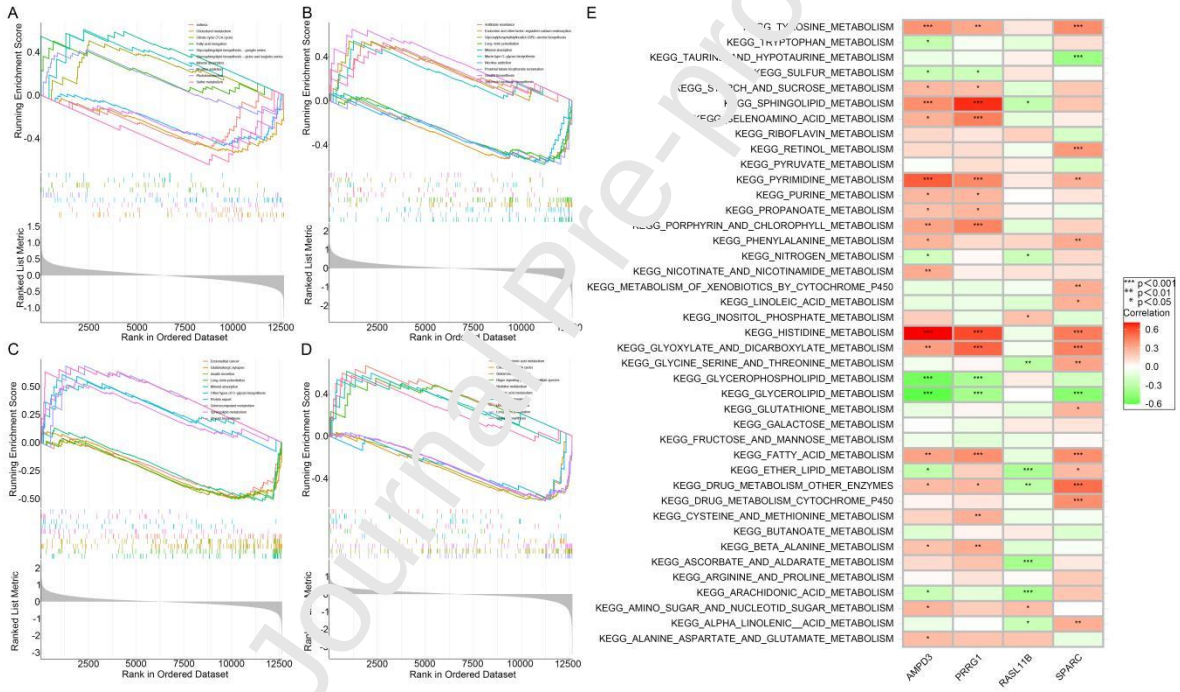

Figure 6. GSEA for expression levels of potential biomarkers (PASL11B, AMPD3, PRRG1 and SPARC). Single-gene GSEA-KEGG pathway analysis of PASL11B(A), AMPD3(B), PRRG1 (C) and SPARC(D). (E)Single gene correlation test for KEGG gene sets.

3.7. Development of a diagnostic chart for Parkinson's disease

A nomogram was developed by integrating the four SADEGs into a single predictive model. The AUC for the ROC curve **(Figure 7B)** was 0.833, while for the validation set **(Figure 7C)** it was 0.850, demonstrating excellent diagnostic precision. A score was assigned to each SADEG, and the total score was calculated by summing the scores of all four genes. **Figure 7A** depicted the correlation between overall scores and various levels of risk for Parkinson's

disease. ROC curves were generated for each SADEG in the test set **(Figure 7D-G)** :RASL11B(AUC:0.952)、AMPD3(AUC:0.829)、PRRG1(AUC:0.834) and SPARC(AUC:0.879) , and validation set **(Figure 7H-K)** :RASL11B(AUC:0.713)、AMPD3(AUC:0.544)、PRRG1(AUC:0.850) and SPARC(AUC:0.625). When an AUC value was higher than 0.7, the hub gene was regarded as having outstanding specificity and sensitivity. RASL11B and PRRG1 emerged as characteristic SADEGs.

