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ARCHIVES OF HELLENIC MEDICINE: ISSN 11-05-3992

ORIGINAL PAPER
ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

In silico analysis of the *TERT* gene identifies common and unique regulators of Alzheimer's and Parkinson's disease

ARCHIVES OF HELLENIC MEDICINE 2026, 43(1):101–105
ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2026, 43(1):101–105

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OBJECTIVE **Investigation of the regulatory role of the *TERT* gene by miRNAs in Alzheimer's disease (AD) and Parkinson's disease (PD) through *in silico* analysis of the TERT interactome to identify novel regulators of the TERT network at the transcriptional and post-transcriptional levels.** METHOD **We first identified the TERT interaction network. Subsequently, we predicted novel miRNAs targeting the TERT interactome and then validated their association with AD and PD by both disease enrichment analysis and exploration of miRNA experimental verification data. Finally, we predicted the transcription factors (TFs) that regulate the common and unique miRNA clusters of interest. To this end, we used four bioinformatic tools (FunRich, RNADisease, string-db, and TransmiR version 2.0).** RESULTS **We found that 89 miRNAs target the TERT interaction network. RNA disease analysis validated the prediction that the retrieved miRNAs are associated with AD and PD. A total of 83 miRNAs were common between the two diseases. Six miRNAs were uniquely associated with PD. Enrichment analysis of the common and unique miRNA elements identified distinct sets of transcription factors (TFs).** CONCLUSIONS **Our data pointed to *TERT* transcriptional and post-transcriptional regulators that have yet to be functionally validated in the context of neurodegeneration. The identified biomolecules offer promising targets for future research aimed at understanding the molecular changes underlying neurodegeneration and developing novel therapeutic strategies.**

In silico ανάλυση του γονιδίου *TERT* αναδεικνύει κοινούς και μοναδικούς ρυθμιστές στις νόσους Alzheimer και Parkinson

Περίληψη στο τέλος του άρθρου

Key words

Alzheimer's disease
miRNAs
Neurodegeneration
Parkinson disease
TERT

Submitted 9.12.2024
Accepted 18.1.2025

The telomerase reverse transcriptase (*TERT*) gene encodes the catalytic component of the telomerase enzyme, which plays a critical role in telomere maintenance and cellular senescence. The various roles of the TERT protein, which is the key determining factor of telomerase activity, include but are not limited to anti-inflammation, tumor suppression, and immunomodulation.[1] TERT exerts a protective effect on the neurons and brain and increased TERT expression has been reported to improve motor function in the brain of a Parkinson's disease (PD) mouse model.[2] Studies on patients with Alzheimer's disease (AD) and mendelian randomization studies have signified the accelerated rate of telomere shortening in AD, as well as the strong influence of gender and ApoE status on telomere length pointing to the connection between short telomeres and AD.[3,4] In PD, differences in telomere length have been reported as a possible risk factor for the disease with remarks outlining telomerase activation as a potential therapeutic strategy for neurological disorders.[5,6] Although available data have increased our understanding of telomere-related dysfunction in neurodegeneration,[1] the underlying role of microRNAs (miRNAs) regulation of *TERT* in AD and PD remains under investigation. In this study, we first identified the TERT interaction network, subsequently, we found the miRNAs that target the TERT interaction network and then validated their association with AD and PD by both disease enrichment analysis and exploration of miRNA experimental data. Finally, we predicted the transcription factors (TFs) that regulate the common and unique miRNA clusters of interest.

MATERIAL AND METHOD

Identification of the *TERT* interactome

The STRING biological database was used to identify the human proteins interacting with *TERT* (https://string-db.org/). STRING integrates protein-protein physical and functional interactions.[7] The settings highest confidence (minimum required interaction score: 0.900) and no more than 50 interactors were selected. Analysis was performed in April 2024.

Prediction and Disease Enrichment Analysis (FEA) of the miRNAs targeting the *TERT* interactors

The software tool FunRich v3.1.3. was accessed for the prediction of the miRNAs that target the *TERT* gene interaction network (http://www.funrich.org/). FunRich performs functional enrichment and interaction network analysis on genome and proteome datasets.[8] To validate the connection between miRNA-mediated *TERT* regulation and neurodegeneration we used RNADisease v4.0 (http://www.rnadisease.org/). The RNADisease database analyzes diseases at the RNA level and provides disease prediction and enrichment tools for various RNA types.[9] Analyses were done in April 2024 using the default parameters.

