

Original Article

Assessing the role of LRRC15 as a prognostic and therapeutic biomarker in glioblastoma

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Abstract: Objectives: Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by rapid progression and poor prognosis. Identifying novel biomarkers and therapeutic targets is crucial for improving GBM outcomes. This study aimed to explore the expression, prognostic value, therapeutic significance, and functional role of Leucine-Rich Repeat Containing 15 (LRRC15) in GBM. Methods: We utilized data from multiple online databases to analyze LRRC15 expression and its prognostic significance. Mutational and methylation profiles were examined, followed by survival analyses. Experimental validation was conducted using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and Western blotting in GBM cell lines. Functional assays, including colony formation, proliferation, and wound healing, were used to assess the effects of LRRC15 knockdown. Results: LRRC15 expression was significantly elevated in GBM. High LRRC15 levels were associated with shorter overall survival (OS) and disease-free survival (DFS) in GBM patients. Methylation analysis indicated that promoter hypermethylation may regulate LRRC15 expression. Knockdown of LRRC15 in GBM cell lines led to reduced cell proliferation, colony formation, and migration, along with a reversal of epithelial-mesenchymal transition (EMT), characterized by decreased N-cadherin and vimentin and increased E-cadherin expression. Conclusion: LRRC15 is highly expressed in GBM and correlates with poor patient prognosis. Its role in enhancing cell proliferation, migration, and EMT suggests that LRRC15 contributes to GBM aggressiveness. These findings highlight LRRC15 as a promising biomarker and potential therapeutic target for GBM, warranting further investigation into LRRC15-targeted therapies.

Keywords: Glioblastoma, LRRC15, diagnosis, biomarker, prognosis

Introduction

Glioblastoma (GBM), a grade IV astrocytic tumor according to the World Health Organization (WHO), is the most common and lethal primary brain tumor in adults [1-4]. GBM is characterized by highly aggressive behavior, including rapid cellular proliferation, extensive angiogenesis, and remarkable invasive capacity, which leads to the infiltration of tumor cells into adjacent brain tissue [5-8]. Standard treatment for GBM combines maximal safe resection, radiotherapy, and temozolomide chemotherapy [9, 10]; however, these treatments are often hindered by the tumor's intrinsic resistance to therapy and the protective environment of the blood-brain barrier [11-13]. Consequently, the prognosis for GBM patients re-

mains dismal, with a median survival time of only 12 to 15 months post-diagnosis and a five-year survival rate of less than 10% [14-16]. These clinical challenges highlight the critical need to identify biomarkers that could not only predict disease progression and therapeutic response but also serve as potential molecular targets for improved treatment strategies.

Under normal physiological conditions, Leucine-Rich Repeat Containing 15 (LRRC15) plays a role in tissue remodeling and immune response, where it is typically expressed in stromal cells and implicated in processes such as wound healing and extracellular matrix (ECM) interactions [17]. As a type I transmembrane protein within the leucine-rich repeat superfamily, LRRC15 is involved in cellular adhesion, migra-

tion, and ECM remodeling, making it crucial for maintaining tissue integrity and responding to cellular stress [18, 19]. However, recent studies have implicated LRRC15 in various cancers, where it appears to adopt a more pathological role, potentially enhancing tumor invasiveness and resistance to apoptosis. In breast cancer, high LRRC15 expression correlates with increased invasiveness and poorer patient outcomes, suggesting its potential as a prognostic biomarker [20]. Similarly, in pancreatic and non-small cell lung cancer, elevated levels of LRRC15 have been associated with greater tumor aggression and resistance to cell death, pointing to its involvement in tumor progression and therapeutic resistance [21-23]. These findings are supported by studies in sarcoma and ovarian cancer, where LRRC15 overexpression has been linked to disease progression and adverse clinical outcomes, reinforcing the potential role of this protein as a universal marker of tumor aggressiveness [24, 25].

Despite this emerging evidence, LRRC15's role in GBM remains largely unexplored. Given its established functions in promoting invasiveness, cell survival, and extracellular matrix remodeling in other cancer types, LRRC15 may similarly contribute to the highly invasive nature and treatment resistance of GBM. Here, we conducted an in-depth analysis of LRRC15 in GBM, employing both in silico and in vitro approaches [26-28] to investigate its expression profile and functional impact on GBM cell behavior. Our study aims to elucidate the prognostic significance and therapeutic potential of LRRC15 in GBM, potentially identifying it as a novel target in the ongoing search for effective GBM treatments.

Methodology

Differential expression of LRRC15 in GBM

The RNA sequencing data used in this study were obtained from the UCSC XENA platform (<https://xenabrowser.net/datapages/>), encompassing paired normal and cancerous tissue samples from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project [29]. Besides this, UALCAN, GSCA, GEPIA2, and OncoDB databases were also used to analyze the expression of LRRC15 in GBM tissue samples. UALCAN (<http://ualcan.path.uab.edu/>) provides interactive data analysis and

visualization of TCGA data, enabling the exploration of gene expression, survival, and methylation profiles [30, 31]. GSCA (<http://bioinfo.life.hust.edu.cn/GSCA/>) integrates genomic and chemical screening data, allowing users to explore cancer genomics, drug responses, and immune infiltrations [32]. GEPIA2 (<http://gepia2.cancer-pku.cn/>) focuses on RNA sequencing expression data, offering tools for survival analysis, correlation studies, and differential expression [33, 34]. OncoDB (<https://oncodb.org/>) is a comprehensive database for exploring cancer-related genes, pathways and mutations [35, 36].

Prognostic significance of LRRC15 in GBM

GEPIA2 [33], GSCA [32], UALCAN, and GENT2 (<http://gent2.appex.kr/>) [37] provide powerful tools for analyzing gene expression, survival, and immune infiltration across various cancers, aiding in biomarker and therapeutic target discovery. In this work, GEPIA2, GSCA, UALCAN, and GENT2 were used to analyze the prognostic significance of LRRC15 in GBM.

Mutational and promoter methylation analysis of LRRC15 in GBM

OncoDB [38], MEXPRESS (<https://mexpress.be/>) [39], and GSCA [32] are robust bioinformatics platforms for cancer research. OncoDB offers insights into cancer-related genes, mutations, and pathways. MEXPRESS integrates DNA methylation and gene expression data, enabling visualization and correlation analyses. GSCA provides tools to explore genomic alterations, immune infiltrations, and drug sensitivity. This work utilized OncoDB for mutational analysis, and MEXPRESS and GSCA databases for promoter methylation analysis of LRRC15 in GBM.

Association of LRRC15 expression with clinical parameters and molecular pathways in GBM

To evaluate correlations of LRRC15 with distinct clinical parameters in GBM, UALCAN [30], GEPIA2 [33, 40], and GSCA [32] databases were utilized. To explore the interactions among LRRC15 and co-regulated differentially expressed genes (DEGs), we employed STRING (<https://string-db.org/>) database [41]. Subsequently, all genes were ranked in descending order based on fold change (high-risk vs. low-

risk groups) and used as input for GSEA-GO and GSEA-KEGG analyses. These analyses were conducted using the org.Hs.eg.db (v3.14.0) and clusterProfiler (v4.2.2) R packages, along with the KEGG database (<https://www.kegg.jp/kegg/>).

Association of LRRC15 expression with immune, molecular subtypes, and immune inhibitory genes in GBM

TISIDB (<http://cis.hku.hk/TISIDB/>) is an integrative platform designed for cancer immunology research [42]. It compiles data from multiple sources, including TCGA, Uniprot, and DrugBank, to explore tumor-immune system interactions. TISIDB provides tools to analyze gene expression, immune cell infiltration, immune-related pathways, and the association of genes with clinical outcomes. In our study, this source was used to analyze the correlation of LRRC15 with immune, molecular subtypes, and immune inhibitory genes in GBM.