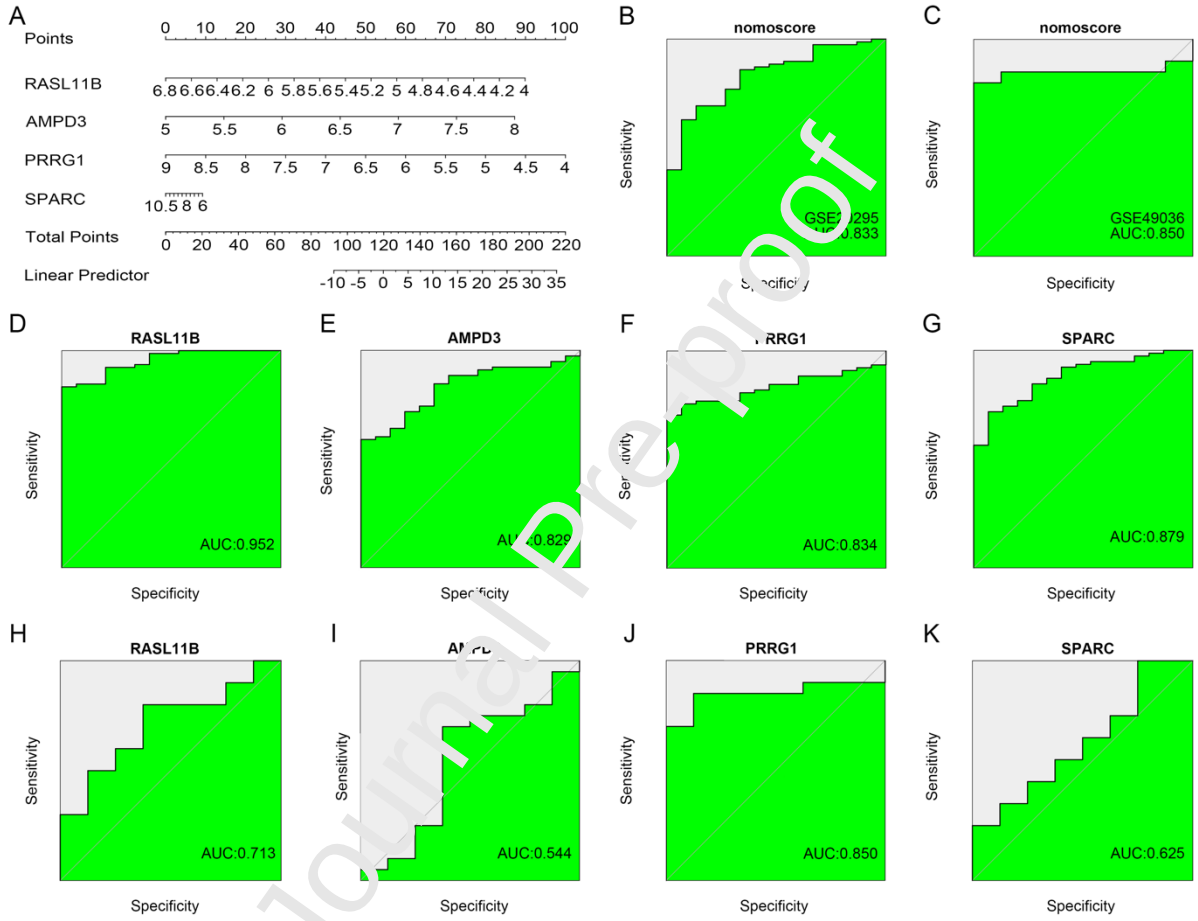

Figure 7. Construction of column line graph based on SADEGs. (A) Construction of column line graph integrating SADEGs for PD. in the column line graph, each variable corresponds to a score, and the total score can be calculated by summing the scores of all variables. (B–C)ROC curve for the GSE20295 dataset and GSE49036 dataset. (D-G)ROC analysis of SADEGs for diagnosing PD in the GSE20295 dataset. (H-K)ROC analysis of SADEGs for diagnosing PD in the GSE49036 dataset.

