ՀԱՅԱՍՏԱՆԻ ՀԱՆՐԱՊԵՏՈՒԹՅԱՆ ԳԻՏՈՒԹՅՈՒՆՆԵՐԻ ԱԶԳԱՅԻՆ ԱԿԱԴԵՄԻԱ
ՄՈԼԵԿՈՒԼԱՅԻՆ ԿԵՆՍԱԲԱՆՈՒԹՅԱՆ ԻՆՍՏԻՏՈՒՏ

ՀԱԿՈԲՅԱՆ ՄԵԼԻՆԵ ԱՆԴՐԱՆԻԿԻ

ԹԵԼՈՄԵՐՆԵՐԻ ԵՐԿԱՐՈՒԹՅԱՆ ՊԱՀՊԱՆՄԱՆ ՄԵԽԱՆԻԶՄՆԵՐԻ ԱԿՏԻՎՈՒԹՅԱՆ ԵՎ ԹԵԼՈՄԵՐՆԵՐԻ ԵՐԿԱՐՈՒԹՅԱՆ ԴԻՆԱՄԻԿԱՆ

Գ.00.03. – «Մոլեկուլային և բջջային կենսաբանություն» մասնագիտությամբ կենսաբանական գիտությունների թեկնածուի գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ – 2026

НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК РЕСПУБЛИКИ АРМЕНИЯ
ИНСТИТУТ МОЛЕКУЛЯРНОЙ БИОЛОГИИ

АКОПЯН МЕЛИНЕ АНДРАНИКОВНА

АКТИВНОСТЬ МЕХАНИЗМОВ ПОДДЕРЖАНИЯ ДЛИНЫ ТЕЛОМЕР И ДИНАМИКА ТЕЛОМЕРНОЙ ДЛИНЫ

АВТОРЕФЕРАТ

диссертации на соискание ученой степени
кандидата биологических наук по специальности
03.00.03 – «Молекулярная и клеточная биология»

ЕРЕВАН – 2026

Ատենախոսության թեման հաստատվել է ՀՀ ԳԱԱ մոլեկուլային կենսաբանության ինստիտուտի գիտական խորհրդում:

Գիտական ղեկավար՝	կ.գ.դ. Առաքելյան Արսեն Արտաշեսի
Պաշտոնական ընդդիմախոսներ՝	կ.գ.դ. Հովհաննիսյան Գալինա Գեորգիի կ.գ.թ. Ներսիսյան Լիլիթ Ռոբերտի
Առաջատար կազմակերպություն՝	ՀՀ ԱՆ «Ակադեմիկոս Ս.Ավդալբեկյանի անվան Առողջապահության ազգային ինստիտուտ» ՓԲԸ

Ատենախոսության պաշտպանությունը տեղի կունենա 2026 թ. հուլիսի 17-ին, ժամը 14:00-ին, ՀՀ ԳԱԱ մոլեկուլային կենսաբանության ինստիտուտում, 042 մասնագիտական խորհրդի նիստում (ՀՀ 0014, ք. Երևան, Հասրաթյան 7):

Ատենախոսությանը կարելի է ծանոթանալ Մոլեկուլային կենսաբանության ինստիտուտի գրադարանում և https://imb.am/ կայքում:

Ատենախոսության սեղմագիրն առաքվել է 2026 թ. հունիսի 16-ին:

042 մասնագիտական խորհրդի գիտական քարտուղար,
կենս. գիտ. թեկնածու Ռ.Վ. Զախարյան

Тема диссертации утверждена на заседании ученого совета Института молекулярной биологии НАН РА.

Научный руководитель:	д.б.н. Аракелян Арсен Арташесович
Официальные оппоненты:	д.б.н. Оганесян Галина Георгиевна к.б.н. Нерсисян Лилит Робертовна
Ведущая организация:	МЗ РА, «Национальный институт здравоохранения имени академика С. Авдалбекяна» ЗАО

Защита диссертации состоится 17-го июля 2026 г. в 14:00, на заседании специализированного совета 042, в Институте молекулярной биологии НАН РА (РА, 0014, г. Ереван, ул. Асратяна 7).
С диссертацией можно ознакомиться в библиотеке Института молекулярной биологии и на сайте https://imb.am/.

Автореферат диссертации разослан 16-го июня 2026 г.

Ученый секретарь специализированного совета 042
кандидат биол. наук. Захарян Р.В.

INTRODUCTION

Background and Context. Telomere maintenance mechanisms (TMM) are activated in the majority of human malignancies and represent a key enabling feature of sustained tumor proliferation [Sung & Cheong, 2021], yet comprehensive pan-cancer assessments of their activity remain limited. Telomeres are specialized nucleoprotein structures at the ends of linear eukaryotic chromosomes that protect genome integrity by preventing chromosomal end-to-end fusions and DNA damage responses [Blackburn, 1991]. In most somatic cells, telomeres shorten with each cell division, eventually triggering replicative senescence or apoptosis, whereas cancer cells overcome this limitation by reactivating TMMs, which is recognized as a hallmark of cancer [Hanahan & Weinberg, 2011].

Two principal mechanisms have been characterized to date. The telomerase-dependent mechanism (TEL) relies on activation of the telomerase ribonucleoprotein complex, comprising the catalytic subunit TERT, the RNA template TERC, and accessory factors [Cohen et al., 2007]. The alternative lengthening of telomeres (ALT) is a telomerase-independent, homologous recombination-based pathway that maintains telomere length through templated synthesis using telomeric DNA from sister chromatids, non-sister chromosomes, or extrachromosomal telomeric repeats [Reddel, 2003; Cesare & Reddel, 2010]. Although these mechanisms have historically been considered mutually exclusive, increasing evidence indicates that tumors may co-activate both TEL and ALT, or display the hallmarks of neither, suggesting that telomere maintenance is more diverse and context-dependent than previously assumed [Gocha et al., 2013; Barthel et al., 2017].

Despite their fundamental role in tumor biology, TMM activity has been most often inferred indirectly from canonical mutational markers, such as *TERT* promoter mutations or *ATRX* loss, which only partially capture the underlying pathway dynamics. Functional, transcriptome-level evaluation of TEL and ALT pathway activity across cancer types is therefore needed to characterize their heterogeneity, to identify subgroups defined by distinct TMM phenotypes, and to evaluate their prognostic and therapeutic implications.

Low-grade gliomas (LGG) constitute a particularly informative model for studying telomere biology. These tumors are characterized by recurrent mutations in *IDH1*/*IDH2*, *ATRX,* and *TP53*, by molecular subgroups defined by *IDH* status and 1p/19q co-deletion, and by marked variation in telomere length across subtypes [Louis et al., 2021]. Such genetic heterogeneity provides a unique context for examination of how molecular background shapes telomere length and TMM pathway activation, and for evaluation of the prognostic value of telomere-related features in clinically annotated cohorts [Sung et al., 2020].

Considering these aspects, a systematic pan-cancer evaluation of TMM pathway activity at the transcriptome level, combined with an in-depth study of telomere length dynamics and TMM regulation in LGG, is needed to resolve the heterogeneity of telomere maintenance and its prognostic value.

Objectives and Tasks

The research objective of this study is to conduct a pan-cancer evaluation of TMM across various cancer types using RNA sequencing data. Furthermore, we delve deeper into TMM aspects in the context of genetic subtypes, specifically within LGG tumors.

To achieve this aim, the following tasks were set:

1. Pan-cancer analysis of telomere length maintenance mechanisms and TMM phenotyping of cancer subtypes.
2. Identification of specific characteristics of telomere maintenance mechanisms across cancers.
3. Assessment of the association of TMM phenotypes with disease clinical characteristics and outcome.

4. Assessment of the association of TMM pathway branches, telomere length, with tumor molecular subtypes in low-grade glioma, and their impact on disease clinical characteristics and outcomes.

Scientific Novelty

1. For the first time, we conducted a pan-cancer analysis of TEL and ALT pathway activity using a Pathway Signal Flow (PSF)-based approach, enabling functional characterization beyond mutation-based inference.
2. We showed that canonical genetic markers, such as *TERT* promoter mutations and *ATRX* loss, only partially explain TEL and ALT pathway activity, revealing substantial heterogeneity across and within tumor types.
3. We introduced a novel tumor classification approach based on TEL and ALT PSF activity profiles, enabling functional phenotyping of tumors beyond traditional genomic subtypes. Moreover, we demonstrated that TMM-based phenotypes are associated with patient survival, highlighting their potential prognostic relevance.
4. We demonstrated that IDH-wildtype (IDH-wt) tumors exhibit significant ALT pathway activity, largely driven by *RAD51*, despite the prevailing view that this subtype is primarily associated with TEL-mediated telomere maintenance.

Practical Significance of the Work. This pan-cancer analysis across 33 tumor types revealed heterogeneous, context-dependent activation of both TEL and ALT pathways. TMM activity was not strictly mutually exclusive, as both mechanisms could be co-activated within tumors and even at the single-cell level, indicating that telomere maintenance is a dynamic rather than binary process. The identification of distinct TEL-dominant, ALT-dominant, co-activated, and TMM-inactive phenotypes underscores the value of TMM profiling in research and clinical settings. These phenotypes, together with their strong association with molecular alterations, may serve as a basis for patient stratification and risk assessment. In low-grade gliomas, telomere length and pathway activity were strongly associated with *IDH* and *ATRX* status. Elevated *RAD51* transcriptional signal was the principal contributor to the PSF-inferred ALT signature, particularly in IDH-wt tumors, suggesting that *RAD51*-mediated homologous recombination may underlie ALT-type telomere maintenance in this subtype. Shorter telomeres were linked to increased TEL pathway activation, mainly through "TERT", while overall ALT activity remained stable across telomere length categories, varying only at specific pathway nodes. TMM phenotypes also showed clinically relevant survival differences, with high combined ALT and TEL activity associated with the poorest prognosis. Overall, this work establishes TMMs as a dynamic, heterogeneous hallmark of malignancy and provides a framework for translating telomere maintenance profiles into diagnostic, prognostic, and therapeutic practice.

Dissertation Approval. The main results of the research were presented at "Science and Technology Convergence Conference" 28-29 September 2022, Yerevan; "International Congress on Informatics: Information Systems and Technologies" 27-28 October 2022, Belarus; "17th RAU annual conference" 06 December 2023, Yerevan; "Genome Bioinformatics for Health" 12-14 June 2024, Germany, and at the meetings of the Scientific Council of the Institute of Molecular Biology NAS RA.

The Volume and Structure of the Dissertation. This dissertation comprises 116 pages of computer-formatted English text, including 2 tables and 40 figures, consisting of the following sections: Introduction, Literature Review, Materials and Methods, Results and Discussion, Conclusions, Inferences, References (including 187 sources).