Correlation of LRRC15 expression with functional states, immune infiltration, and drug sensitivity in GBM

CancerSEA (<http://biocc.hrbmu.edu.cn/CancerSEA/>) [37], GSCA [26], and TIMER2.0 (<http://cistrome.org/TIMER2/>) [43] are valuable resources for cancer research. CancerSEA focuses on single-cell data, providing functional states of cancer cells across various tumor types, such as proliferation, metastasis, and inflammation, facilitating insights into tumor heterogeneity. GSCA integrates multi-omics data and chemical screening, enabling analysis of genomic alterations, immune cell infiltration, and drug sensitivity across cancer types. TIMER2.0 further enhances immune infiltration analysis, providing a more comprehensive view of immune cell types and their interactions within the tumor microenvironment. Herein, CancerSEA was used to decipher correlations between LRRC15 and 14 diverse states of GBM. Moreover, GSCA was utilized to conduct immune infiltration and drug sensitivity analyses of LRRC15 in GBM, and TIMER2.0 was employed to validate immune cell infiltration patterns associated with LRRC15 expression.

Purchase and cultivation of cell lines

Three GBM cell lines (SNB19, U251, and U87) and two normal brain tissue cell lines (NHAs

and HBMECs) were purchased from the Chinese Academy of Sciences Cell Bank (China). The cells were cultured according to the supplier's recommendations. All cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cell cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂ and regularly monitored for contamination and confluency before subsequent experiments.

RNA extraction and RT-qPCR

Total RNA was extracted from the cells using the TRIzol™ Reagent (Invitrogen, Cat. No. 15596026) according to the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop™ spectrophotometer. cDNA was synthesized from 1 µg of RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat. No. 4368814). RT-qPCR of LRRC15, N-cadherin, vimentin, E-cadherin, and GAPDH was conducted using the PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Cat. No. A25742) in a 96-well plate on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems). Relative gene expression was calculated using the 2^{-ΔΔCt} method. All reactions were performed in triplicate to ensure reliability. The following primers were used in this study.

GAPDH-F: 5'-ACCCACTCCTCCACCTTTGAC-3'; GAPDH-R: 5'-CTGTTGCTGTAGCCAAATTTCG-3'; LRRC15-F: 5'-GAGGCAGGAAAAGAGTCCGAGA-3'; LRRC15-R: 5'-CTCCGACGTTTGGAGGACAACA-3'; ECAD-F: 5'-CAGAAAGTTTTCCACCAAAG-3'; ECAD-R: 5'-ACTGAACCTGACCGTACAAAATGTGAGCAATTCTGCTT-3'; vimentin-F: 5'-AGGAAATGGCTCGTCACCTTCGTGAATA-3'; vimentin-R: 5'-GGAGTGTGCGTTGTTAAGAACTAGAGCT-3'; NCAD-F: 5'-GCCCCCTCAAGTGTACCTCAA-3'; NCAD-R: 5'-AGCCGAGTGATGGTCCAATTT-3'.

Knockdown of LRRC15 in GBM cell lines

LRRC15 knockdown in SNB19, U251, and U87 cells was performed using siRNA-mediated gene silencing. Cells were seeded in 6-well plates at an appropriate density to achieve 50-70% confluency at the time of transfection. Specific siRNAs targeting LRRC15 were purchased from Thermo Fisher Scientific (Assay ID: 129061). Transfection was carried out using Lipofecta-

mine™ RNAiMAX Transfection Reagent (Thermo Fisher Scientific, Cat. No. 13778150), following the manufacturer's protocol. For each well, 5 pmol of siRNA and 5 µL of RNAiMAX reagent were diluted separately in 250 µL of Opti-MEM™ Reduced Serum Medium (Thermo Fisher Scientific, Cat. No. 31985062), mixed gently, and incubated for 5 minutes at room temperature. The mixtures were combined, incubated for 20 minutes, and added dropwise to the cells.

After 48-72 hours of transfection, cells were harvested for downstream analyses, including RNA extraction for RT-qPCR or protein extraction for Western blotting, to validate LRRC15 knockdown efficiency. RT-qPCR was performed following abovementioned conditions. For Western blot analysis, protein was extracted from cells using RIPA buffer (Biovision, USA), and their concentrations were measured using the BCA protein assay kit (Beyotime Institute of Biotechnology). Proteins were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes (Millipore Corp., Billerica, MA, USA). The membranes were blocked with 5% non-fat milk for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies. Following this, the membranes were treated with secondary anti-rabbit antibodies for 1 hour at 4°C. After washing with TBST (0.05% Tween 20), protein bands were visualized using an ECL chemiluminescence kit (Beyotime), and their intensities were quantified using ImageJ software.

Colony formation assay

Following transfection, cells were plated at a density of 800 cells per well in 6-well plates and incubated at 37°C in a 5% CO₂ environment for two to three weeks. For fixation, cells were treated with 10% formaldehyde for 20 minutes and subsequently stained with 0.1% crystal violet for 10 minutes. Colony counts were performed using ImageJ software.

Cell proliferation assay

Cell proliferation was assessed using a Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan). Briefly, cells were seeded into 96-well plates at a density of 3,000 cells per well and cultured under standard conditions. At specified time

points (0, 24, 48, and 72 hours), 10 µL of CCK-8 reagent was added to each well, followed by incubation at 37°C for 2 hours. The absorbance was measured at 450 nm using a microplate reader to quantify cell proliferation.

Wound healing assay

Cells were cultured in 6-well plates until they reached full confluence. A linear scratch was made across the monolayer using a pipette tip to simulate a wound. Images of the wound were captured immediately (0 hour) and 24 hours after the scratch to observe healing. The degree of wound closure, reflecting cell migration, was quantified using ImageJ software.

Statistical analysis

We performed statistical analyses using GraphPad Prism software, version 8.0. Survival outcomes were assessed using Kaplan-Meier survival plots. Additionally, Pearson correlation analysis was conducted to assess the strength of linear relationships between variables, and receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic performance of specific markers. *P*-values < 0.05, 0.01, and 0.001 were considered significant.

Results

LRRC15 is significantly overexpressed in GBM compared to normal tissues

In our study, we analyzed the expression of the LRRC15 gene across various tumor types, with a particular focus on GBM, using data from multiple databases, including TCGA, UALCAN, GEPIA, and OncoDB. Our analysis revealed that LRRC15 was significantly overexpressed in 20 different tumor types relative to normal tissues, as shown in both TCGA and GTEx pan-cancer datasets. These tumor types included bladder cancer (BLCA), breast cancer (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), and glioblastoma (GBM), among others (**Figure 1A**). Specifically, in GBM, LRRC15 expression was found to be markedly elevated in primary tumor samples when compared to normal tissues. This was consistently supported by data from various platforms, including the UALCAN, GSCA, GEPIA2, and OncoDB databases, which confirmed the statistically significant increase in LRRC15 expression in GBM tumor