3.8. Immune infiltration analysis using CIBERSORT

The CIBERSORT algorithm assessed immune cell proportions in PD and control samples **(Figure 8A)**. Comparisons revealed that individuals with Parkinson's disease exhibited elevated levels of inactive natural killer cells and M2 macrophages, while experiencing reduced levels of active natural killer cells and inactive dendritic cells (as depicted in **Figure**

8B). Correlation analysis revealed the characteristic SADEGs were more strongly associated with M2 macrophages **(Figure 8C)**.

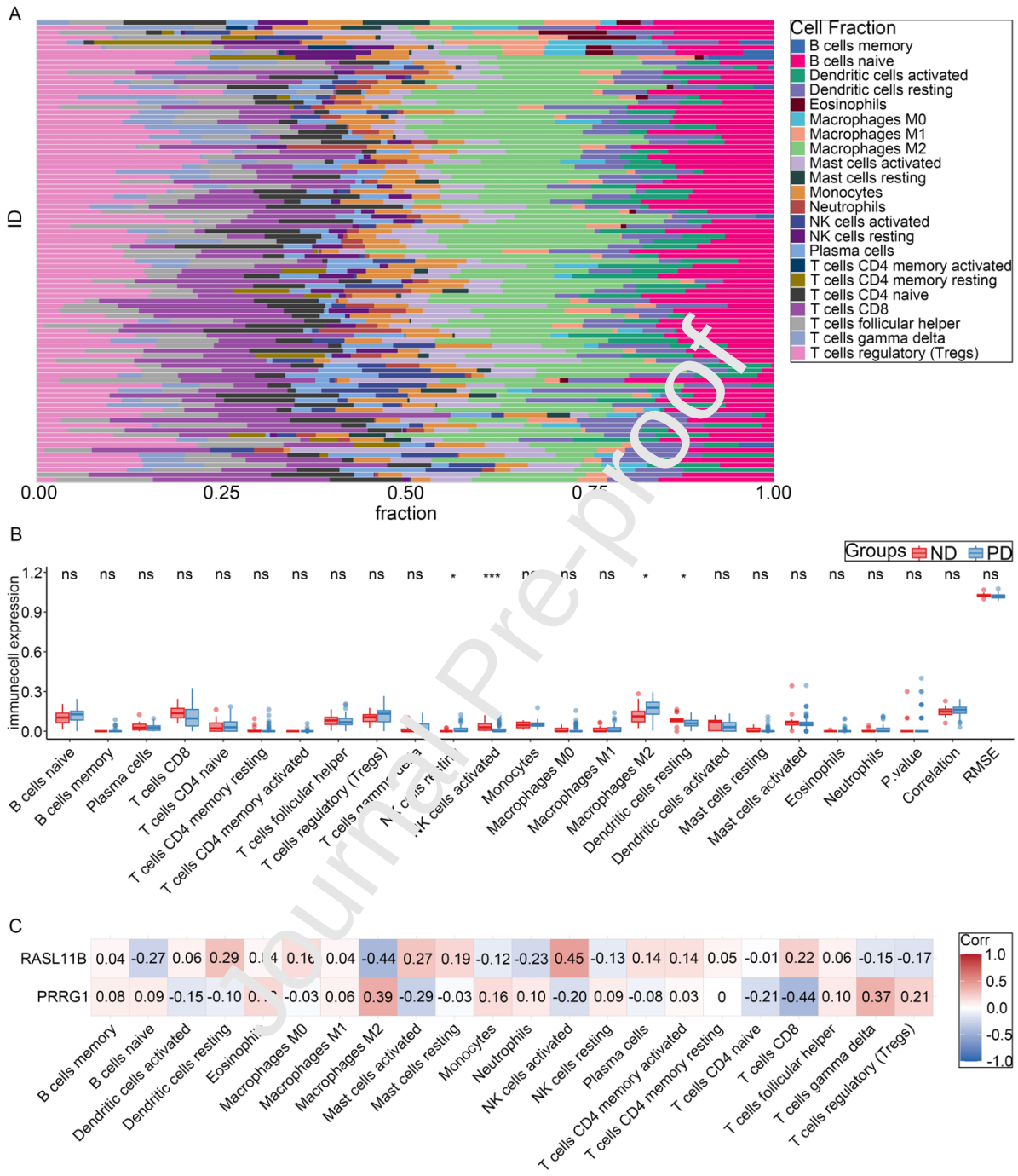

Figure 8. Immune cell infiltration analysis between PD and ND. (A) The proportion of 22 kinds of immune cells in different samples visualized from the barplot. (B) Comparison regarding the proportion of 22 kinds of immune cells between PD and ND visualized by the boxplot. (C) Pearson correlation analysis of immune cell infiltration with hub genes. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