Publications. The results of the work are presented in 3 scientific publications.

MATERIALS AND METHODS

Data Sources. The pan-cancer analysis was based on RNA-sequencing, somatic mutation, and clinical data obtained through the Genomic Data Commons (GDC) portal for 33 The Cancer Genome Atlas Program (TCGA) tumor types, comprising 11,123 samples, including both primary tumors and tumor-adjacent normal tissues. Detailed in-depth analysis of telomere length dynamics and TMM regulation was performed on the TCGA-LGG cohort (506 samples with available telomere length estimates from [Barthel et al., 2017]). Validation in an independent cohort used 176 LGG samples from the Chinese Glioma Genome Atlas (CGGA). Single-cell RNA-sequencing data of glioblastoma (GEO accession GSE84465) were used to assess TMM heterogeneity at the single-cell resolution, with 3,567 cells classified into six cell types. The independent Chronic Obstructive Pulmonary Disease (COPD) (GSE124180) dataset was used as a supporting and negative-control cohort.

Data Preprocessing. Raw RNA-seq counts were normalized for library size using DESeq2 (v 1.34.0) and log-transformed. Batch effects were assessed and corrected using SVA (v 3.42.0) for the pan-cancer cohort and Limma (v 3.50.3) for the supporting cohorts. Normalized data were mean-centered and converted into fold-change (FC) values. For the LGG cohort, tumors were classified as Long TL or Short TL based on the tumor-to-blood telomere length ratio (>1 or <1, respectively). Samples with ambiguous or discordant *IDH* and transcriptomic subtype annotations were excluded; 182 samples with concordant *IDH* classification and transcriptomic subtype were retained for IDH-stratified analyses. Single-cell GBM data were processed in Seurat (v 5.0.1) with SCTransform normalization, cell-type annotation via ScType, and pseudobulk aggregation per sample and cell type before PSF computation.

Pathway Signal Flow (PSF) Algorithm. Pathway activity was quantified using the PSF algorithm [Nersisyan et al., 2017], which integrates relative gene expression with pathway topology to estimate node-level activity. The algorithm propagates signals from input nodes through pathway branches to terminal (sink) nodes, weighting interactions by activation or inhibition effects and the strength of upstream contributions. Unlike over-representation analysis or gene set enrichment analysis, PSF explicitly accounts for signal propagation, activation, inhibition, and complex formation, providing a structure-aware estimate of pathway activity. To assess TMM states in cancer cells, we applied the TMM evaluation strategy developed by our research group [Nersisyan et al., 2019; Hakobyan et al., 2023], which employs the PSF algorithm. TMM pathways were reconstructed through extensive literature curation, identifying 37 ALT-related and 26 TEL-related genes across 19 and 13 studies, respectively.

TMM Phenotyping. TMM phenotypes were defined by mapping samples into a two-dimensional ALT versus TEL PSF space. Activity thresholds for low, middle, and high categories were determined separately for TEL and ALT using segmented regression on sorted PSF values, identifying breakpoints in the activity distribution. Five phenotypes were defined: $ALT^{low}TEL^{low}$, $ALT^{low}TEL^{high}$, $ALT^{high}TEL^{low}$, $ALT^{high}TEL^{high}$, and $ALT^{middle}TEL^{middle}$. The same approach was applied to single-cell GBM data using cohort-specific thresholds.

Additional Bioinformatic and Statistical Analyses. Tumor purity was evaluated using five computational methods (including ESTIMATE, ABSOLUTE, LUMP, IHC, and a combined approach) as described by Aran et al. [2015]. Microsatellite instability (MSI-high (MSI-H), MSI-low (MSI-L), microsatellite-stable (MSS)) status was retrieved using TCGAbiolinks for seven applicable TCGA cohorts. American Joint Committee on Cancer (AJCC) clinical stage information was obtained from TCGA, with stages consolidated into Stage I-IV groups. Mutation data for TEL- and ALT-pathway genes were obtained from cBioPortal, and samples were classified as mutated if

at least one pathway gene carried a mutation. Survival analyses used Kaplan-Meier curves with the log-rank test, and hazard ratios were estimated with Cox proportional hazards regression (univariate for the pan-cancer cohort; multivariate for LGG, with covariates including age, treatment, telomere length, and TMM phenotype). Differential gene expression was performed with DESeq2 using ALT ^{low} TEL ^{low} as the reference group, and Gene Ontology enrichment was performed with clusterProfiler (v 4.2.2) (Benjamini-Hochberg $q < 0.05$). Proteomic validation was performed using the Cancer Proteome Atlas for 153 genes, with mRNA-protein correlation evaluated by Pearson correlation. Statistical significance was assessed by the Mann-Whitney U test, Kruskal–Wallis with Dunn's post hoc, or Fisher's exact test as appropriate, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

Pan-cancer Assessment of TEL and ALT Pathway Activity. Application of the PSF-based framework to 33 TCGA tumor types and matched normal tissues revealed that both TEL and ALT pathways show significantly higher activity in tumors than in matched normal samples in the majority of cancer types, with the most pronounced difference observed in cervical squamous cell carcinoma and endocervical adenocarcinoma (Figures 1, 2).

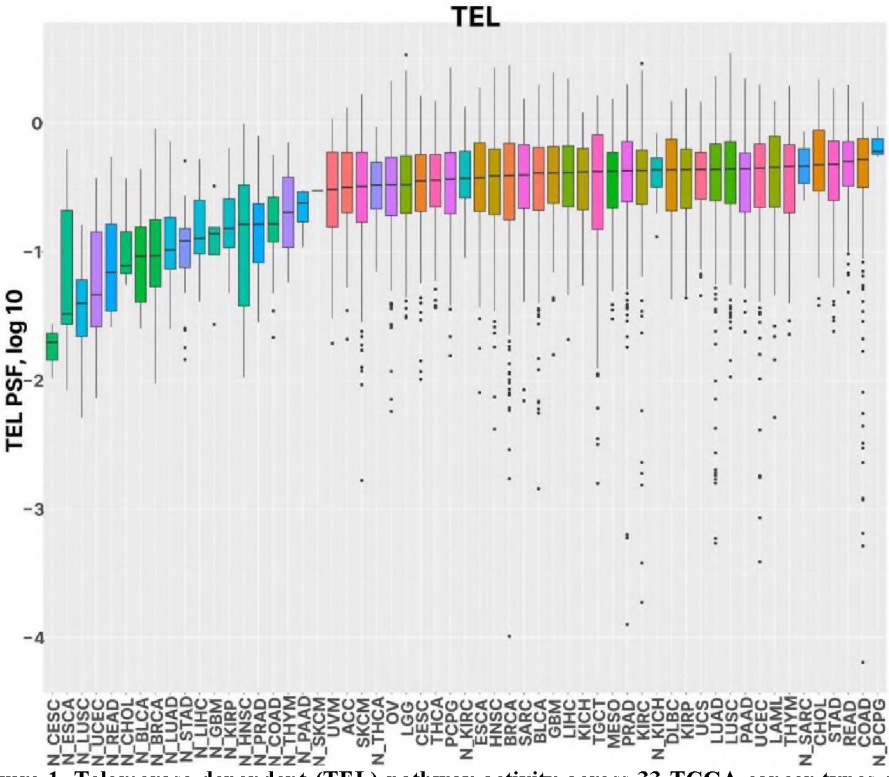

Figure 1. Telomerase-dependent (TEL) pathway activity across 33 TCGA cancer types and matched normal tissues. Box plots display the distribution of TEL PSF scores (log_{10}-transformed; y-axis) across all primary tumor samples and their tumor-adjacent normal counterparts. Each cancer type is shown as a colored box; matched normal tissues are denoted by the prefix "N_".

The TEL pathway showed elevated activity in colon, rectum, stomach, and cholangiocarcinoma samples, while the ALT pathway exhibited markedly greater variability across cancer types, with the highest activity in cholangiocarcinoma, stomach adenocarcinoma, and acute myeloid leukemia (Figures 1, 2). In a subset of tumor types, including pheochromocytoma and paraganglioma, sarcoma, prostate adenocarcinoma, melanoma, and thymoma, adjacent normal tissues displayed elevated TEL or ALT activity, suggesting molecular changes in histologically normal tissue within the tumor field.

Importantly, simultaneous activation of TEL and ALT pathways was observed in several tumor types, supporting the conclusion that telomere maintenance is not a strictly binary process and that co-activation may contribute to telomere maintenance flexibility in pathway usage and potentially to therapeutic resistance.

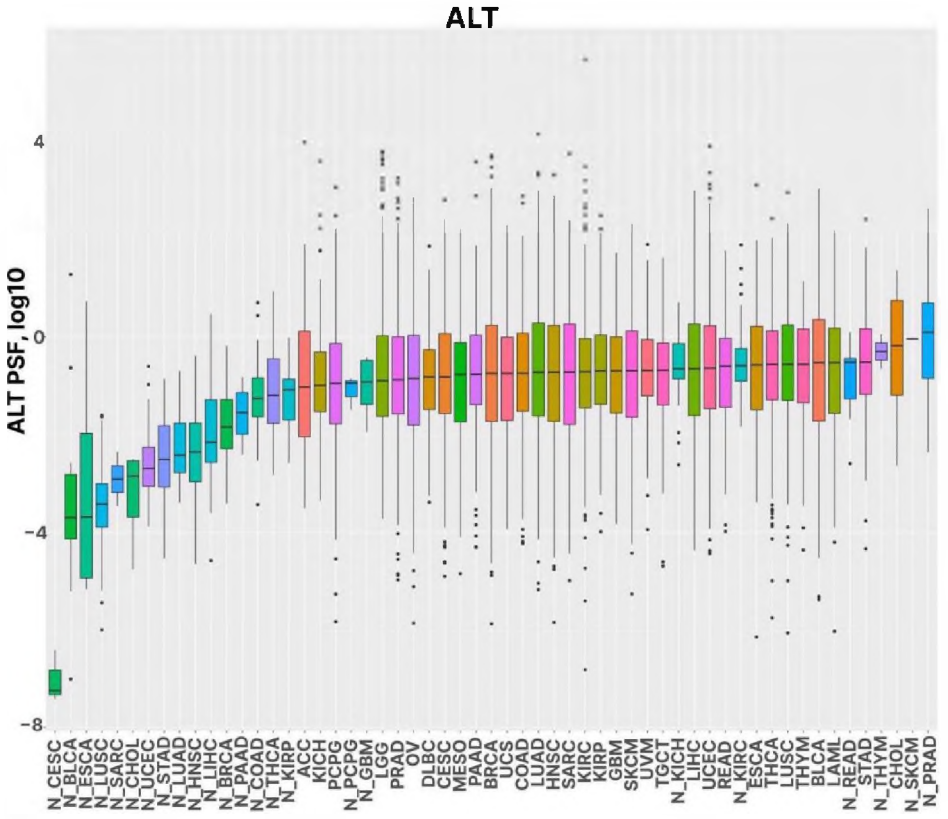

Figure 2. Alternative lengthening of telomeres (ALT) pathway activity across 33 TCGA cancer types and matched normal tissues. Box plots display the distribution of ALT PSF scores (log_{10}-transformed; y-axis) across all primary tumor samples and their tumor-adjacent normal counterparts. Each cancer type is shown as a colored box; matched normal tissues are denoted by the prefix "N_".