Exploration of miRNA experimental verification data

To further enhance confidence in the *in silico* findings, we accessed miRNA experimental data in RNADisease v4.0 (http://www.rnadisease.org/).[9] The miRNAs targeting the predicted *TERT* interactors were entered as a list (batch search) and strong experimental evidence was selected as the data source. The investigation was performed in May 2024.

Enrichment analysis of the predicted miRNAs for transcription factors

The enrichment analysis module of the TransmiR v2.0 database was used to identify the significant TFs that regulate the miRNA clusters of interest (https://www.cuilab.cn/transmir).[10] TransmiR v2.0 contains 3,730 literature-curated TF-miRNA regulations, also providing 1,785,998 TF-miRNA regulations derived from ChIP-seq evidence in five species. TF-miRNA regulations were investigated separately for the common and unique miRNA elements associated with AD and or PD. Analysis was done in May 2024 using the default parameters.

RESULTS

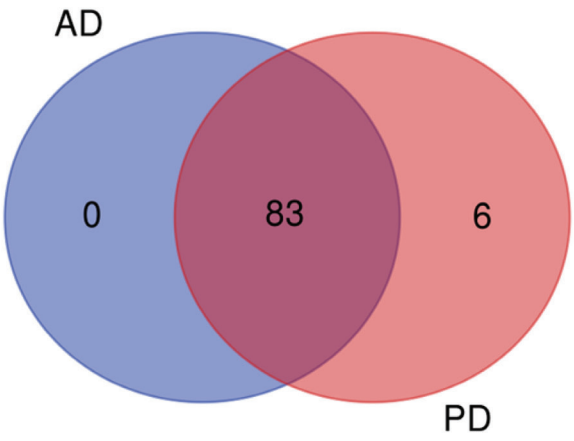
The 35 unique proteins that were found to interact with the highest confidence with *TERT* are provided in table 1. FunRich analysis resulted in the identification of 89 miRNAs targeting the predicted *TERT* interactors. RNA disease enrichment analysis validated the prediction that the retrieved miRNAs are associated with AD (83 out of 89 miRNAs; p value: 2.44e-5; false discovery rate: 1.84e-6) and PD (89 miRNAs; p value: 1.11e-11; false discovery rate: 1.41e-38). A total of 83 miRNAs were common between the two disease categories. Six unique miRNAs were identified for PD (hsa-miR-4458; hsa-miR-520f-3p; hsa-miR-6893-3p; hsa-miR-6866-3p; hsa-miR-4319; hsa-miR-4295) (fig. 1). The query of miRNA experimental verification data returned 67 and 31 results associated with AD and PD, respectively. Of note, 15 miRNAs were strongly associated with both diseases (tab. 2). The results of the subsequent enrichment for TFs of the miRNAs that were over-represented only in PD (6 unique elements), as well as in both diseases (83 common elements) are depicted in table 3.

Table 1. The 35 unique proteins that were found to interact with the highest confidence with *TERT*.

TEP1	HSP90AB1	CLPTM1L
NHP2	SMG6	GNL3L
WRAP53	ACD	MYC
NOP10	RUVBL1	TCF7L2
DKC1	TERF1	RTEL1
HSP90AA1	GAR1	TCF7
SULT1E1	TINF2	NAT10
CTNNB1	XRCC6	KPNA1
RUVBL2	TCF7L1	PIF1
SMARCA4	XRCC5	AKT1
POT1	NVL	TP53
PTGES3	SMG5	

DISCUSSION

The transcriptional and post-transcriptional regulation of *TERT* in the context of AD and PD has been previously assessed to a limited extent.[2–4] In this study, we aimed at identifying miRNAs and TFs that target the *TERT* gene interactors and are over-represented in neurodegeneration. Our investigation yielded 89 miRNAs of which 83 were common between the two diseases of interest while six miRNAs were uniquely associated with PD. This substantiates previous findings in the literature which have suggested commonalities in genetics, and cellular mechanisms between neurodegenerative diseases.[11] Recently, Awuson-David et al identified 15 common miRNAs between AD and PD from two independent systematic reviews.[12] Of note, six of these miRNAs (hsa-miR-22-3p; hsa-miR-30b-5p; hsa-miR-181c-5p; hsa-let-7f-5p; hsa-miR-150-5p; hsa-miR-222-3p) are