3.9. Regulatory network analysis reveals miRNAs targeting characteristic SADEGs

A network in Cytoscape was created to identify miRNA regulators of the central genes. **Figure S3** shows that RASL11B and PRRG1 have common miRNA targets, such as hsa-mir-93-5p, hsa-mir-106a-5p, hsa-mir-106b-5p, hsa-mir-20a-5p, hsa-mir-20b-5p, and hsa-mir-17-5p.

4. Discussion

The global burden of neurodegenerative diseases is increasing alarmingly as the population ages. Currently, there is no successful treatment for Parkinson's disease due to a lack of comprehensive understanding of its cause and progression. The state of permanent cell growth arrest known as cellular senescence is thought to play a significant role in the process of aging and aging-related diseases, including Alzheimer's disease and cancer(37-39). Furthermore, studies have revealed aging-related effects, such as DNA damage and inflammatory response, in the brains of both PD patients and PD animal models(40-41). Despite the growing evidence highlighting the importance of cellular senescence in aging and PD, the specific mechanisms and how senescent cells contribute to the pathophysiology of PD neurodegeneration are still largely unknown. In recent times, certain research has highlighted the possible involvement of genes associated with senescence (SAGs) in various diseases related to aging. For instance, the study conducted by Xiao and colleagues investigated the possible involvement of SAGs in glioblastoma(42). It was discovered that SAGs have significant impacts on the onset and progression of glioblastoma. Furthermore, Xu and Chen emphasized the significance of SAGs in predicting the prognosis of lung adenocarcinoma(43). Their study unveiled the correlation between specific SAGs and the prognosis of patients with lung adenocarcinoma. In addition to cancer, SAGs have a strong connection to the development and advancement of neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, and Huntington's disease(44-46). These studies revealed that SAGs participated in the pathological processes and neural injury mechanisms of these diseases.In addition, SAGs also play important roles in cardiovascular diseases. Research has discovered that certain SAGs are linked to the susceptibility of illnesses such as cardiovascular disease, stroke, and atherosclerosis(47-49). These studies suggest that SAGs have a significant impact on the development and advancement of these conditions. Nevertheless, there has been no bioinformatic examination of SAGs in relation to PD, and the crucial SAGs responsible for the pathogenesis of PD are still unknown. Through the utilization of the limma package, WGCNA, and the inclusion of 1302 SAGs from the Human Aging Genomic Resources and MSigDB gene sets, we successfully identified SADEGs

among the DEGs. Furthermore, three machine learning algorithms identified four ARDEGs (RASL11B, AMPD3, PRRG1, and SPARC), and their diagnostic capability was confirmed using an external dataset. Eventually, two characteristic SADEGs (RASL11B and PRRG1) were obtained.

The analysis of bioinformatic enrichment indicated that SADEGs were highly concentrated in the metabolic process of phosphatidylcholine and the regulation of cellular senescence. Phosphatidylcholine, a crucial neurotransmitter, has a notable impact on diverse neurological disorders. The development of Parkinson's disease may be linked to an unusual breakdown of phosphatidylcholine, as indicated by a study that discovered a significant decrease in phosphatidylcholine levels in the brain tissue of patients with Parkinson's disease compared to controls(50). Additionally, recent findings(51-52) suggest that impaired phosphatidylcholine metabolism can contribute to cellular senescence. Researchers have found that impaired phosphatidylcholine metabolism during cell senescence leads to declined cell function. Additional research into the genetic factors and pathways associated with this metabolic abnormality have provided insights into possible treatment strategies. Similar results were also noted in mice(52), suggesting that alterations in phosphatidylcholine metabolism may contribute to cellular senescence and the aging process. Hence, the outcomes of these GO BP concepts might have significance for cellular aging and Parkinson's disease; however, additional investigation is necessary to ascertain their influence on these mechanisms. These types of research can assist in the creation of innovative treatment approaches that can postpone the initiation and advancement of cellular senescence and Parkinson's disease.