This variability may reflect the tissue-specific regulation of recombination-based telomere maintenance and aligns with earlier reports suggesting that tumors of mesenchymal origin are more

likely to activate the ALT pathway compared to epithelial tumors [Cesare et al., 2010; Henson et al., 2010].

TMM Phenotyping and Stratification Across Cancer Types. Stratification of samples by segmented regression-based thresholds defined five TMM phenotypes: ALT ^{low} TEL ^{low}, ALT ^{low} TEL ^{high}, ALT ^{high} TEL ^{low}, ALT ^{high} TEL ^{high}, and ALT ^{middle} TEL ^{middle} (Figure 3).

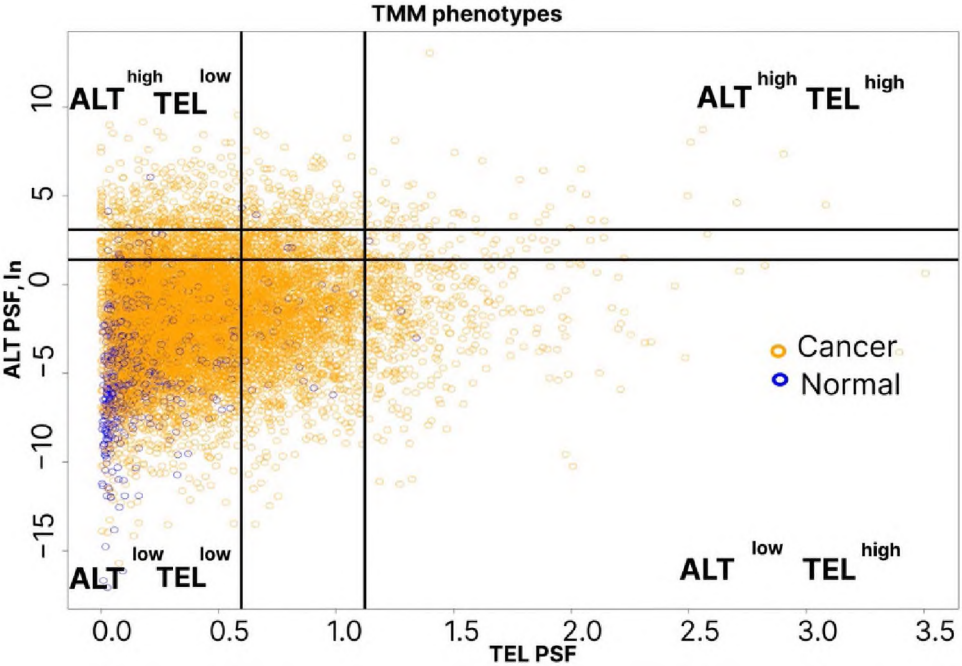

Figure 3. Classification of ALT and TEL TMM into five phenotypes. Cancer (orange) and **normal** (blue) samples were categorized into five phenotypes based on their TEL PSF and ln-transformed ALT PSF values. The vertical and horizontal lines indicate the thresholds used for stratification. ALT-high threshold (ALT_ln PSF > 3.10), ALT-low threshold (ALT_ln PSF < 1.35), TEL-high threshold (TEL PSF > 1.12), and TEL-low (TEL PSF < 0.61).

The ALT ^{low} TEL ^{low} phenotype was the most prevalent (approximately 64.5% of cancer samples), the ALT ^{middle} TEL ^{middle} phenotype was identified in 28% of samples, and at least one "high" phenotype was observed in approximately 8% of cases. One possible explanation for this observation is the presence of ALT-positive cells that maintain telomeres independently of ALT-associated PML bodies (APB) formation and in the absence of telomerase activity. Colorectal adenocarcinoma and cholangiocarcinoma showed the highest proportions of dual-activated phenotypes (~40%), while several tumor types (ACC, GBM, CHOL, DLBC, MESO, LIHC, OV, READ, UVM) lacked the ALT ^{high} TEL ^{high} phenotype. This suggests potential lineage- or context-specific constraints on the co-activation of both TMM pathways.

Single-cell GBM Phenotyping. Single-cell analysis of glioblastoma identified a co-active ALT ^{high} TEL ^{high} state in approximately 2.5% of cells, indicating that TEL and ALT signatures can coexist

within individual cells, and 32% of cells fell into the dual-active ALT ^{high} TEL ^{high}, ALT ^{high} TEL ^{low}, or ALT ^{low} TEL ^{high} categories (Figure 4).

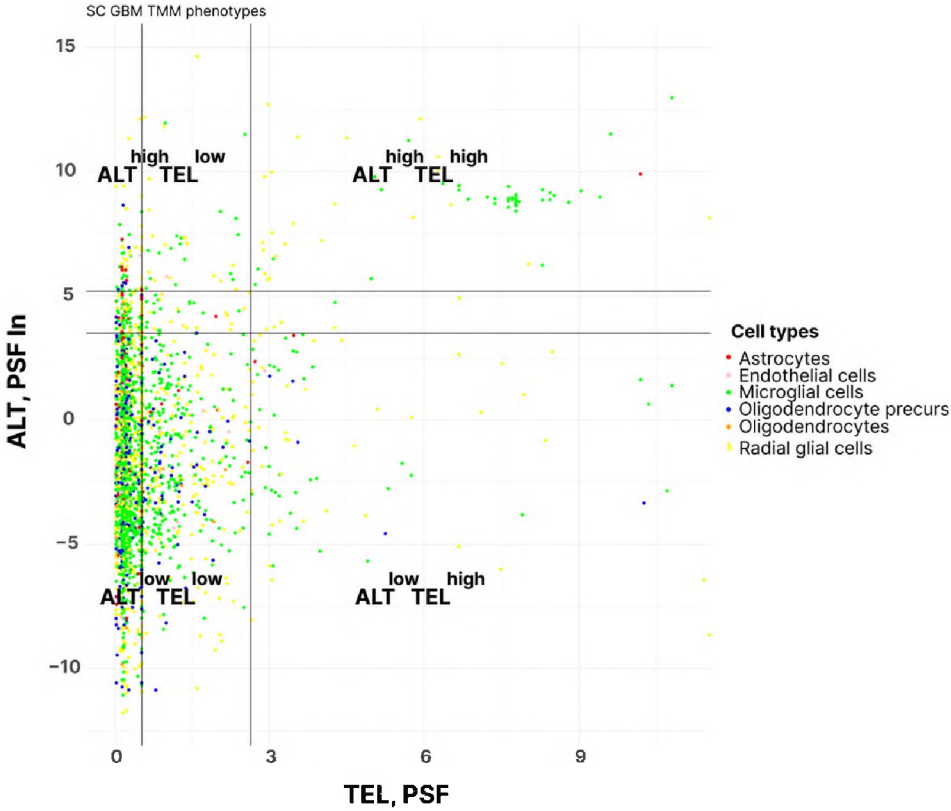

Figure 4. Stratification of ALT and TEL TMM phenotypes for single-cell GBM. The color coding corresponds to the cell type. SC GBM is divided into five groups based on their TEL PSF and ALT (ln) PSF values: ALT ^{high} TEL ^{low}, ALT ^{low} TEL ^{low}, ALT ^{middle} TEL ^{middle}, ALT ^{high} TEL ^{high}, and ALT ^{low} TEL ^{high} phenotypes. The vertical and horizontal lines show the thresholds used: ALT-high threshold (ALT_ln PSF > 5.19), ALT-low threshold (ALT_ln PSF < 3.50), TEL-high threshold (TEL PSF > 2.63), and TEL-low (TEL PSF < 0.52).

Tumor Purity, Microsatellite Status, and Clinical Stage Association with TMM Pathway Branch Activity. TMM phenotype classification was found to be largely independent of tumor purity, as estimated by five computational approaches, supporting the robustness of phenotype assignment (Figure 5 A, B). All TMM phenotypes were represented across a broad range of tumor purity values, suggesting that phenotype assignment is largely independent of tumor purity.

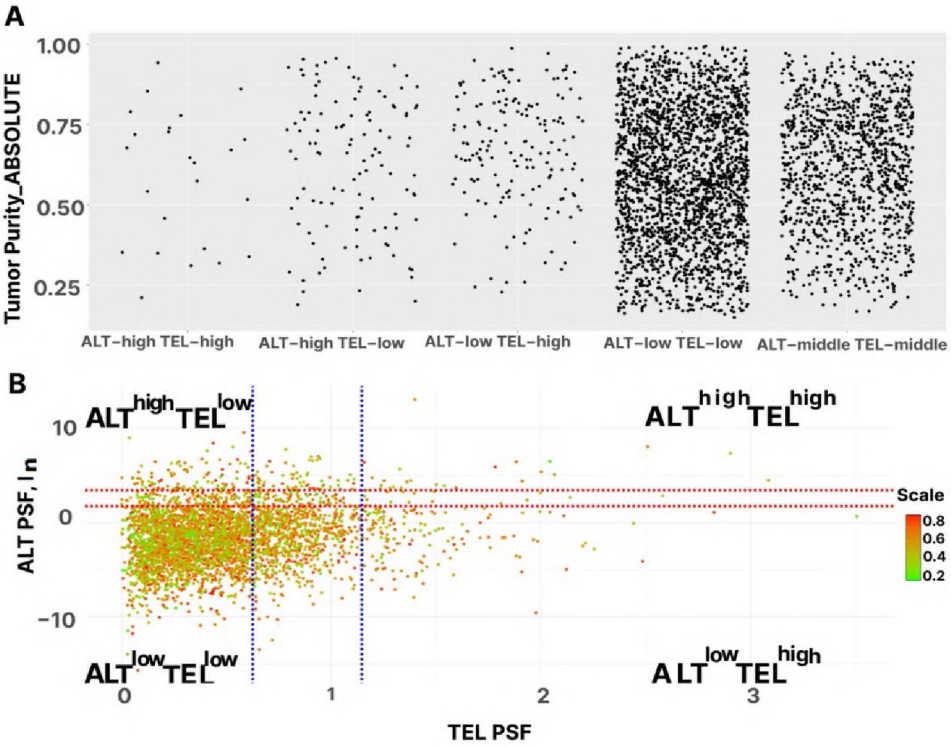

Figure 5. Distribution of tumor purity across TMM phenotypes. A, Tumor purity estimates (ABSOLUTE method) are shown for five TMM phenotypic groups. **B,** Samples are mapped in the TMM space based on their TEL PSF and ln-transformed ALT PSF values, stratified into five phenotypic categories using defined threshold lines. Color represents tumor purity levels. ALT-high threshold (ALT_ln PSF > 3.10), ALT-low threshold (ALT_ln PSF < 1.35), TEL-high threshold (TEL PSF > 1.12), and TEL-low (TEL PSF < 0.61).