Disease	Total	miRNAs
AD AND PD	83	hsa-miR-181b-5p hsa-miR-361-5p hsa-miR-188-5p hsa-miR-148a-3p hsa-miR-370-3p hsa-miR-20b-5p hsa-miR-133a-3p hsa-miR-30d-5p hsa-miR-409-5p hsa-miR-4500 hsa-miR-30c-5p hsa-let-7b-5p hsa-miR-376b-3p hsa-miR-376a-3p hsa-miR-455-5p hsa-miR-19b-3p hsa-miR-101-3p hsa-miR-129-2-3p hsa-let-7f-5p hsa-miR-221-3p hsa-miR-455-3p hsa-miR-423-5p hsa-miR-181c-5p hsa-miR-144-3p hsa-miR-30a-5p hsa-let-7d-5p hsa-miR-652-3p hsa-miR-183-5p hsa-miR-17-5p hsa-miR-873-5p hsa-miR-30b-5p hsa-miR-30e-5p hsa-miR-1271-5p hsa-miR-98-5p hsa-miR-181a-5p hsa-let-7i-5p hsa-miR-494-3p hsa-miR-129-1-3p hsa-miR-212-3p hsa-miR-133b hsa-miR-379-5p hsa-miR-3666 hsa-miR-382-3p hsa-miR-135a-5p hsa-miR-181d-5p hsa-miR-125b-5p hsa-let-7g-5p hsa-miR-93-5p hsa-miR-519d-3p hsa-let-7a-5p hsa-miR-155-5p hsa-miR-330-3p hsa-miR-3184-5p hsa-miR-96-5p hsa-miR-431-5p hsa-miR-9-5p hsa-miR-106a-5p hsa-miR-1306-5p hsa-miR-130a-3p hsa-miR-130b-3p hsa-miR-150-5p hsa-let-7c-5p hsa-miR-222-3p hsa-miR-491-5p hsa-let-7e-5p hsa-miR-135b-5p hsa-miR-148b-3p hsa-miR-377-3p hsa-miR-195-5p hsa-miR-142-3p hsa-miR-125a-5p hsa-miR-454-3p hsa-miR-22-3p hsa-miR-182-5p hsa-miR-483-3p hsa-miR-301a-3p hsa-miR-3529-5p hsa-miR-1-5p hsa-miR-328-3p hsa-miR-19a-3p hsa-miR-301b-3p hsa-miR-152-3p hsa-miR-191-5p
PD	6	hsa-miR-4458 hsa-miR-520f-3p hsa-miR-6893-3p hsa-miR-6866-3p hsa-miR-4319 hsa-miR-4295

Figure 1. The common and unique *TERT* miRNA regulators associated with Alzheimer's disease (AD) and Parkinson's disease (PD).

Table 2. The 15 miRNAs that were strongly associated with both Alzheimer's disease (AD) and Parkinsons' disease (PD), as evidenced by experimental verification data.

hsa-miR-376a-3p	hsa-miR-222-3p
hsa-miR-221-3p	hsa-miR-301b-3p
hsa-miR-370-3p	hsa-miR-130a-3p
hsa-miR-142-3p	hsa-miR-101-3p
hsa-miR-148b-3p	hsa-miR-330-3p
hsa-miR-17-5p	hsa-miR-133b
hsa-miR-19b-3p	hsa-miR-30c-5p
hsa-miR-133a-3p	

Table 3. Results of the TransmiR v2.0 enrichment analysis.

TFs	p-value	False discovery rate
EIF2C2	1.57e-9	6.64e-7
LIN28	4.82e-8	5.10e-6
LIN28B	4.82e-8	5.10e-6
TRIM32	4.82e-8	5.10e-6
IL6	1.13e-6	9.53e-5
GTF2I	1.83e-6	1.29e-4
STAT5	7.85e-6	4.74e-4
AKT1	2.01e-5	1.06e-3
NF-Y	3.56e-5	1.68e-3
CTNNB1	1.89e-4	7.91e-3
RYBP	3.62e-3	0.3654
KDM5A	7.24e-3	0.3654
CDK12	0.0103	0.3654
C17orf49	0.0129	0.3654
CDX2	0.0191	0.4092
GATAD1	0.0243	0.4092
ELK3	0.0253	0.4092
ELK4	0.0341	0.4823
SUMO1	0.0525	0.5649
INTS3	0.0585	0.5649

Italics: TFs that regulate the list of miRNAs that were common between Alzheimer's disease (AD) and Parkinson's disease (PD). *Non italics:* Transcription factors (TF) that are likely to regulate the list of miRNAs that were uniquely associated with PD

also included in our list of predicted post-transcriptional *TERT* regulators associated with both neurodegenerative diseases. Most interestingly, our query of miRNA experimental verification data showed that the hsa-miR-222-3p is significantly dysregulated in the two brain disorders further enhancing confidence in our *in silico* findings. The results in regard to the six miRNAs uniquely associated with PD point to *TERT*-related regulatory mechanisms specific to PD that may not be as prominent in AD. Therefore, further research will be required to demonstrate the specific roles of these non-coding RNAs in PD.