LRRC15 as a prognostic biomarker in glioblastoma

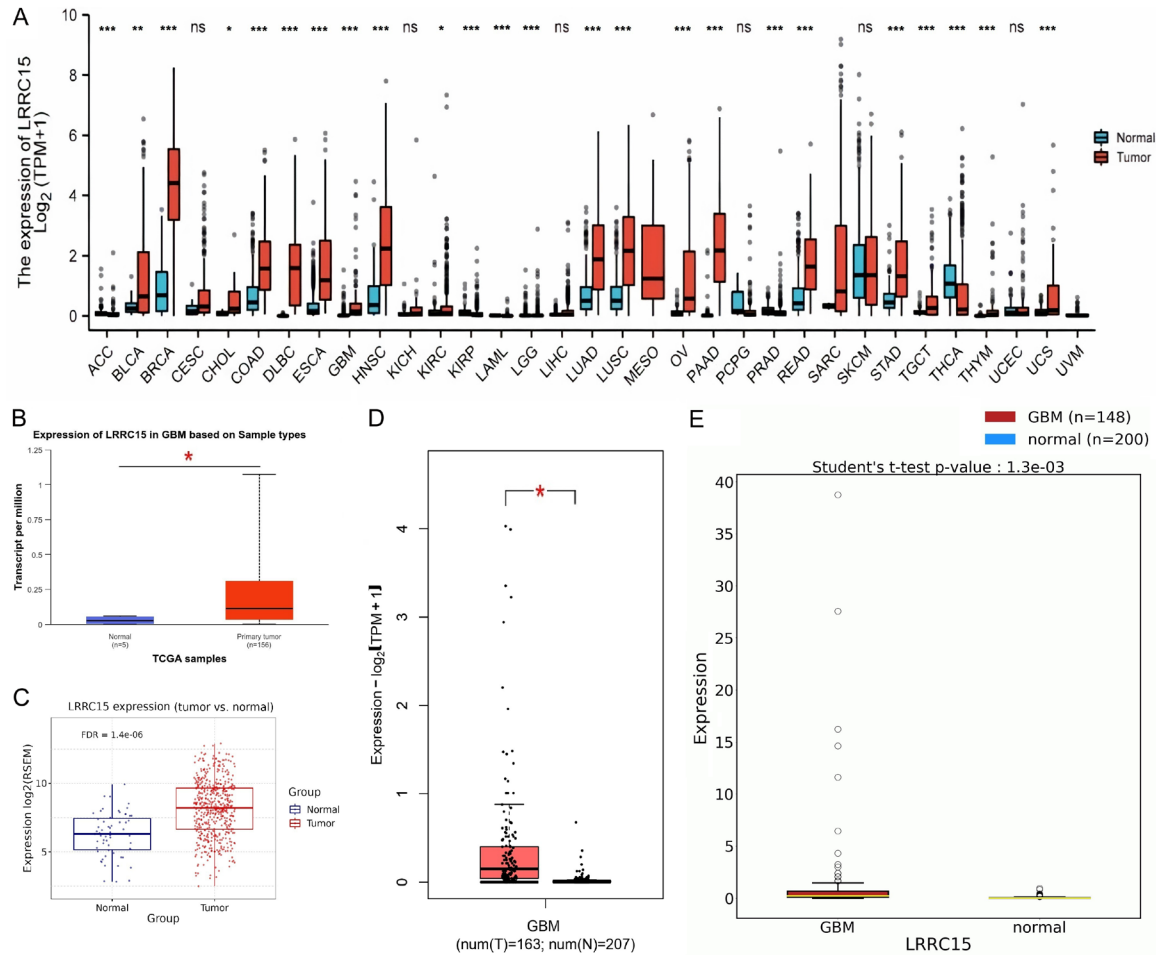


Figure 1. Leucine-Rich Repeat Containing 15 (LRRC15) expression analysis across multiple tumor types with a focus on glioblastoma (GBM) using various cancer databases. A. LRRC15 expression in 33 different tumor types derived from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) pancancer datasets. B. LRRC15 expression data in GBM from the UALCAN database. C. LRRC15 expression comparison in GBM tumor tissues and normal tissues using GSCA database. D. Expression analysis from the GEPIA2 database. E. LRRC15 expression difference between GBM and normal tissues as shown by the OncoDB database. *P < 0.05, **P < 0.01, and ***P < 0.001.

samples relative to normal control tissues (Figure 1B-E).

Higher LRRC15 expression is significantly associated with poorer survival outcomes in GBM

We investigated the prognostic significance of LRRC15 expression in GBM using survival analysis data from multiple databases, including GEPIA2, GSCA, GENT2, and UALCAN. Our analysis revealed that higher LRRC15 expression was significantly associated with poorer survival outcomes, including shorter overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS), and progression-free survival (PFS) in GBM patients (Figure 2A-D). Mo-

reover, survival analysis using UALCAN explored the effects of LRRC15 expression levels on GBM survival based on race and gender. **Figure 2E** demonstrated that high LRRC15 expression in African American patients correlated with a worse survival prognosis, with a *p*-value of 0.081 (Figure 2E). Similarly, it was shown that high LRRC15 expression in female GBM patients was associated with poor survival outcomes, with a *p*-value of 0.084, further emphasizing the prognostic impact of LRRC15 expression in specific subgroups of GBM patients (Figure 2F). Further research and validation studies are required to accurately determine the true prognostic value of LRRC15 in relation to clinical variables in GBM.