Analysis of MSI status across seven TCGA cohorts (n = 1,759 samples) revealed significantly elevated TEL and ALT pathway activity in MSI-H tumors relative to MSI-L and MSS tumors (Kruskal-Wallis test, TEL: $p = 5.1 \times 10^{-10}$; ALT: $p = 4.8 \times 10^{-16}$) (Figure 6). Pairwise comparisons revealed that MSI-H tumors exhibited significantly higher TEL activity compared to MSI-L ($p = 0.018$) and MSS ($p = 1.8 \times 10^{-10}$). MSI-L tumors also showed elevated TEL activity compared to MSS ($p = 0.0088$). ALT pathway activity was also significantly different across groups. MSI-H tumors had significantly higher ALT activity than MSI-L ($p = 2.3 \times 10^{-7}$) and MSS ($p < 2.2 \times 10^{-16}$) (Figure 6). These findings suggest a broad activation of telomere maintenance mechanisms in tumors with high MSI, likely reflecting genomic instability and a heightened requirement for telomere elongation. These findings align with prior observations that associate high telomerase activity with MSI tumors [Cortes-Ciriano et al., 2017; Vidaurreta et al., 2007; Vilar et al., 2010].

In the stratified analysis across seven cancer types, the following trends were observed: TEL Pathway (Figure 6 C): MSI-H tumors consistently exhibited higher TEL PSF scores compared to MSS, with significant differences observed particularly in UCEC ($p = 0.0015$) and STAD ($p < 0.0001$). Some cancers, like COAD and UCS, showed moderate increases, though not always

statistically significant, and for the PAAD cancer type, the MSI-H phenotype was missing (Figure 6C, D).

ALT Pathway (Figure 6 D): MSI-H samples showed markedly higher ALT activity in COAD and STAD (all $p < 0.0001$). A significant increase in ALT activity was observed in MSI-L samples in certain cancer types, including UCEC ($p = 0.0027$).

TEL activation in MSI-H tumors was driven predominantly by the "TERT" and "TERC" branches, whereas ALT activation in MSI-H tumors involved a broader set of branches, including "Holliday junction processing", "strand invasion", "templated synthesis", and "APB recruitment".

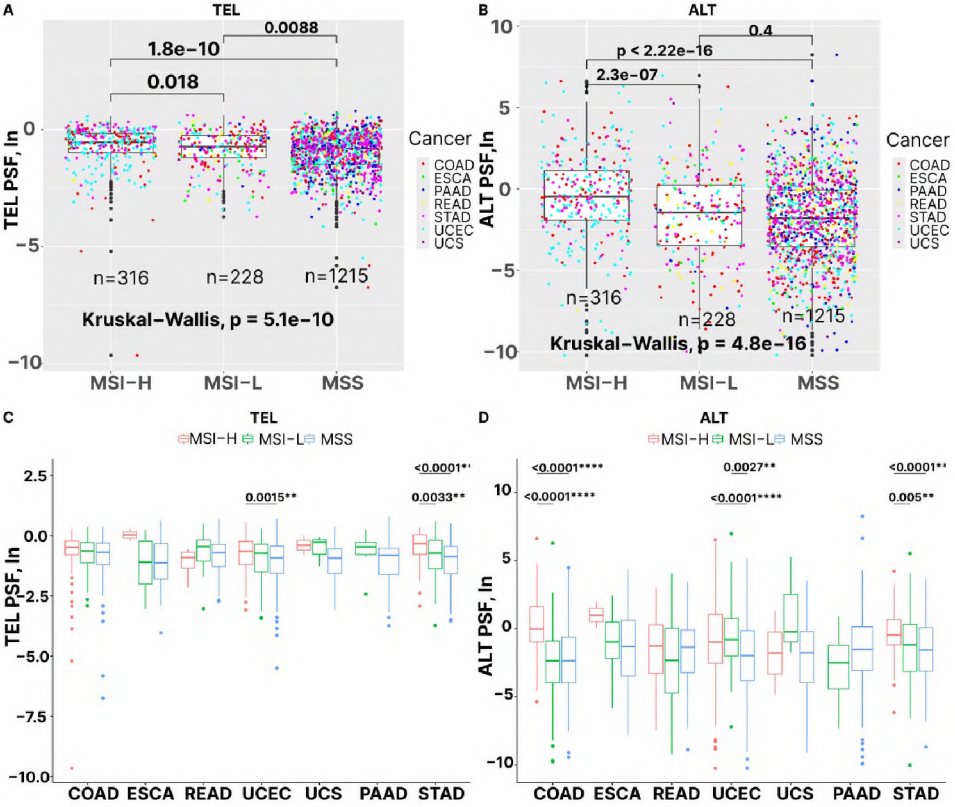

Figure 6. PSF activity plots of TEL and ALT TMM pathways for MSI and MSS tumors. Comparison of **A,** the TEL, and **B,** the ALT TMM PSF activities for MSI and MSS tumors. Statistical significance between the MSS and MSI groups was assessed using the Kruskal-Wallis test followed by Dunn's test for multiple comparisons. **C,** TEL, and **D,** ALT pathway activity across seven cancer types. The color coding corresponds to the MSS/MSI status. Statistical analysis for MSS/MSI comparison used Dunn's test.

TEL pathway activity also showed a clear stage-dependent increase across clinical stages I-IV, with the most pronounced increase observed for the "TERT" branch ($p < 0.0001$ between Stage I and Stage IV) (Figure 7). Higher TEL pathway activity in advanced clinical stages is consistent with

a role for telomerase in tumor progression in cancers, and with the role of *TERT* expression and telomerase enzymatic activity [Hanahan et al., 2011; Shay et al., 2011].
The ALT pathway remained largely stable across stages.

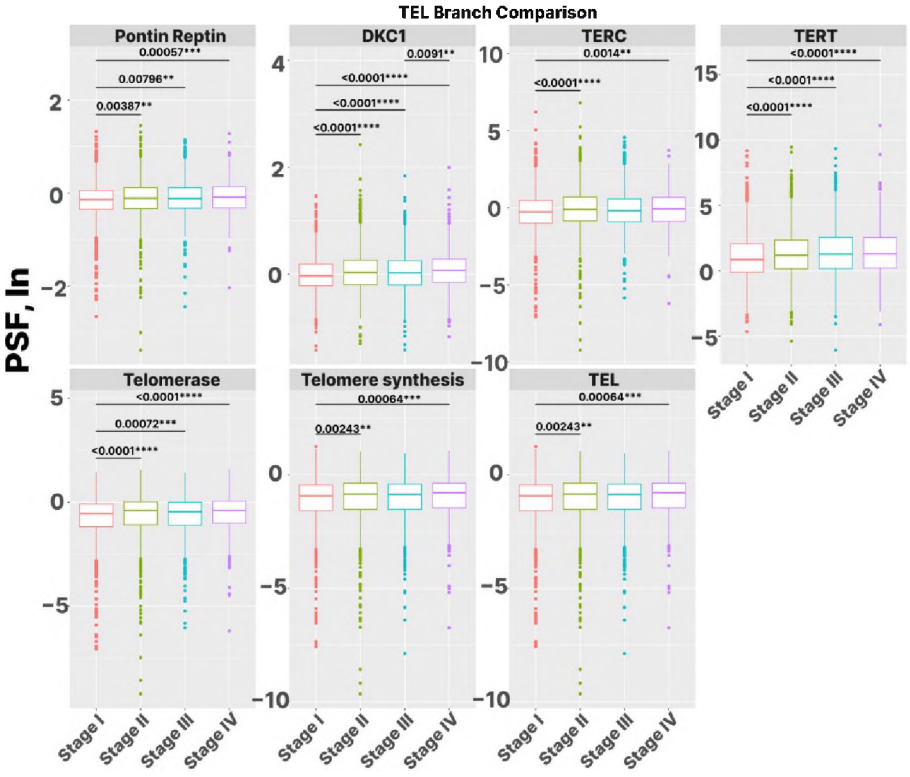

Figure 7. TEL pathway branch activity across tumor clinical stages. Boxplots display the ln-transformed PSF scores for individual TEL branches and overall TEL activity across clinical stages (Stage I to Stage IV). Statistical analysis for Stage comparison used Dunn's test.

Mutational Landscape of TEL and ALT Pathways. Samples harbouring mutations in TEL- or ALT-pathway-associated genes exhibited slightly elevated pathway activity compared with wildtype (WT) samples (Mann-Whitney U test, TEL $p = 7.4 \times 10^{-6}$; ALT $p = 3.1 \times 10^{-14}$) (Figure 8 A, B). However, mutations in the canonical TMM regulators *TERT* and *ATRX* did not produce uniform effects at the pan-cancer level. *TERT* mutations were not significantly associated with TEL activity in the global analysis ($p = 0.49$), and *ATRX* mutations did not significantly alter ALT activity ($p = 0.13$) (Figure 8 C, D). At the level of individual cancer types, statistically significant associations were observed in specific contexts: TERT-mutated TGCT samples showed elevated TEL activity ($p = 0.023$), while *ATRX*-mutated GBM and KICH tumors displayed elevated ALT activity ($p = 0.0011$ and $p = 0.0075$, respectively). These findings indicate that canonical mutations only partially explain pathway activity, and that transcriptome-level analysis provides additional information beyond mutational status.