RASL11B, being a part of the RAS clan, functions as a GTPase activator involved in cellular growth and development(53).While RAS proteins play a significant role in essential cellular functions like growth, survival, and apoptosis(54-55), the precise physiological function of RASL11B remains unknown. The highly homologous RASL11A is thought to possibly have tumor suppressive roles(56). In a particular investigation, it was discovered that RASL11B exhibited distinct expression patterns while macrophages matured and reacted to TGF-β1 within tumor tissues(57). The study revealed that RASL11B expression in tumor tissues was either comparable or marginally reduced when compared to healthy tissues, which aligns with our observation of RASL11B downregulation in M2 macrophages. These clues can be used to investigate the physiological function of RASL11B. Additionally, considering that macrophages are the primary supplier of TGF-β1 in the walls of atherosclerotic vessels, and TGF-β1 has the ability to prompt macrophages to enhance the synthesis of smooth muscle

cell matrix(58), it is logical to hypothesize that RASL11B might play a role in the development of atherosclerosis by regulating TGF-β1 signaling. Nevertheless, further investigations are necessary to clarify the precise functional mechanisms of RASL11B. Taking into account previous studies, RASL11B might contribute to conditions such as developmental disorders, inflammation, cancer, and atherosclerosis due to its participation in TGF-β1 mediated pathways. Nevertheless, further investigation and verification are required to fully understand the precise functioning of RASL11B.

PRRG1, also known as PRGP1, is a vitamin K-dependent protein containing a glutamate carboxylation domain. TMG3, a transmembrane γ-carboxylase 3 protein, is encoded by a gene situated on the 2p12 region of the human chromosome. Its γ-carboxylation function is related to calcium homeostasis regulation(59), and calcium homeostasis imbalance can lead to cell dysfunction. Furthermore, PRRG1 is linked to numerous conditions including heart disease(60) and brittle bones(61). Research has indicated that a lack of vitamin K may result in a rise in uncarboxylated proteins, which can indirectly disturb the balance of calcium within cells, ultimately impacting their ability to survive(62). In a study by Sun X et al.(63), PRRG1 was shown to be a risk-increasing gene in macrophage-related genes that can predict prognosis in glioblastoma patients. In liver cancer(64), it was discovered that PRRG1 could potentially be among the genes targeted by the miR-17-92 cluster, leading to the promotion of liver cancer development by suppressing PRRG1 expression and unleashing its tumor-inhibiting properties. Furthermore, it is necessary to conduct additional research and confirmation on the precise mechanisms. Moreover, the miR-17-92 cluster plays a role in controlling neurogenesis(65–71) by stimulating the proliferation of neural stem cells (NSCs) and hindering their differentiation and programmed cell death. This is primarily achieved by repressing antineurogenic genes such as PTEN, Tp53, inp1, and p21(66). Moreover, the upregulation of miR-17-92 and miR-106b-25 has the ability to prompt the differentiation of NSCs into neurons(72). Taking into account the present discoveries, PRRG1 might have significant functions in illnesses by regulating calcium balance and neurogenesis, although further investigation is necessary to clarify the precise mechanisms involved.

MicroRNAs, also known as miRNAs, are RNA molecules that are naturally present in the body and do not code for proteins. They play a crucial role in controlling gene expression after the process of transcription and are significant controllers of cellular conditions and activities during instances of acute and chronic damage to the central nervous system. miR-20 is a member of the miR-17-92 family and has important functions. Ghosh et al. investigated the role of miR-20 in normal growth by studying its impact on cyclin D1 expression and the