LRRC15 as a prognostic biomarker in glioblastoma

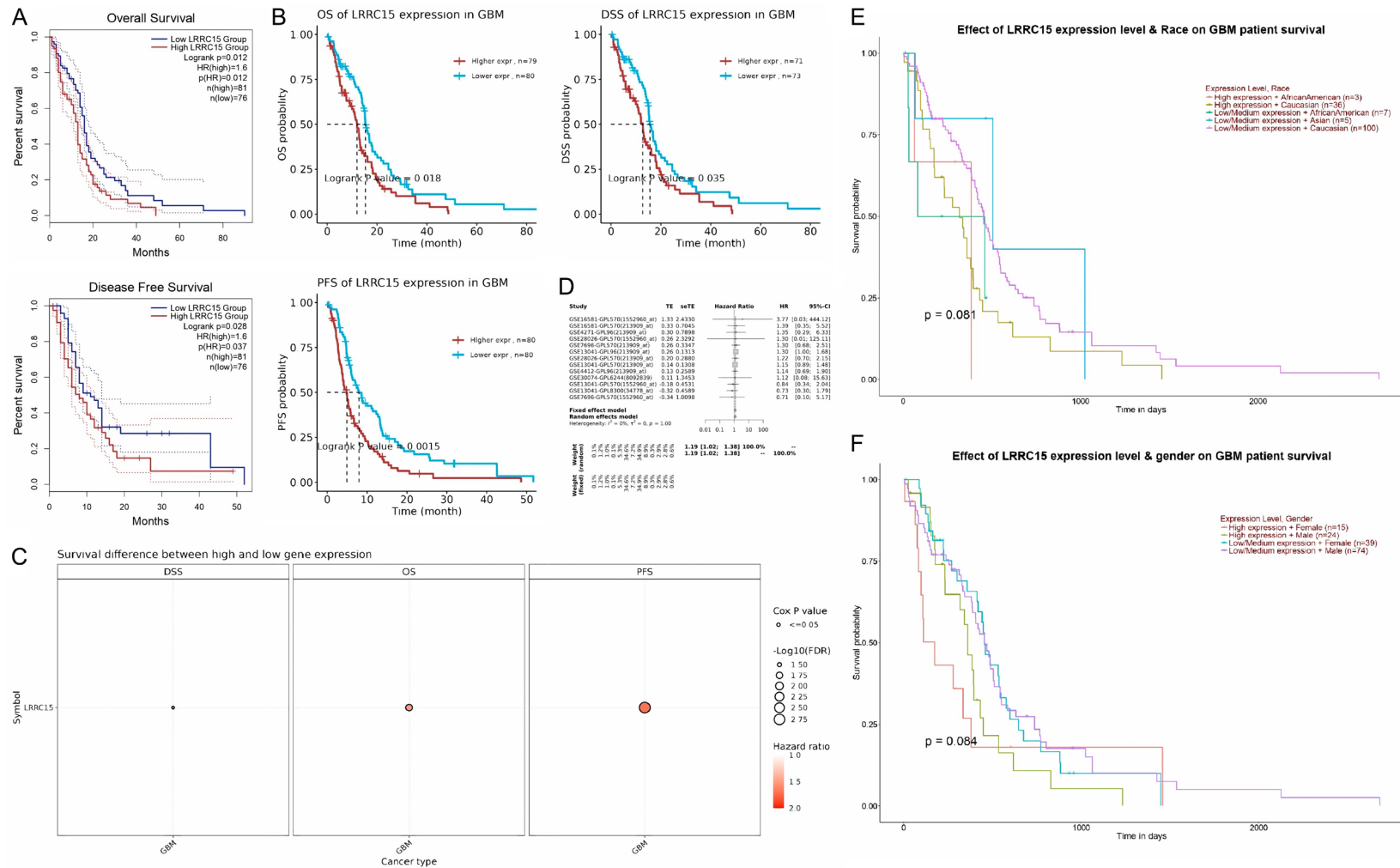


Figure 2. Prognostic impact of Leucine-Rich Repeat Containing 15 (LRRC15) expression on survival outcomes in glioblastoma (GBM) patients across multiple databases. A. Kaplan-Meier survival curves for overall survival (OS) and disease-free survival (DFS) in GBM patients from GEPIA2 database. B. Survival analysis from GSCA database. C. Visual summary of survival differences in disease-specific survival (DSS), OS, and progression-free survival (PFS) based on LRRC15 expression levels in GBM patients from the GSCA database. D. Forest plot of meta-analysis results from GENT2 database. E. Kaplan-Meier survival curves for OS across GBM patients classified based on different races from UALCAN. F. Kaplan-Meier survival curves for OS across GBM patients classified based on different genders from UALCAN. P -value < 0.05.

LRRC15 as a prognostic biomarker in glioblastoma

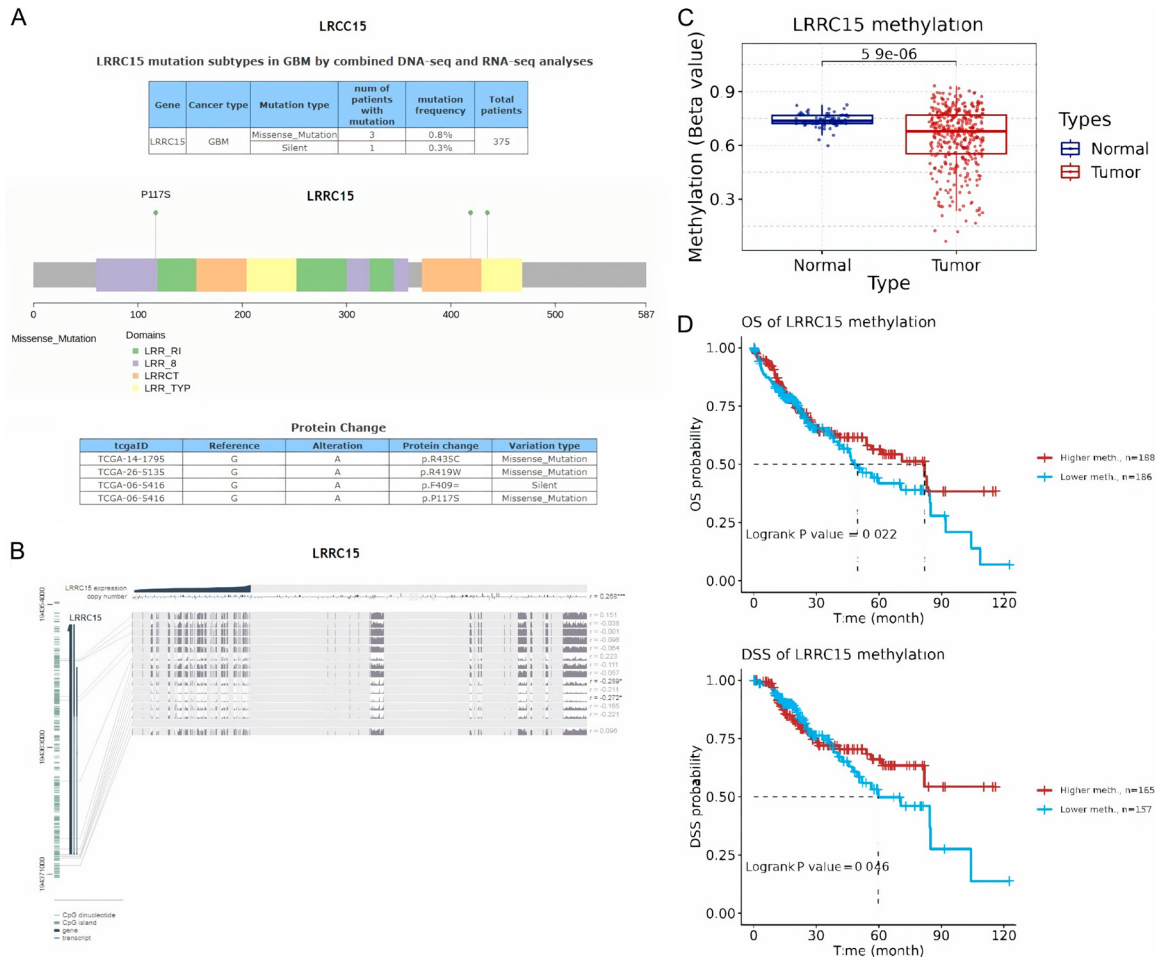


Figure 3. Mutational and methylation analysis of Leucine-Rich Repeat Containing 15 (LRRC15) in glioblastoma (GBM) and their association with tumor progression. A. Mutation analysis of LRRC15 in GBM using the OncoDB database. B. Promoter methylation profile of LRRC15 from the MEXPRESS database. C. Box plot from the GSCA database comparing LRRC15 methylation levels between normal and GBM tumor tissues. D. Survival analysis based on LRRC15 methylation levels in GBM patients using data from the GSCA database. P -value < 0.05.

LRRC15 exhibits low mutation frequency and hypomethylation in GBM

We analyzed the mutational and methylation profiles of the LRRC15 gene in GBM to explore its potential role in tumor progression and prognosis. Mutation analysis from the OncoDB database revealed a low mutation frequency for LRRC15 in GBM, with only a few patients exhibiting missense mutations, such as p.R483C and p.P117S (**Figure 3A**). Promoter methylation analysis from the MEXPRESS and GSCA databases highlighted hypomethylation across CpG sites in the LRRC15 promoter in GBM (**Figure 3B, 3C**). However, survival analysis based on LRRC15 methylation levels did not show a significant association with OS and DSS in GBM (log-rank p -values of 0.022 and 0.046, respectively) (**Figure 3D**).

LRRC15 expression is associated with clinical parameters and molecular pathways in GBM

We analyzed the expression of LRRC15 in GBM across various clinical parameters using UALCAN, GEPIA2, and GSCA, and conducted gene enrichment analysis. Our findings from UALCAN revealed that LRRC15 expression was significantly (p -value < 0.05) higher in GBM tissues compared to normal tissues across several clinical categories, including race, gender, age, and TP53 mutation status (**Figure 4A**). Specifically, Caucasian and African American patients exhibited notably higher LRRC15 expression compared to normal controls. No significant gender-based difference was observed, though LRRC15 expression was higher in patients over 40 years of age. Additionally, samples with non-