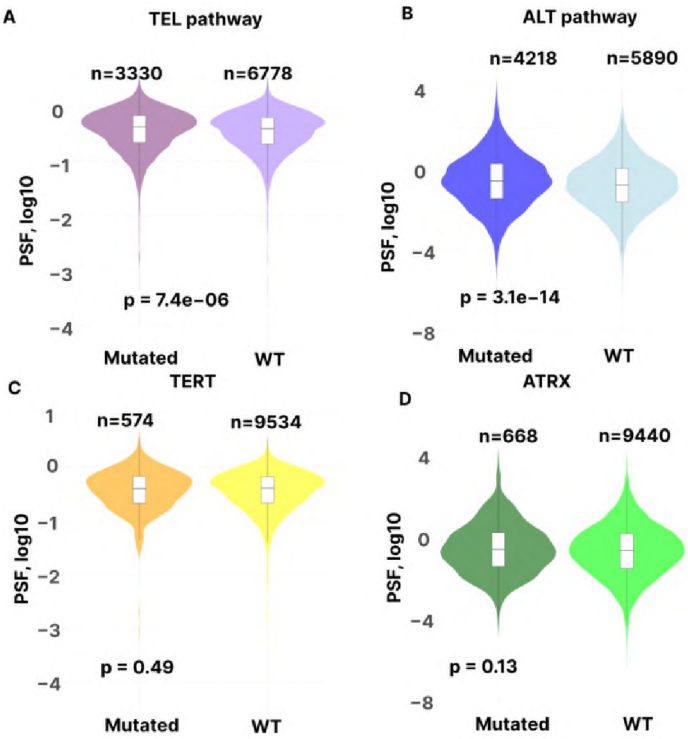

Figure 8. Activity plots of ALT and TEL pathways for TEL/ALT pathway genes mutated/WT and *TERT/ATRX* mutated/WT genes samples. **A,** The TEL and **B,** the ALT pathway genes mutated/WT sample plots for 33 cancer types. **C,** The *TERT* mutated/*TERT* WT samples TEL pathway activity patterns across all cancer types. **D,** The *ATRX* mutated/*ATRX* WT samples ALT pathway activity patterns across all cancer types. Statistical significance between mutated and WT samples for each gene and pathway was assessed using the Mann-Whitney U test.

Survival Outcomes and TMM Phenotypes. Survival analysis across the pan-cancer cohort revealed that the ALT ^{high}TEL ^{high} phenotype was consistently associated with the poorest overall survival (OS) and progression-free survival (PFS). Pairwise log-rank comparisons demonstrated significantly worse OS in the ALT ^{high}TEL ^{high} group compared with ALT ^{low}TEL ^{low}, ALT ^{middle}TEL ^{middle}, and ALT ^{low}TEL ^{high} phenotypes (Figure 9 A, B). Cox proportional hazards regression confirmed an elevated hazard of death for the ALT ^{high}TEL ^{high} group (HR = 1.9, 95% CI: 1.3-2.7, $p < 0.001$) and a modest but significant increase for the ALT ^{middle}TEL ^{middle} group (HR = 1.1, $p = 0.008$) (Figure 9 C, D). Similar trends were observed in individual cancer types also. Our findings, consistent with prior reports, suggest that TMM activity delays cellular senescence and enhances proliferative potential, contributing to more aggressive tumor behavior and reduced patient survival. A study by Roderwieser et al. [2019] demonstrated that the activation of TEL and ALT pathways defines distinct neuroblastoma subgroups, both linked to poor prognosis.

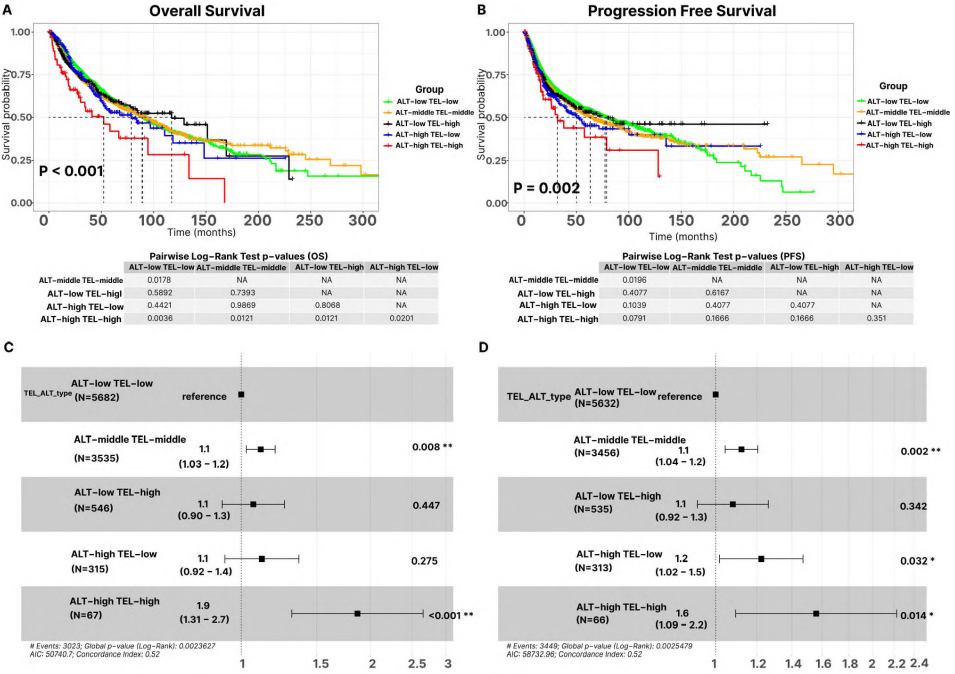

Figure 9. Survival outcomes and Hazard ratios for ALT and TEL tumors. Survival and hazard ratio forest plots for ALT and TEL tumors **A,** and **C**, Overall survival. **B,** and **D,** Progression-free survival curves. Significance was calculated using a log-rank test for K-M plots, and a Cox proportional hazards regression model was used to estimate hazard ratios. Reference group ALT ^{low} TEL ^{low}.

Differential Gene Expression and Gene Ontology Enrichment and Protein-Level Expression Analysis of Differentially Expressed Genes. Differential gene expression analysis across TMM phenotypes, using ALT ^{low} TEL ^{low} as reference, identified a recurrent set of genes upregulated in dual-active and high-activity phenotypes, including *MCM7*, *MCM10*, *TRIP13*, *TPX2*, *RAD51*, *RAD51AP1*, *POLQ*, *ASPM*, *HMMR*, *GTSE1*, and *KIF2C* genes involved in DNA replication, mitotic progression, and homologous recombination. Conversely, *ADAMTS8*, *TSPAN7*, *MYH11*, *ZBTB16*, *OGN* and *MTND1P23* were consistently downregulated. Gene Ontology enrichment confirmed strong over-representation of cell-cycle checkpoint signaling, DNA replication, chromosome segregation, and DNA recombination across multiple phenotype comparisons. Protein-level validation in TCGA confirmed elevated FOXM1 protein expression across TMM phenotypes (Kruskal-Wallis $p < 2.2 \times 10^{-16}$), with moderate and strong correlation between mRNA and protein levels for *FOXM1* (PCC = 0.515) and *BCL2* (PCC = 0.784) in pan-cancer samples (Figure 10).

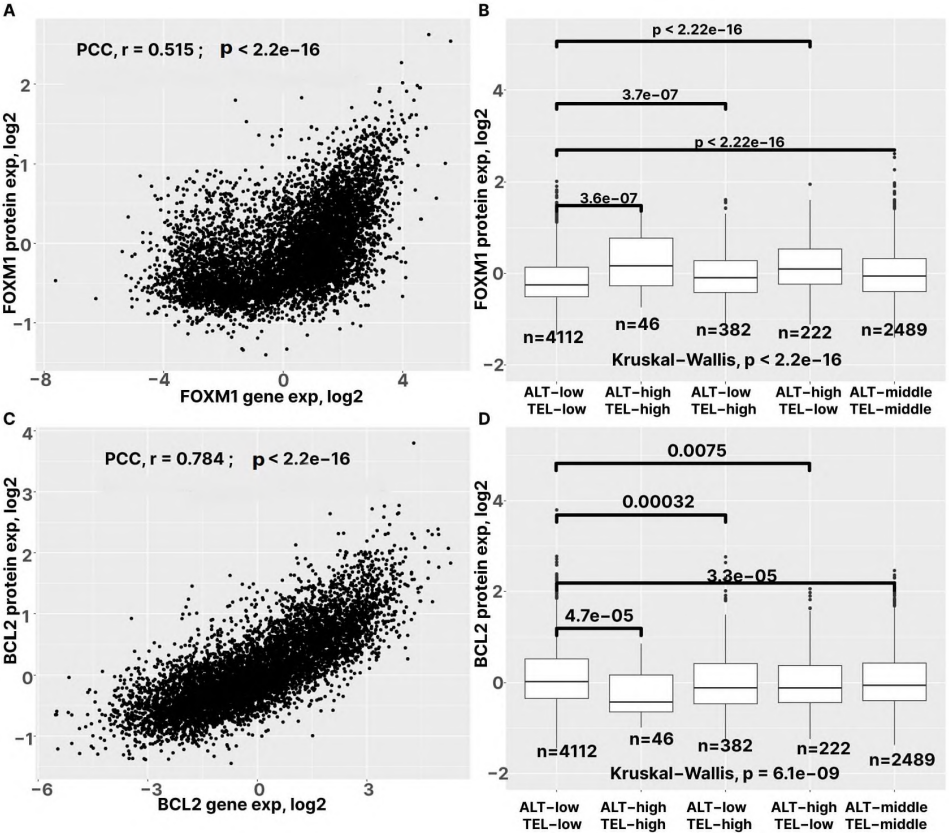

Figure 10. Correlation plots of the relationship between protein expression versus RNA-seq gene expression, and proteome-level TMM phenotype activity plots for all cancer types. A, Scatter plot showing the Pearson correlation between *FOXM1* mRNA expression and protein abundance (both $\log_2$-transformed) across pan-cancer samples. **B,** FOXM1 protein expression levels stratified by TMM phenotype. **C,** Scatter plot showing the Pearson correlation between *BCL2* mRNA expression and protein abundance (both $\log_2$-transformed) across pan-cancer samples. **D,** BCL2 protein expression levels stratified by TMM phenotype. **B,** and **D,** Global differences across TMM phenotypes were assessed using the Kruskal-Wallis test; pairwise comparisons were performed using the Mann-Whitney U test. PCC and corresponding p-values are indicated within panels **A,** and **C**.

Telomere Length, IDH status, and TMM Regulation in Low-grade Glioma. In the TCGA-LGG cohort, TEL pathway activity was significantly higher in tumors with short telomeres compared with long-telomere tumors (Mann-Whitney U test, $p = 0.034$), driven predominantly by the "TERT" branch ($p = 2.9 \times 10^{-15}$). In contrast, overall ALT pathway activity did not differ significantly between Long-TL and Short-TL groups, suggesting that ALT activation is not strictly governed by telomere length but is shaped by broader genomic and recombinational context (Figure 11). The lack of a significant correlation between TL ratio and either TEL or ALT PSF values further suggests that TMM activation may not scale linearly with telomere length in LGG. This could reflect

the threshold-based or switch-like nature of TMM activation, particularly for ALT, which is often activated in response to broader genomic instability rather than telomere shortening alone.

Interestingly, while ALT activity did not differ significantly between Long TL and Short TL tumors, previous reports indicate that ALT-positive cells often harbor highly heterogeneous telomere lengths, sometimes even longer than those in telomerase-positive cells. Thus, the presence of ALT activity may not directly mirror telomere erosion but may reflect regulatory triggers.