balance between stem cell proliferation and differentiation(73). In recent research, it has been discovered that miR-20a-5p possesses neuroprotective properties by reducing the expression of interferon regulatory factor 9 (IRF9), hindering the activation of IRF9/NF-κB, and mitigating mitochondrial dysfunction, inflammation, and apoptosis caused by 1-methyl-4-phenylpyridinium ion (MPP+) in models of Parkinson's disease(74-76). During experiments on rats, specific miRNAs such as miR-17-5p, miR-20a, miR-93, and miR-106 exhibited dysregulation in neurodegenerative mechanisms(77). Furthermore, the transfer of miR-106b through extracellular vesicles derived from mesenchymal stem cells has the potential to alleviate neuronal apoptosis caused by MPP+ and promote autophagy by reducing the expression of CDKN2B(78). To summarize, miR-20 has significant regulatory functions in both normal development and neurodegenerative disorders by influencing crucial elements associated with cellular activities such as growth, specialization, inflammation, and programmed cell death. Further research on miR-20 may provide new therapeutic targets for neurodegenerative diseases.

In summary, while the screened genes' roles in tumorigenesis are relatively clear, their functions and mechanisms in non-tumor diseases need further elucidation. This highlights the necessity for more research to fully uncover these genes' functions. Although miRNAs like miR-20 show differential expression changes across neurological diseases, likely due to their multi-targeting and synergistic effects, their involvement in these diseases is increasingly evident. This warrants in-depth investigations into miRNA mechanisms to identify optimal therapeutic windows for different diseases. In the progression, the utilization of miRNA-based treatment has surfaced as an extremely encouraging approach for the management of neurological disorders. We will continue our research to enable the screened genes' potential in therapeutics. The discovery of RASL11B and PRRG1's strong connections to Parkinson's disease and cellular senescence in our research is groundbreaking, laying crucial groundwork for future investigations into their underlying mechanisms. The discovery of these results has the potential to enhance our comprehension of Parkinson's disease and cellular aging, providing fresh perspectives on the creation of specific therapies. Further investigation can unlock the vast potential of the identified genes and miRNAs, leading to the development of effective treatments for diseases that are currently considered untreatable.

5. Conclusion

To summarize, we have discovered and confirmed two genes (RASL11B and PRRG1) that are strongly linked to PD and are involved in the process of senescence. The results of our

study indicate that these genes could have significant functions in the onset and advancement of PD pathology. Our study reveals the participation of RASL11B and PRRG1 in PD, offering fresh perspectives on their potential as biomarkers for early detection and monitoring of the disease. Additional investigation is necessary to explore the mechanisms by which these genes contribute to the development of PD, as it has the potential to unveil novel therapeutic targets. In general, our discovery of the connections between genes related to aging and PD signifies a significant progress in comprehending the causes and development of PD.

There are some limitations to our study:Although we validated our results with other datasets, there may be some bias in our interpretation due to the relatively small sample size and data from mRNA expression. In future work, we intend to perform ELISA experiments (at the protein level) to validate the diagnostic role of these two SADEGs in larger sample sizes.

Supporting Information

Figure S1.(A) GO for SADEGs. (B) KEGG pathways of SADEGs.
Figure S2.(A-D) The expression levels of SADEGs in PD and ND of the GSE20295 dataset.
Figure S3.(A) The Predicted microRNA for the selected two characteristic SADEGs. The circular node shape represent the SADEGs, and the V node shape represent miRNA.
Figure S4.(F) Distribution of module significance and errors in the modules associated with the development of PD.
Table S1.Genes associated with the senescence-related genes.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

Publicly available datasets were analyzed in this study. This data can be found here: GSE20295 and GSE49036 were downloaded from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/). NetworkAnalyst 3.0 (https://www.networkanalyst.ca/) for mapping interactions.Aging and Senescence-Associated Genes were obtained from the Human Aging Genomic Resources (https://genomics.senescence.info/) and MSigDB gene sets (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp).

Abbreviations

DEGs, differential expression genes; PCA, principal component analysis; WGCNA, weighted gene co-expression network analysis; GSEA, gene set enrichment analysis; ssGSEA, single sample gene set enrichment analysis; SAGs,senescence-related genes; SADEG, senescence-related differential expression genes; GSVA, gene set variation analysis; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; LASSO, least absolute shrinkage and selection operator; RF, random Forest; SVM-RFE,support vector machine recursive feature elimination; ROC,receiver operating characteristic; CIBERSORT,cell-type identification by estimating relative subsets of RNA transcripts;AUC,area under the curve

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.