LRRC15 as a prognostic biomarker in glioblastoma

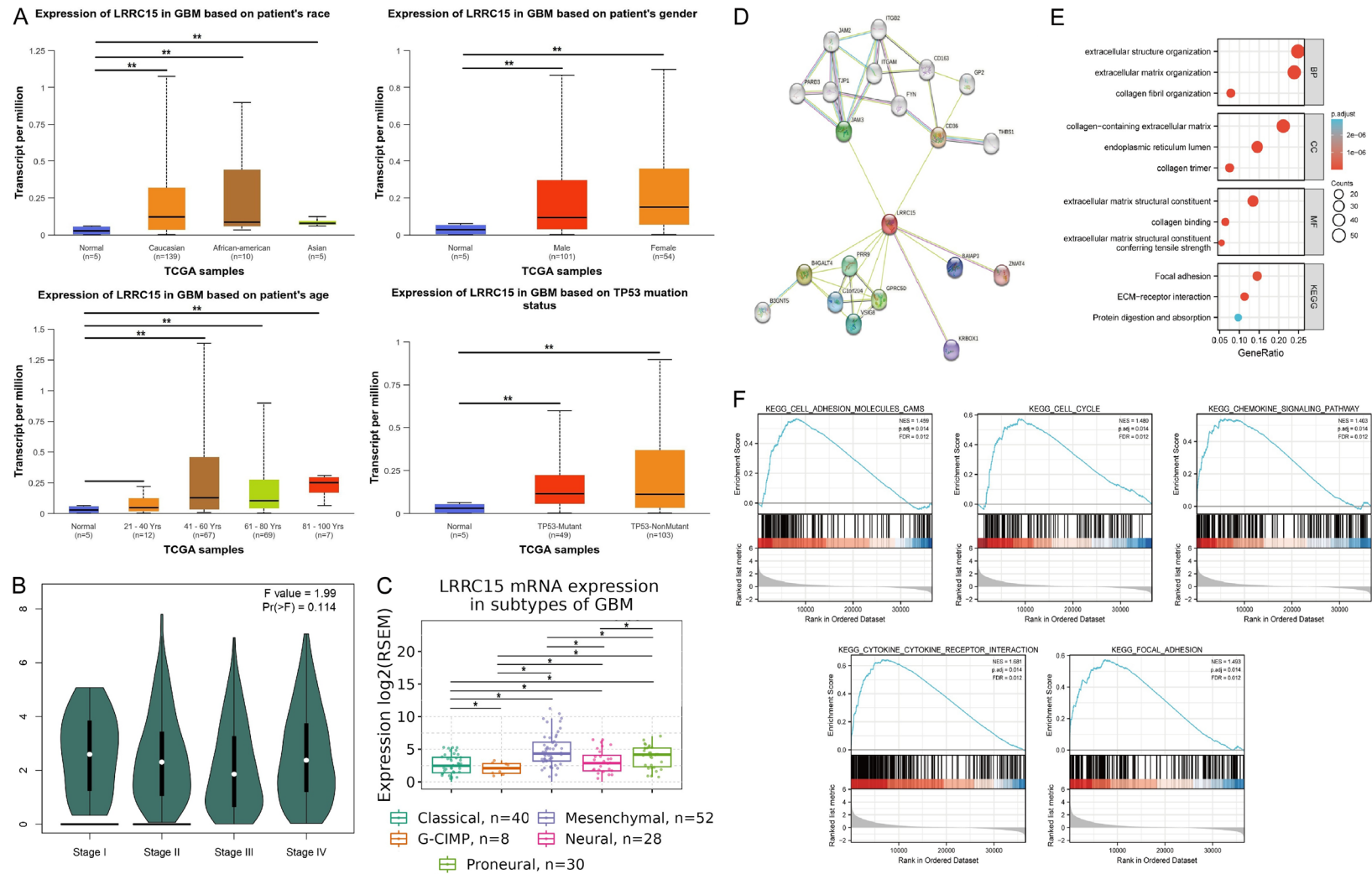


Figure 4. Clinical parameter analysis and gene enrichment profiling of Leucine-Rich Repeat Containing 15 (LRRC15) expression in glioblastoma (GBM) across multiple datasets. A. LRRC15 expression analysis in GBM samples based on race, gender, age, and TP53 mutation status using the UALCAN database. B. Stage-wise comparison of LRRC15 expression in GBM using GEPIA2 database. C. Molecular subtype analysis of LRRC15 mRNA expression in GBM from GSCA database. D. Interaction network of LRRC15 with twenty co-interacting proteins using the STRING database. E. Gene Ontology (GO) enrichment analysis of LRRC15-associated proteins. F. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA) of LRRC15. *P < 0.05 and **P < 0.01.

mutated TP53 showed increased LRRC15 expression compared to TP53-mutated cases. Using GEPIA2, we found that LRRC15 expression increased with GBM stage progression, though the differences were not statistically significant ($P = 0.114$) (**Figure 4B**). GSCA analysis showed significant variations in LRRC15 expression across GBM molecular subtypes, with the mesenchymal subtype displaying the highest levels, followed by the classical subtype, while the G-CIMP subtype exhibited the lowest expression (**Figure 4C**).

Further, we explored the interaction network of LRRC15 using the STRING database, which identified twenty potential co-interacting proteins. Gene Ontology (GO) analysis revealed that these proteins are significantly involved in biological processes such as extracellular structure remodeling, matrix organization, and collagen fiber arrangement (**Figure 4D**). The key cellular components associated with LRRC15 include the collagen-rich extracellular matrix and the endoplasmic reticulum lumen (**Figure 4E**). Molecular functions affected by LRRC15 primarily involve structural roles in the extracellular matrix, including collagen binding and enhancing tensile strength. KEGG pathway analysis indicated that LRRC15 participates in focal adhesion, ECM-receptor interactions, and protein digestion and absorption pathways. Additionally, Gene Set Enrichment Analysis (GSEA) via the GSCA database identified key LRRC15-associated signaling pathways, such as CAM signaling, cell cycle regulation, chemokine signaling, cytokine-cytokine receptor interactions, and the FAK signaling pathway (**Figure 4F**).

LRRC15 expression is associated with immune and molecular subtypes and correlates with immune inhibitory genes in GBM

We further investigated the correlation of LRRC15 expression with immune and molecular subtypes of GBM, as well as its association with immune inhibitory genes. Our analysis using the GSCA database revealed that LRRC15 expression varied significantly (p -value < 0.05) across different immune subtypes, with particularly higher expression observed in the C4 subtype, which is typically associated with lymphocyte-depleted tumors (**Figure 5A**). This suggests that LRRC15 may be linked to immune suppression in specific GBM immune subtypes. Additionally, when examining LRRC15 expres-

sion across GBM molecular subtypes, significant (p -value < 0.05) differences were observed, with notably higher expression in the mesenchymal-like subtype, followed by the classical-like subtype (**Figure 5B**). Survival analysis conducted via the GENT2 database across different GBM subtypes, classified by LRRC15 expression levels, showed no significant difference in survival outcomes, as indicated by a log-rank p -value of 0.542 (**Figure 5C**). This suggests that while LRRC15 expression is associated with specific molecular subtypes, its impact on patient survival might be influenced by other factors or is more complex than initially thought. Furthermore, we observed positive correlations between LRRC15 and several immune inhibitory genes, including TGFB1, TGFRB1, and IL10 (**Figure 5D, 5E**). These findings suggest that LRRC15 may play a role in immune inhibitory signaling within the GBM microenvironment, potentially contributing to immune escape mechanisms in GBM.