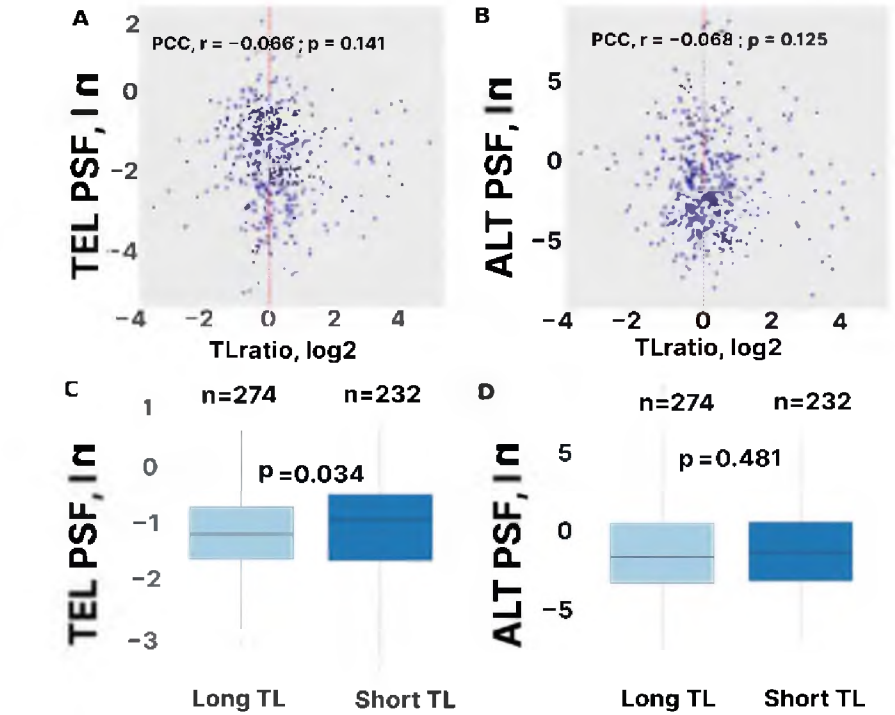

Figure 11. Relationship between telomere length and PSF scores for TEL and ALT pathways. Scatter plots showing the correlation between the telomere length ratio (TL ratio, $\log_2$) and **A,** the TEL PSF, and **B,** the ALT PSF. Comparison of **C,** the TEL PSF, and **D,** the ALT PSF scores between samples with long and short telomere lengths. Statistical significance was evaluated using the Mann-Whitney U test. PCC and corresponding p-values are indicated within panels **A,** and **B**.

Previous studies have linked IDH mutation status to telomere length variation [Waitkus et al., 2024], but our results suggest a more complex interplay between IDH subtype, telomere length, and TMM pathway activation. While IDH mutation status shapes telomere biology, no direct, consistent correlation between IDH subtype and TL was observed in our dataset.

Analysis across IDH subtypes revealed that IDH-wt tumors displayed the most pronounced TEL pathway activation under short-telomere conditions and the highest overall ALT pathway activity, particularly through *RAD51*-related branches (Figure 12). These results were validated in the independent CGGA cohort, where IDH-wt tumors again showed the highest ALT pathway activity, with TERT driving TEL activation and *RAD51* emerging as the principal contributor to ALT activity.

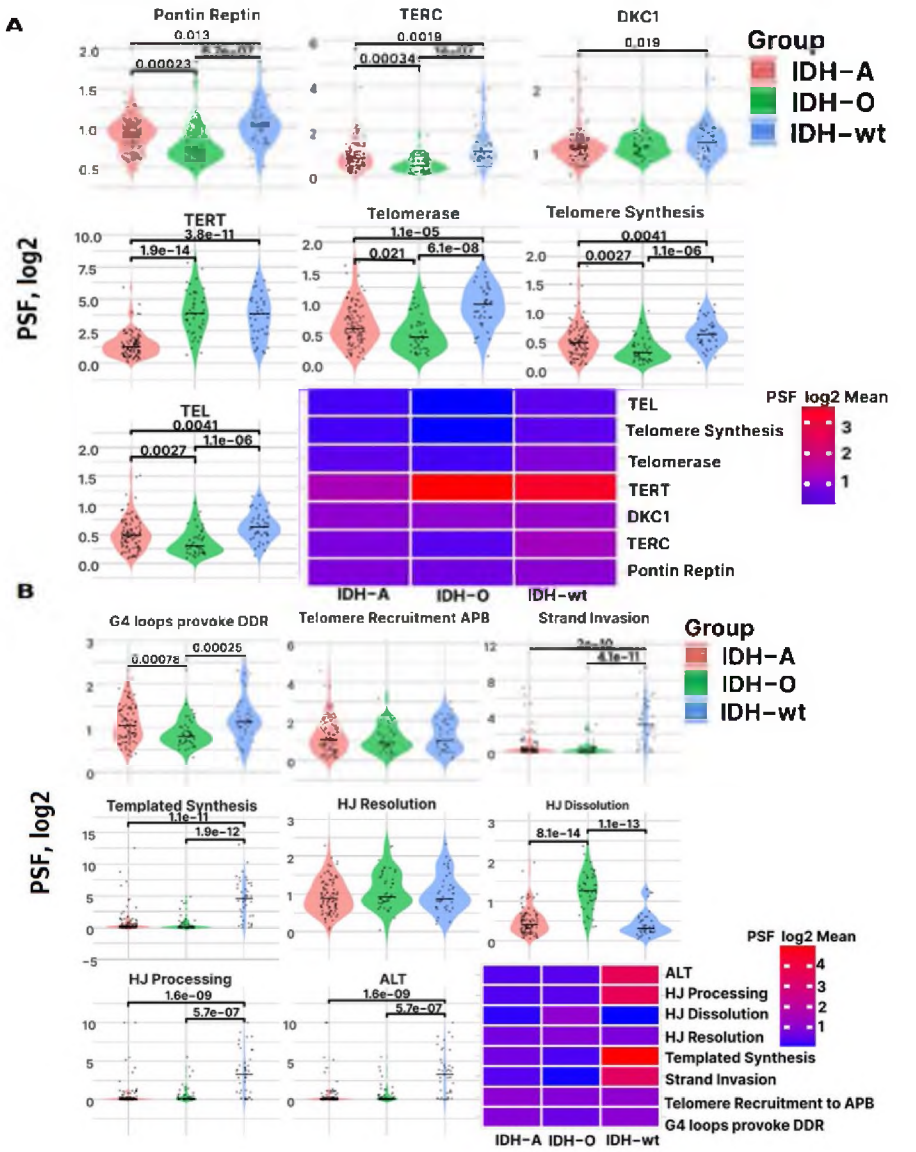

Figure 12. Comparison of TEL and ALT pathway branch activities across *IDH* molecular subtypes in TCGA LGG samples. A, The TEL pathway branches (log_2-transformed PSF values) show activity patterns among IDH-A, IDH-O, and IDH-wt gliomas. **B,** The ALT pathway branches. P-values indicate pairwise comparisons (Mann-Whitney U test). IDH group (IDH-A = red, IDH-O = green, IDH-wt = blue). The heatmap (right panel) shows the mean log_2-transformed PSF values for each branch within each *IDH* subgroup, with color intensity representing the magnitude of pathway activity (scale bar: PSF log_2 Mean)

Telomere Length, ATRX Status, and Clinical Outcome in LGG. Analysis of genetic subtypes defined by IDH and *ATRX* status revealed that IDH-A/*ATRX*-Mutant tumors displayed the highest telomere length ratios, whereas IDH-wt tumors (both *ATRX*-WT and *ATRX*-mutant) exhibited the lowest telomere length ratios and the highest ALT pathway activity (Mann-Whitney U test, $p = 6.9 \times 10^{-14}$ and $p = 0.001$) (Figure 13). These observations indicate that *ATRX* mutations are a key determinant of telomere length and ALT activation, but that *ATRX* status alone does not fully account for ALT pathway activity.

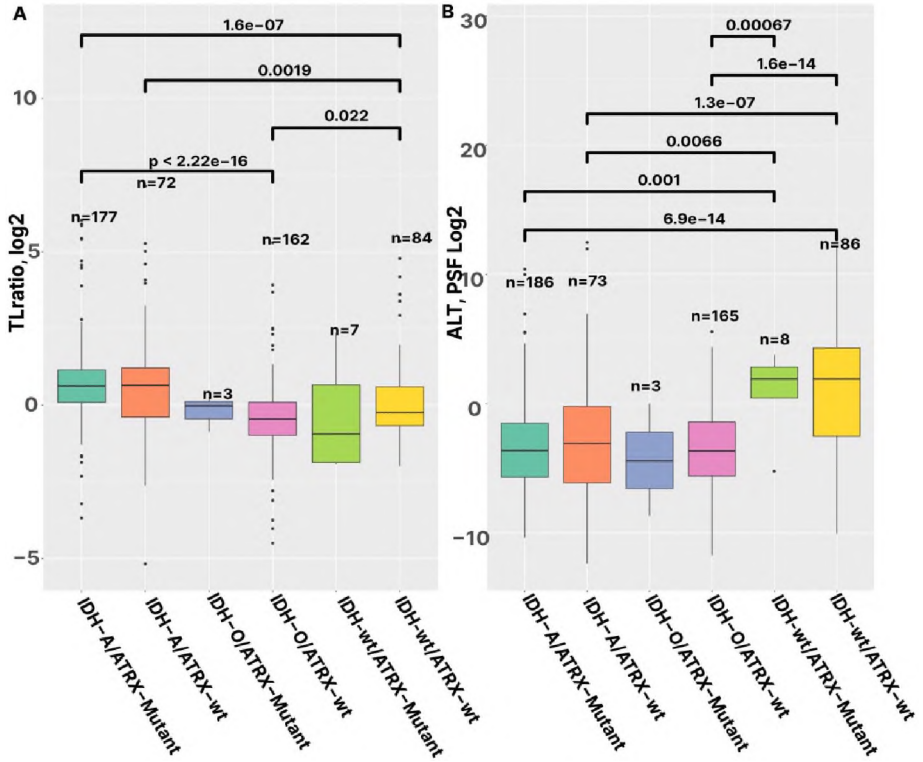

Figure 13. Comparison of telomere length ratios and ALT pathway PSF scores among *IDH* genetic subtypes with different *ATRX* statuses.
A, TL ratio (log_2) variations across genetic subtypes defined by *IDH* and *ATRX* mutation status. **B,** The ALT pathway PSF score (log_2) variations across *IDH* genetic subtypes and ATRX status. Statistical significance was determined using the Mann-Whitney U test.