As to TFs, several studies have highlighted their crucial roles in inflammation, oxidative stress, and protein homeostasis, pathological processes underlying neurodegeneration.[13] The importance of investigating proteins that control the rate of genes' transcription in the context of AD and PD lies in their therapeutic potential.[13,14] In this study, we have considered the consequences of miRNAs' regulation by TFs, as this approach remains briefly addressed in the literature.[10] Our results point to specific proteins regulating the common and unique miRNA clusters of interest.

The scrutiny of miRNA experimental verification data returned 67 and 31 results associated with AD and PD,

respectively, along with 15 miRNAs strongly associated with both diseases. The evidence we found further supports our notion that the predicted *TERT* interactome and its associated post-transcriptional regulators function during the pathological processes of the two most common neurodegenerative diseases. However, more research is still necessary to validate the interactions between all identified biomolecules. Future work should also involve the potential therapeutic implications of our findings.

In conclusion, this study provides additional support for shared molecular mechanisms between AD and PD whilst also suggesting potential PD-specific regulatory mechanisms of *TERT* transcription. The identified *TERT* interactors, miRNAs, and TFs offer promising targets for future research aimed at understanding the molecular changes underlying neurodegeneration and developing novel therapeutic strategies.

ΠΕΡΙΛΗΨΗ

In silico ανάλυση του γονιδίου *TERT* αναδεικνύει κοινούς και μοναδικούς ρυθμιστές στις νόσους Alzheimer και Parkinson

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Αρχεία Ελληνικής Ιατρικής 2026, 43(1):101–105

ΣΚΟΠΟΣ Διερεύνηση του ρυθμιστικού ρόλου του γονιδίου *TERT* από τα miRNAs στις νόσους Alzheimer (AD) και Parkinson (PD) με *in silico* ανάλυση του διαδραστώματος του *TERT* για τον εντοπισμό νέων ρυθμιστών του δικτύου TERT σε μεταγραφικό και μετα-μεταγραφικό επίπεδο. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Προσδιορίσαμε πρώτα το διαδράστωμα του γονιδίου *TERT*. Στη συνέχεια, προβλέψαμε νέα miRNAs που στοχεύουν το ρυθμιστικό δίκτυο του *TERT* και στη συνέχεια επιβεβαιώσαμε τη συσχέτισή τους με την AD και την PD τόσο με ανάλυση εμπλουτισμού όσο και με τη διερεύνηση πειραματικών δεδομένων επικύρωσης των miRNA. Τέλος, προβλέψαμε τους μεταγραφικούς παράγοντες (TFs) που ρυθμίζουν τα κοινά και μοναδικά miRNA ενδιαφέροντος. Χρησιμοποιήσαμε τέσσερα βιοπληροφορικά εργαλεία (FunRich, RNADisease, string-db και TransmiR v2.0). **ΑΠΟΤΕΛΕΣΜΑΤΑ** Διαπιστώσαμε ότι 89 miRNAs στοχεύουν το διαδράστωμα του *TERT*. Η ανάλυση RNADisease επικύρωσε την πρόβλεψη ότι τα miRNAs που ανακτήθηκαν σχετίζονται με την AD και την PD. Συνολικά, 83 miRNAs ήταν κοινά μεταξύ των δύο ασθενειών. Με την PD συσχετίστηκαν αποκλειστικά 6 miRNAs. Η ανάλυση εμπλουτισμού των κοινών και μοναδικών miRNA προσδιόρισε διακριτά σύνολα μεταγραφικών παραγόντων (TFs). **ΣΥΜΠΕΡΑΣΜΑΤΑ** Τα δεδομένα μας υποδεικνύουν μεταγραφικούς και μετα-μεταγραφικούς ρυθμιστές του *TERT* που δεν έχουν ακόμη επικυρωθεί λειτουργικά στο πλαίσιο του νευροεκφυλισμού. Τα βιομόρια που εντοπίστηκαν προσφέρουν πολλά υποσχόμενους στόχους για μελλοντική έρευνα, με σκοπό την κατανόηση των μοριακών αλλαγών που διέπουν τον νευροεκφυλισμό και την ανάπτυξη νέων θεραπευτικών στρατηγικών.

Λέξεις ευρετηρίου: Γονίδιο *TERT*, ΜικροRNAs, Νευροεκφυλισμός, Νόσος Alzheimer, Νόσος του Parkinson

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