LRRC15 expression correlates with functional states, immune cell infiltration, and drug sensitivity in GBM

We explored the correlations between LRRC15 expression and various functional states, immune cell infiltration, and drug sensitivity in GBM using the CancerSEA database. Specifically, we observed that LRRC15 expression positively correlated with angiogenesis, EMT, and DNA damage, while it showed a negative correlation with apoptosis (**Figure 6A, 6B**). Furthermore, we utilized the GSCA database to assess the correlation between LRRC15 expression and immune cell infiltration within the GBM microenvironment. Our analysis demonstrated a significant positive correlation between LRRC15 expression and various immune cells, including macrophage monocyte infiltration (**Figure 6C**), indicating that LRRC15 may play a role in shaping the immune landscape of GBM by affecting the recruitment of these cells. We further validated the correlation of LRRC15 expression with macrophage and monocyte infiltration in GBM using the TMEER2.0 database. Results showed that LRRC15 expression correlated positively with macrophage infiltration ($\text{Rho} = 0.181$, $P = 3.42\text{e-}02$) and monocyte infiltration ($\text{Rho} = 0.191$, $P = 2.56\text{e-}02$), reinforcing the potential involvement of LRRC15 in the immune modulation of GBM (**Figure 6D**). Lastly, we explored the association between LRRC15

LRRC15 as a prognostic biomarker in glioblastoma

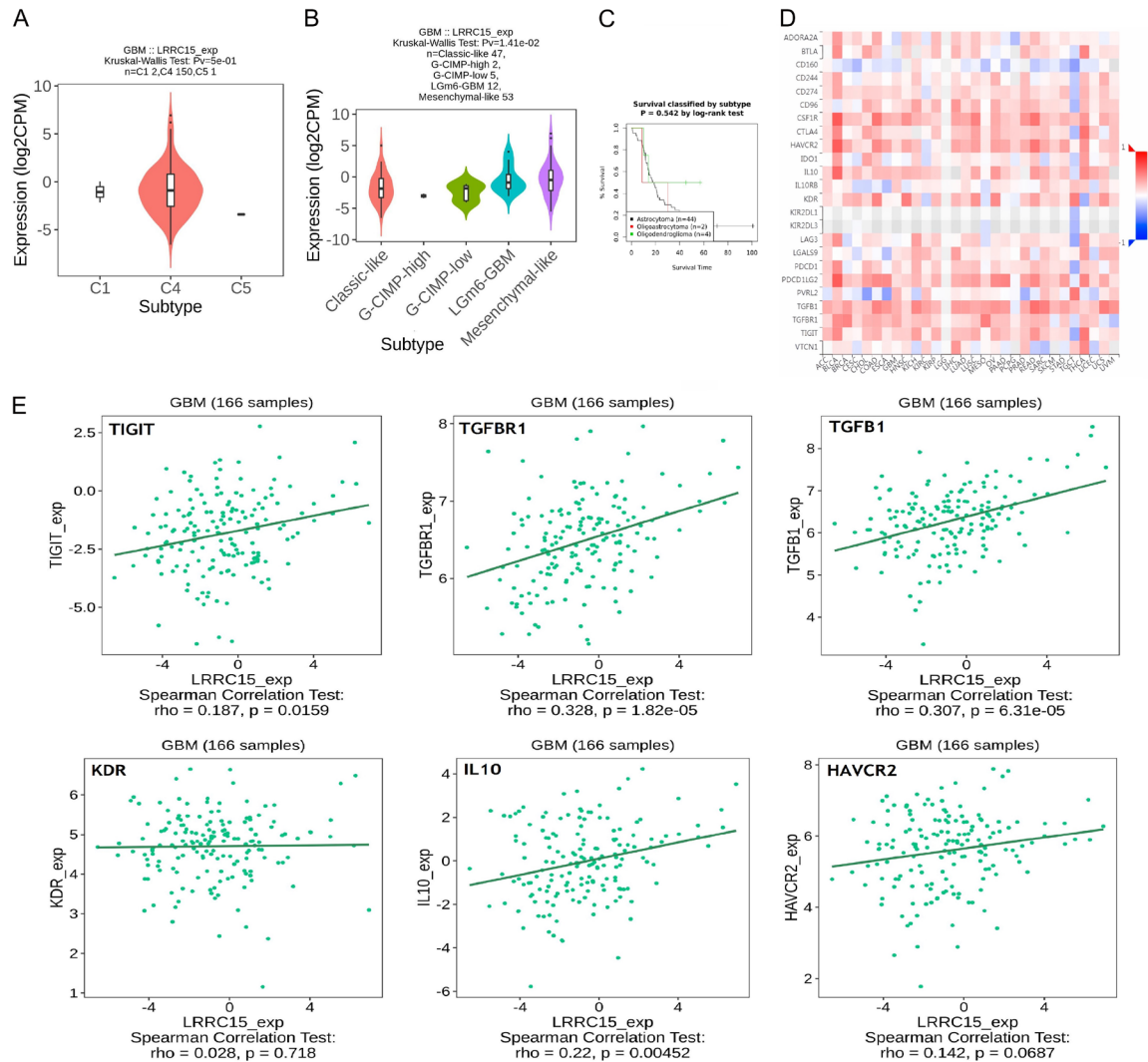


Figure 5. Correlation of Leucine-Rich Repeat Containing 15 (LRRC15) expression with immune and molecular subtypes in glioblastoma (GBM) and its association with immune inhibitory genes. A. LRRC15 expression across different immune subtypes of GBM. B. Survival analysis of LRRC15 across different subtypes of GBM using GENT2 database. C. Expression analysis of LRRC15 among GBM molecular subtypes. D. Heatmap illustrating the correlation between LRRC15 expression and various immune inhibitory genes. E. Scatter plot showing the positive correlation between LRRC15 and immune inhibitory genes, suggesting a potential role for LRRC15 in immune inhibitory signaling pathways in GBM. P -value < 0.05.

expression and drug sensitivity using the GS-CA database. However, our results indicated no significant correlation between LRRC15 expression and sensitivity to a range of tested drugs (Figure 6E), suggesting that LRRC15 expression levels might not directly impact GBM's response to these therapies.

LRRC15 expression is validated across GBM cell lines

In the validation part of our study, we confirmed the expression of LRRC15 in GBM and control cell lines using both RT-qPCR and Western

blot analysis. The RT-qPCR results (Figure 7A) showed that LRRC15 expression was significantly higher in GBM cell lines ($n = 3$) compared to normal cell lines ($n = 2$). To further evaluate the diagnostic potential of LRRC15, we performed ROC curve analysis (Figure 7B), which demonstrated an area under the curve (AUC) of 1, indicating excellent discriminatory power of LRRC15 expression between GBM and normal tissues. Additionally, Western blot analysis confirmed LRRC15 expression across different GBM and control cell lines. The results revealed that LRRC15 expression was notably higher in