Survival analysis in LGG demonstrated that the ALT ^{high} TEL ^{high} phenotype consistently linked to the poorest prognosis in patients with short and long telomeres (log-rank test, $p < 0.0001$), while ALT ^{middle} TEL ^{middle} and ALT ^{low} TEL ^{high} phenotypes were associated with more favorable outcomes (Figure 14). Multivariate Cox regression, including age, treatment, telomere length, and TMM phenotype, confirmed TMM phenotype as an independent prognostic factor in LGG.

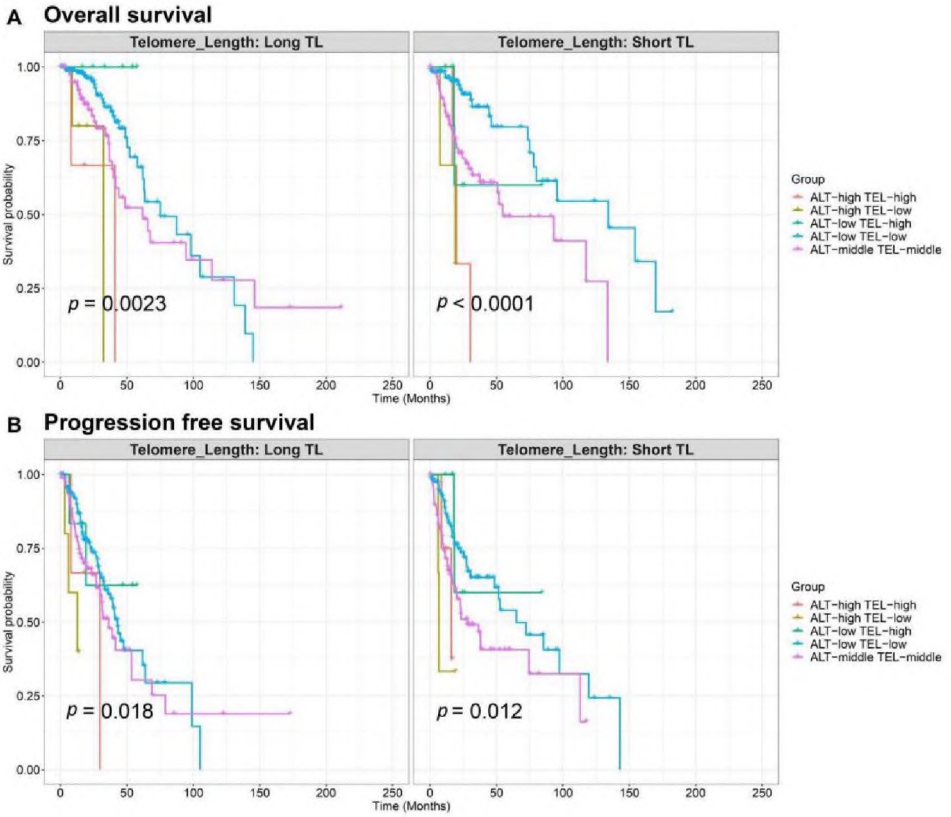

Figure 14. Survival analysis of long- and short-telomere groups in glioma based on TMM phenotype. A, Overall survival and **B,** progression-free survival curves. Significance between TMM phenotype groups ALT ^{high} (ALT_ln PSF > 3.64), ALT ^{low} (ALT_ln PSF < 1.51), TEL ^{high} (TEL PSF > 0.92), and TEL ^{low} (TEL PSF < 0.43) was evaluated using the log-rank test. Samples falling outside these thresholds were classified as the intermediate group, ALT ^{middle} TEL ^{middle}.

Validation Through Negative-control Analysis. To verify the specificity of the TMM framework, TEL and ALT pathway activity was compared across COPD, LUAD, GBM, LGG, and their corresponding normal tissues. GBM and LGG tumors exhibited markedly higher TEL pathway activity compared with COPD ($p = 5.1 \times 10^{-8}$ and $p = 1.4 \times 10^{-8}$, respectively) and relative to normal tissues, whereas ALT activity in COPD displayed high inter-sample variability consistent with heterogeneous immune and inflammatory infiltration rather than genuine ALT pathway activation (Figure 15). These findings support the capacity of the framework to distinguish biologically meaningful telomere maintenance activity from background variation.

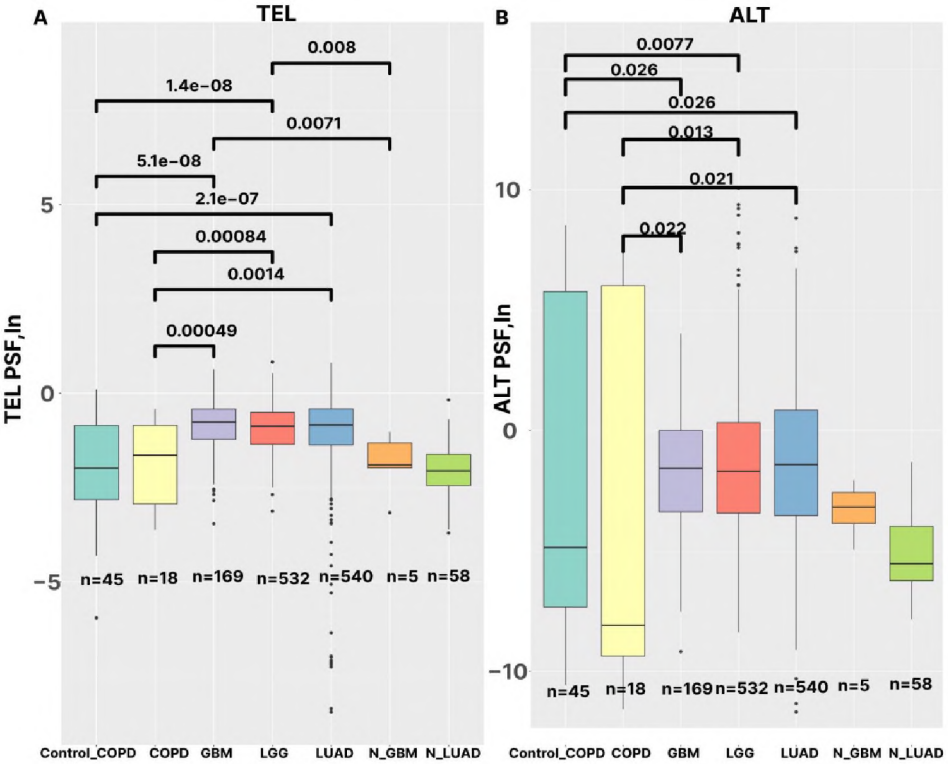

Figure 15. Validation of TEL and ALT pathways using negative controls. A, The TEL pathway and **B,** the ALT pathway PSF scores (ln) across the groups: Control_COPD, COPD, GBM, LGG, LUAD, Normal GBM (N_GBM), and Normal LUAD (N_LUAD).

CONCLUSION

This study provides a comprehensive pan-cancer evaluation of telomere maintenance mechanisms across 33 tumor types using TCGA transcriptomic data, complemented with additional LGG-focused research. By integrating pathway topology with gene expression profiles, we systematically characterized the activity of both TEL and ALT pathways, revealing distinct and heterogeneous patterns of activation among cancers.

Both TEL and ALT pathways exhibited significantly higher activity in tumors compared to matched normal tissues, underscoring their fundamental role in sustaining tumor proliferation and highlighting TMM activation as a hallmark of malignancy. The ALT activity displayed substantial variability, particularly in mesenchymal tumors. Moreover, evidence of co-activation of TEL and ALT pathways was observed in certain cancers, suggesting that tumors may exploit multiple complementary TMM strategies to sustain telomere integrity and support continued proliferation.

Mutations in TEL- and ALT-pathway associated genes were associated with mildly elevated pathway activity, although specific driver mutations such as *TERT* promoter and *ATRX* showed diverse effects depending on tumor type, significant in GBM, KICH, STAD, and TGCT, but not at

the pan-cancer level. These findings indicate that while genetic lesions contribute to TMM activation, they do not fully account for pathway heterogeneity.

Stratification of tumors into TMM phenotypes demonstrated prognostic significance. In particular, tumors with concurrent high ALT and TEL activity were consistently associated with the poorest overall and progression-free survival, emphasizing the clinical relevance of TMM phenotyping in cancer prognosis.

In-depth analysis of LGGs revealed subtype-specific differences in telomere length. IDH-wt tumors showed the strongest ALT pathway activation, largely associated with *RAD51*. Variations in telomere length were strongly affected by both *ATRX* and *IDH* status, with the IDH-wt/*ATRX*-wt and IDH-wt/*ATRX*-Mutant subgroups presenting the lowest telomere length ratios and the highest levels of ALT activity. These results reinforce the pivotal role of telomere biology in gliomagenesis and support the utility of LGG as a model system to study the interplay between genetic background and TMM regulation.

Taken together, the pan-cancer and LGG analyses converge on a conclusion: telomere maintenance in cancer is not a binary choice between telomerase and ALT, but a graded, context-dependent property whose activation pattern is shaped by tissue lineage, mutational background, microsatellite status, and telomere length itself. The TMM phenotypes identified in this work: TEL-dominant, ALT-dominant, co-activated, and TMM-inactive, recur across cancer types and stratify patients by survival, but their boundaries depend on cohort-specific thresholds and on transcriptomic inference rather than direct enzymatic measurement.

INFERENCES

1. TMM activity does not switch in a binary fashion; rather, it forms a continuum of phenotypes, including TEL-TMM-dominant, ALT-TMM-dominant, co-activated in the same cell, or inactivated in the same cell.

2. TMM phenotypes revealed shared transcriptional signatures, indicating that elevated TMM activity, regardless of the dominant pathway, is coupled to broader proliferative and survival programmes in tumors.

3. Co-activation of TEL-TMM and ALT-TMM defines the poorest pan-cancer overall and progression-free survival, establishing TMM phenotyping as an orthogonal prognostic marker.

4. The elevated TMM activity in MSI-high tumors further suggests that the genomic instability characteristic of MMR-deficient tumors is associated with a more complex, multi-branched TMM response than that observed in MSS tumors.

5. In IDH-wt low-grade gliomas, the elevation of *RAD51*-associated strand invasion identifies homologous-recombination-mediated strand invasion as the dominant contributor to high ALT-TMM activity in this lineage, and points to *RAD51* as a potential therapeutic target in IDH-wt tumors.

6. In low-grade gliomas, TEL-TMM is selectively engaged in response to telomere shortening, while ALT-TMM is shaped by the genomic and recombinational context of the tumor rather than by telomere length itself. The combined assessment of telomere length, *IDH* mutation status, and TMM phenotype provides a more informative prognostic framework for survival than any single molecular variable alone.