LRRC15 as a prognostic biomarker in glioblastoma

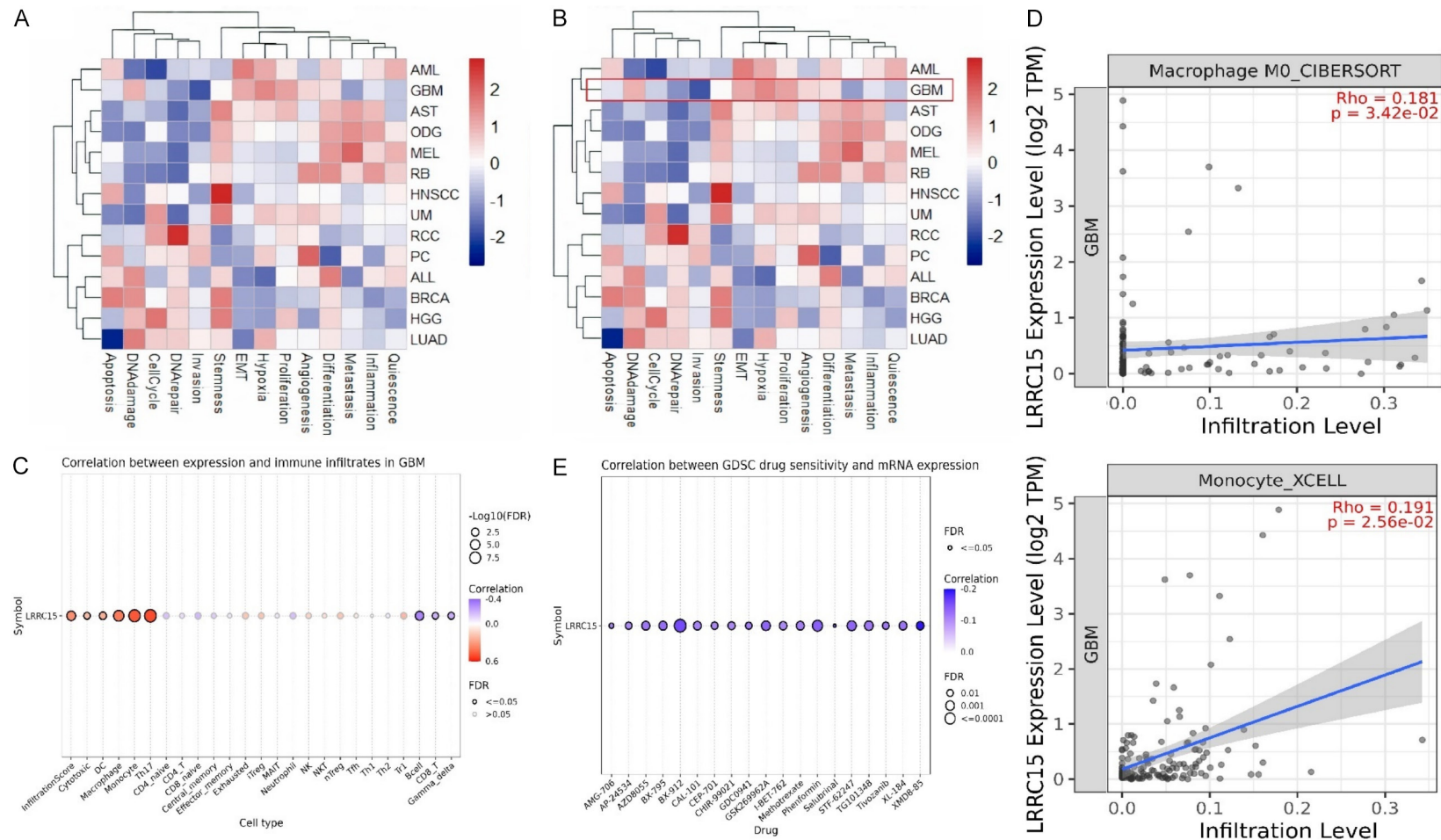


Figure 6. Functional, immune, and drug sensitivity analysis of Leucine-Rich Repeat Containing 15 (LRRC15) expression in glioblastoma (GBM) using CancerSEA and GSCA databases. A. Correlation of LRRC15 expression with various functional states in GBM using the CancerSEA database. B. Scatter plots highlighting the strength of correlations between LRRC15 expression and selected functional states. C. Analysis of the association between LRRC15 expression and immune cell infiltration in the GBM microenvironment using the GSCA database. D. Validation of association between LRRC15 expression and immune cell infiltration of macrophages and monocytes in GBM using TIMER2.0 database. E. Drug sensitivity analysis of LRRC15 expression in GBM using the GSCA database. P -value < 0.05 .

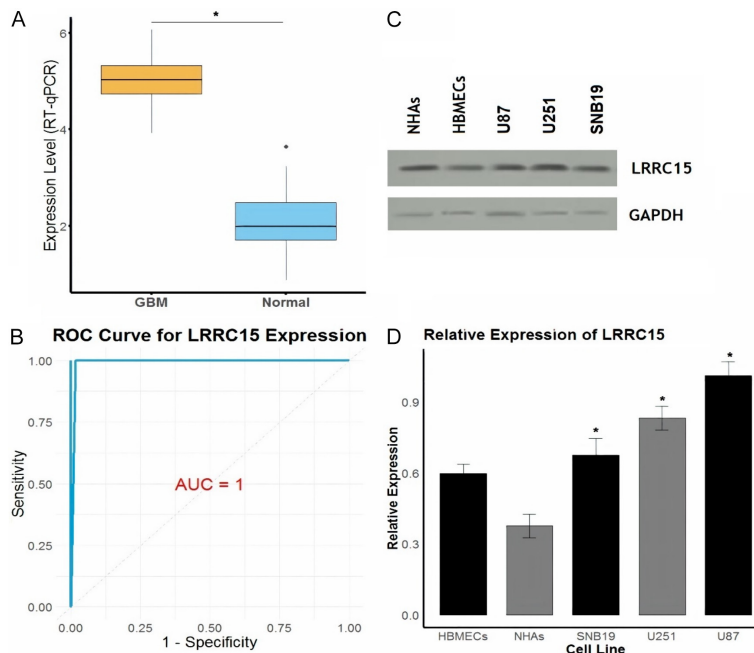


Figure 7. Validation of Leucine-Rich Repeat Containing 15 (LRRC15) expression in glioblastoma (GBM) and control cell lines using reverse transcription quantitative polymerase chain reaction (RT-qPCR), Western blot, and diagnostic receiver operating characteristic (ROC) curve analysis. A. Relative LRRC15 mRNA expression levels in GBM cell lines (n = 3) compared to normal control cell lines (n = 2) as determined by RT-qPCR. B. ROC curve analysis of LRRC15 expression. C. Western blot analysis of LRRC15 protein expression in different GBM cell lines versus control cell lines. D. Quantification of Western blot results, showing relative LRRC15 protein expression levels in GBM and control cell lines. *P < 0.05.

GBM cell lines compared to control cell lines, with U87 showing the highest relative expression (Figure 7C, 7D and Supplementary Figure 1). These findings underscore the potential of LRRC15 as a diagnostic marker for GBM.

LRRC15 knockdown inhibits colony formation, cell proliferation, and migration in GBM cells

In this section of the study, we investigated the effects of LRRC15 knockdown on GBM cell behavior through different assays, including colony formation, cell proliferation, and wound healing. LRRC15 expression was knocked down in three GBM cell lines (SNB19, U251, and U87), and the effects were assessed using RT-qPCR and Western blot. The RT-qPCR analysis (Figure 8A) confirmed that LRRC15 mRNA expression was significantly reduced in the siRNA-treated cells compared to controls. Western blot analysis (Figure 8B and Supplementary Figure 1) further verified the decrease in LRRC15 protein levels, with rela-

tive quantification (Figure 8C) showing a marked reduction in LRRC15 protein expression in the knockdown groups. The colony formation assay results (Figure 8D, 8E) indicated that LRRC15 knockdown led to a significant reduction in colony formation across all three transfected GBM cell lines, suggesting that LRRC15 plays a role in supporting GBM cell clonogenicity. Additionally, the cell proliferation assay (Figure 8F) demonstrated that LRRC15 knockdown resulted in decreased proliferation rates in the transfected GBM cell lines compared to controls, further supporting the hypothesis that LRRC15 promotes GBM cell growth. Lastly, the wound healing assay results (Figure 8G, 8H) revealed a significant reduction in wound closure in the LRRC15 knockdown GBM cells after 24 hours, suggesting that LRRC15 contributes to the migratory capabilities of GBM cells.