LIST OF PUBLISHED WORKS

1. Hakobyan, M.; Binder, H.; Arakelyan, A. Pan-Cancer Analysis of Telomere Maintenance Mechanisms. J. Biol. Chem. 2024, 300, 107392. doi: 10.1016/j.jbc.2024.107392.
2. Hakobyan, M. Pan-Cancer Screening for *TERT* and *ATRX* Mutation-Associated Changes in Telomere Maintenance Mechanisms. Семнадцатая Годичная Научная Конференция Физико-Математические и Естественные Науки 2024, 1, 74–79. doi: 10.24412/cl-37235-2024-1-74-79
3. Hakobyan, M.; Binder, H.; Arakelyan, A. Telomere Maintenance Pathways in Lower-Grade Gliomas: Insights from Genetic Subtypes and Telomere Length Dynamics. Int. J. Mol. Sci. 2025, 26, 4175. doi: 10.3390/ijms26094175

Հակոբյան Մելինե Անդրանիկի

Թելոմերների երկարության պահպանման մեխանիզմների ակտիվության և թելոմերների երկարության դինամիկան

Ամփոփագիր

Բանալի բառեր՝ թելոմերների երկարության պահպանման մեխանիզմներ, թելոմերների այլընտրանքային երկարացում, թելոմերազ, պան-քաղցկեղային վերլուծություն, թելոմերների երկարության դինամիկա:

Թելոմերները ԴՆԹ-ի երկար, հաջորդական կրկնվող հատվածներ են՝ տեղակայված գծային էուկարիոտային քրոմոսոմների ծայրերին: Դրանց հայտնաբերումից ի վեր ենթադրվում է, որ թելոմերները կարևոր դեր են խաղում գենոմային կայունության պահպանման գործում և պաշտպանում են քրոմոսոմային միաձուլումից:

Մարդու սոմատիկ բջիջների մեծամասնությունում թելոմերները աստիճանաբար կրճատվում են բջջի ծերացմանը զուգընթաց: Թելոմերների պահպանման մեխանիզմների (telomere maintenance mechanisms, TMM) ակտիվացումը քաղցկեղի տարբերակիչ նշաններից է, որը թույլ է տալիս ուռուցքային բջիջներին հակազդել թելոմերների կրճատմանը և խուսափել թելոմերային «ճգնաժամի» մեկնարկից՝ էականորեն նպաստելով քաղցկեղի զարգացմանը:

Ներկայումս հայտնի են երկու հիմնական TMM-ներ: Թելոմերազային TMM-ը (TEL-TMM) գործում է ակտիվացված թելոմերազային ուղու միջոցով, որը թելոմերների երկարացման համար օգտագործում է ՌՆԹ մատրիցա պարունակող թելոմերազային ռիբոնուկլեոսպիտակուցային համալիրը: Իսկ թելոմերների այլընտրանքային երկարացման ուղին (ALT-TMM) իրականացվում է հոմոլոգ ռեկոմբինացիայի մեխանիզմով՝ քույր քրոմատիդների թելոմերային հատվածների, ինչպես նաև հեռավոր քրոմոսոմների կամ արտաքրոմոսոմային թելոմերային կրկնվող հաջորդականությունների միջև:

Թեև այս մեխանիզմները ավանդաբար համարվել են փոխադարձ բացառող, հետազոտությունները վկայում են, որ քաղցկեղում թելոմերների պահպանումն ավելի բարդ գործընթաց է: Կանոնական մուտացիոն մարկերներից անկախ՝ TMM ակտիվության համապարփակ պան-քաղցկեղային ֆունկցիոնալ բնութագրումը մնում է բաց խնդիր՝ ունենալով էական ախտորոշիչ և բուժական նշանակություն:

Այսպիսով, սույն ուսումնասիրության նպատակն է իրականացնել TMM-ի պան-քաղցկեղային գնահատում քաղցկեղի տարբեր տեսակներում՝ օգտագործելով ՌՆԹ սեքվենավորման տվյալները: Բացի այդ, ուսումնասիրել TMM-ների առանձնահատկությունները գենետիկական ենթատեսակների համատեքստում՝ մասնավորապես ցածր աստիճանի գլիոմաների (LGG) դեպքում:

Հետազոտության արդյունքները ներկայացնում են թելոմերների պահպանման մեխանիզմների համապարփակ պան-քաղցկեղային գնահատում ուռուցքների 33 տեսակներում՝ հիմնված TCGA-ի տրանսկրիպտոմային տվյալների վրա և համակցված ցածր աստիճանի գլիոմաների թիրախային վերլուծություններով: Թե՛ TEL, թե՛ ALT

ուղիները ցուցաբերել են զգալիորեն ավելի բարձր ակտիվություն ուռուցքներում, քան համապատասխան նորմալ հյուսվածքներում: Սահմանվել են TMM-ի հինգ ֆենոտիպեր: TEL-ի ու ALT-ի համատեղ ակտիվացում դիտվել է ուռուցքների մի մասում և հաստատվել գլիոբլաստոմայի առանձին բջջային (single cell) մակարդակում՝ փաստելով, որ թելոմերների պահպանումը խիստ երկակի (բինար) բնույթ չի կրում:

ALT-ի և TEL-ի միաժամանակյա բարձր ակտիվությամբ ուռուցքները ցուցաբերել են ամենացածր ապրելիությունը պան-քաղցկեղային խմբերում և քաղցկեղի առանձին տեսակներում: TEL և ALT ուղիների գեների մուտացիաները կապված են ուղու ակտիվության չափավոր բարձրացման հետ, սակայն *TERT*-ի և *ATRX*-ի մուտացիաները ամբողջությամբ չեն բացատրում ուղու հետերոգենությունը՝ հաստատելով տրանսկրիպտոմի վրա հիմնված TMM ֆենոտիպավորման հավելյալ արժեքը:

Ցածր աստիճանի գլիոմայում IDH-wildtype (չմուտացված) ուռուցքները ցուցաբերել են ALT ուղու ամենաուժեղ ակտիվացումը՝ պայմանավորված հիմնականում *RAD51* գենի բարձր ակտիվությամբ, ինչպես նաև թելոմերների երկարության ամենացածր հարաբերակցությամբ: TMM ֆենոտիպը LGG-ում դրսևորվել է որպես անկախ կանխագուշակիչ գործոն՝ թելոմերների երկարության հետ մեկտեղ:

Այսպիսով, ստացված արդյունքները վկայում են, որ թելոմերների պահպանման մեխանիզմները հանդիսանում են քաղցկեղի դինամիկ և հետերոգեն բնորոշիչ հատկանիշ: TMM ֆենոտիպերի բացահայտումը՝ ներառյալ TEL-դոմինանտ, ALT-դոմինանտ, համատեղ ակտիվացված (co-activated) և TMM-ինակտիվ վիճակները, ընդգծում է TMM համապարփակ ֆենոտիպավորման անհրաժեշտությունը ինչպես կանխագուշակիչ և կլինիկական նպատակներով, այնպես էլ թերապևտիկ ռազմավարությունների մշակման գործընթացում:

АКОПЯН МЕЛИНЕ АНДРАНИКОВНА

АКТИВНОСТЬ МЕХАНИЗМОВ ПОДДЕРЖАНИЯ ДЛИНЫ ТЕЛОМЕР И ДИНАМИКА ТЕЛОМЕРНОЙ ДЛИНЫ

РЕЗЮМЕ

Ключевые слова: механизмы поддержания длины теломер, альтернативное удлинение теломер, теломераза, пан-онкологический анализ, динамика длины теломер.

Теломеры — тандемно повторяющиеся участки ДНК на концах линейных эукариотических хромосом. С момента их открытия предполагается, что теломеры играют ключевую роль в обеспечении геномной стабильности и защищают хромосомы от слияний.

В большинстве соматических клеток человека теломеры постепенно укорачиваются по мере старения клетки. Активация механизмов поддержания длины теломер (telomere maintenance mechanisms, TMM) является отличительным признаком рака и позволяет опухолям противостоять укорочению теломер, предотвращая наступление теломерного кризиса, что существенно способствует развитию рака.

В настоящее время известно два основных механизма TMM. Теломеразный путь TMM (TEL-TMM) реализуется через активированный теломеразный путь, который для удлинения теломер использует теломеразный рибонуклеопротеиновый комплекс, содержащий РНК-матрицу. Альтернативный путь удлинения теломер (ALT-TMM) осуществляется посредством механизма гомологичной рекомбинации между теломерными участками сестринских хроматид, а также удалённых хромосом или внехромосомных теломерных повторяющихся последовательностей. Хотя эти механизмы традиционно считались взаимоисключающими, накапливающиеся данные свидетельствуют о том, что поддержание теломер при раке является более сложным процессом. За пределами канонических мутационных маркеров комплексная пан-онкологическая функциональная характеристика активности TMM остаётся нерешённой задачей, имеющей существенное диагностическое и терапевтическое значение.

Таким образом, целью настоящего исследования является пан-онкологическая оценка активности TMM при различных типах рака на основе данных секвенирования РНК (RNA-seq), а также изучение особенностей TMM в контексте генетических подтипов опухолей — в частности, при глиомах низкой степени злокачественности (LGG).

Результаты исследования дают комплексную пан-онкологическую оценку механизмов поддержания теломер для 33 типов опухолей на основе транскриптомных данных TCGA, дополненную таргетным анализом глиом низкой степени злокачественности. Как путь TEL, так и путь ALT продемонстрировали значительно более высокую активность в опухолях по сравнению с сопоставимыми нормальными тканями. Было выделено пять фенотипов TMM; одновременная активация TEL и ALT наблюдалась в части опухолей и подтверждена на уровне отдельных клеток при глиобластоме, что свидетельствует о небинарном характере поддержания теломер.

Опухоли с одновременно высокой активностью ALT и TEL демонстрировали наихудшую выживаемость как в пан-онкологических группах, так и при отдельных типах рака. Мутации в генах путей TEL и ALT ассоциировались с умеренным повышением их активности, однако канонические драйверы, такие как мутации *TERT* и мутации *ATRX*, не полностью объясняли гетерогенность путей, что подтверждает прогностическую ценность транскриптомного фенотипирования TMM.

При глиомах низкой степени злокачественности опухоли с диким типом гена IDH (wildtype) демонстрировали наиболее выраженную активацию пути ALT, обусловленную главным образом высокой экспрессией гена *RAD51*, а также наименьшие соотношения длины теломер. При LGG фенотип TMM оказался независимым прогностическим фактором — наряду с длиной теломер.

Таким образом, полученные результаты показывают, что механизмы поддержания теломер представляют собой динамическую и гетерогенную характеристику рака. Выявление фенотипов TMM, включая TEL-доминантное, ALT-доминантное, совместно активированное (co-activated) и TMM-неактивное состояния, подчёркивает необходимость комплексного фенотипирования TMM как в прогностических, так и в клинических целях, а также указывает на его потенциал для направленной разработки терапевтических стратегий.