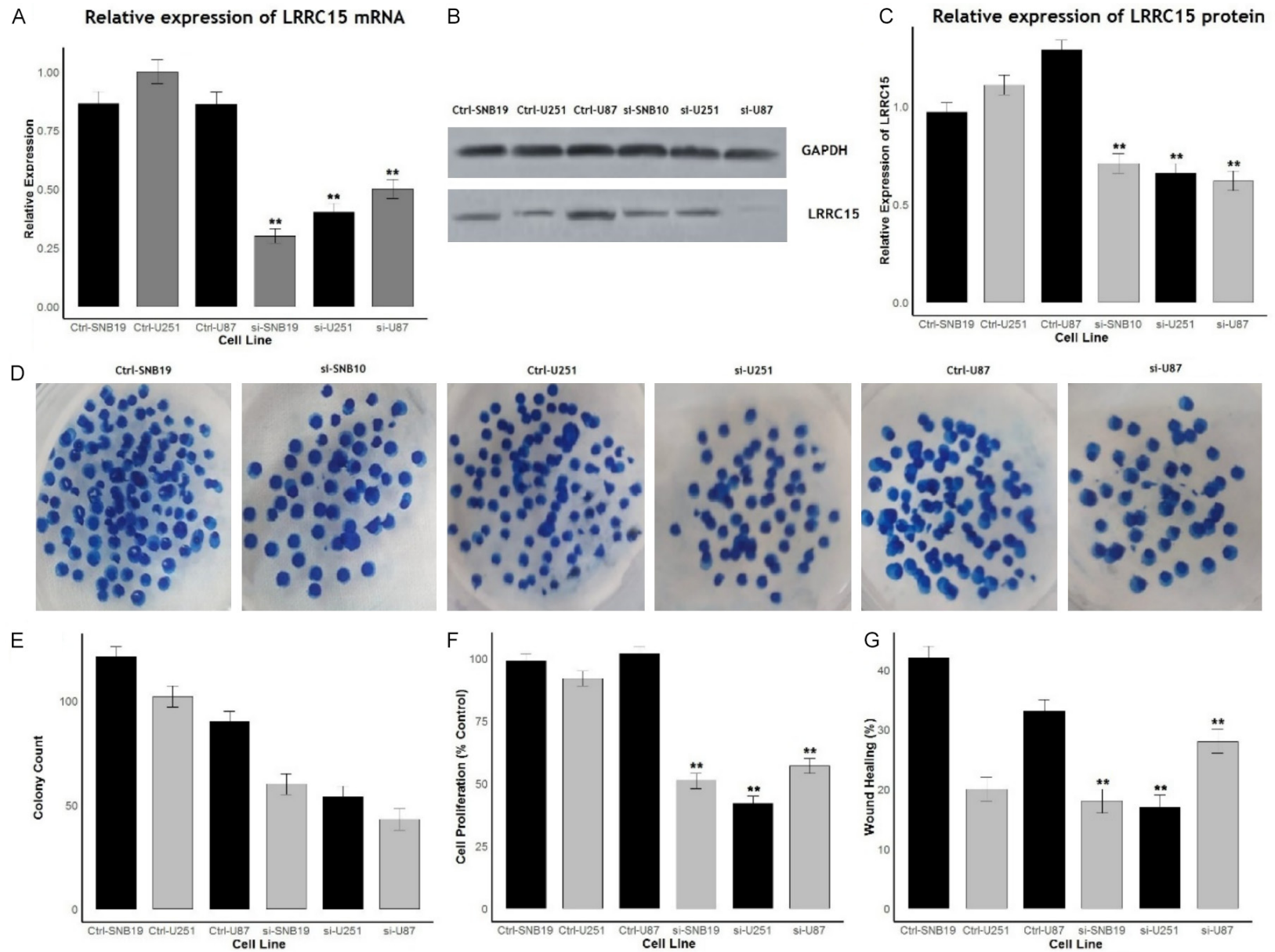
LRRC15 overexpression influences EMT regulation in GBM progression

To explore the role of LRRC15 in GBM progression at the molecular level, we examined its impact on EMT regulation. RT-qPCR analysis of EMT markers showed that LRRC15 knockdown resulted in decreased levels of N-cadherin and vimentin, along with an increase in E-cadherin, indicating a reversal of EMT (Figure 9A-C). These findings highlight the crucial role of LRRC15 in promoting EMT, suggesting it as a potential therapeutic target for reduce the invasiveness and altering the cellular phenotype of GBM cells.

Discussion

GBM is the most common and aggressive primary brain tumor in adults, characterized by rapid progression, high invasiveness, and resistance to conventional therapies [12, 44, 45]. Despite advances in surgical techniques, che-

LRRC15 as a prognostic biomarker in glioblastoma



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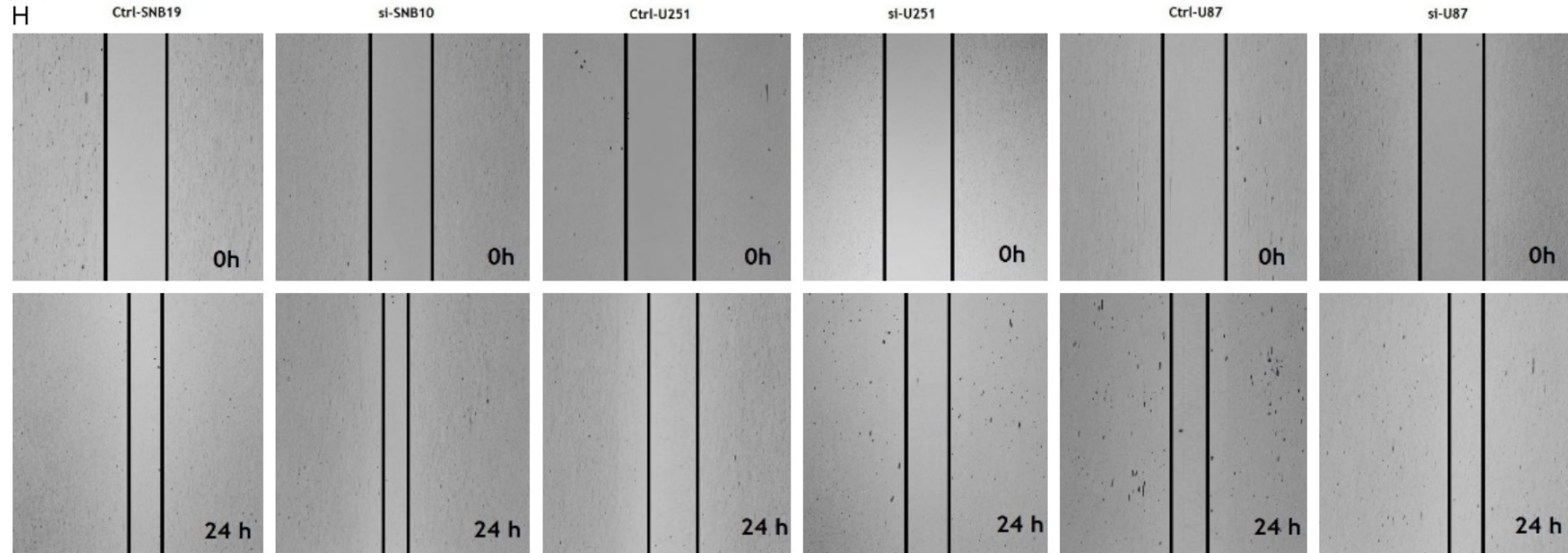


Figure 8. Assessment of the effects of Leucine-Rich Repeat Containing 15 (LRRC15) knockdown on the behavior of glioblastoma (GBM) cell lines (SNB19, U251, and U87) using siRNA-mediated gene silencing. A. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis showing a significant reduction in LRRC15 mRNA expression in siRNA-treated GBM cells compared to control cells. B. Western blot analysis confirming decreased LRRC15 protein levels following siRNA knockdown. C. Quantification of the relative protein expression levels, demonstrating a marked reduction in LRRC15 expression in the knockdown groups. D. Colony formation assay results, illustrating reduced colony formation in LRRC15 knockdown cells compared to controls. E. Quantification indicating a significant decrease in clonogenic potential across all three GBM cell lines. F. Cell proliferation assay showing a notable decline in proliferation rates in LRRC15-silenced GBM cells relative to controls, suggesting that LRRC15 may play a role in promoting GBM cell growth. G. H. Wound healing assay results demonstrating significantly impaired wound closure in LRRC15 knockdown cells after 24 hours, with representative images. $**P < 0.05$.

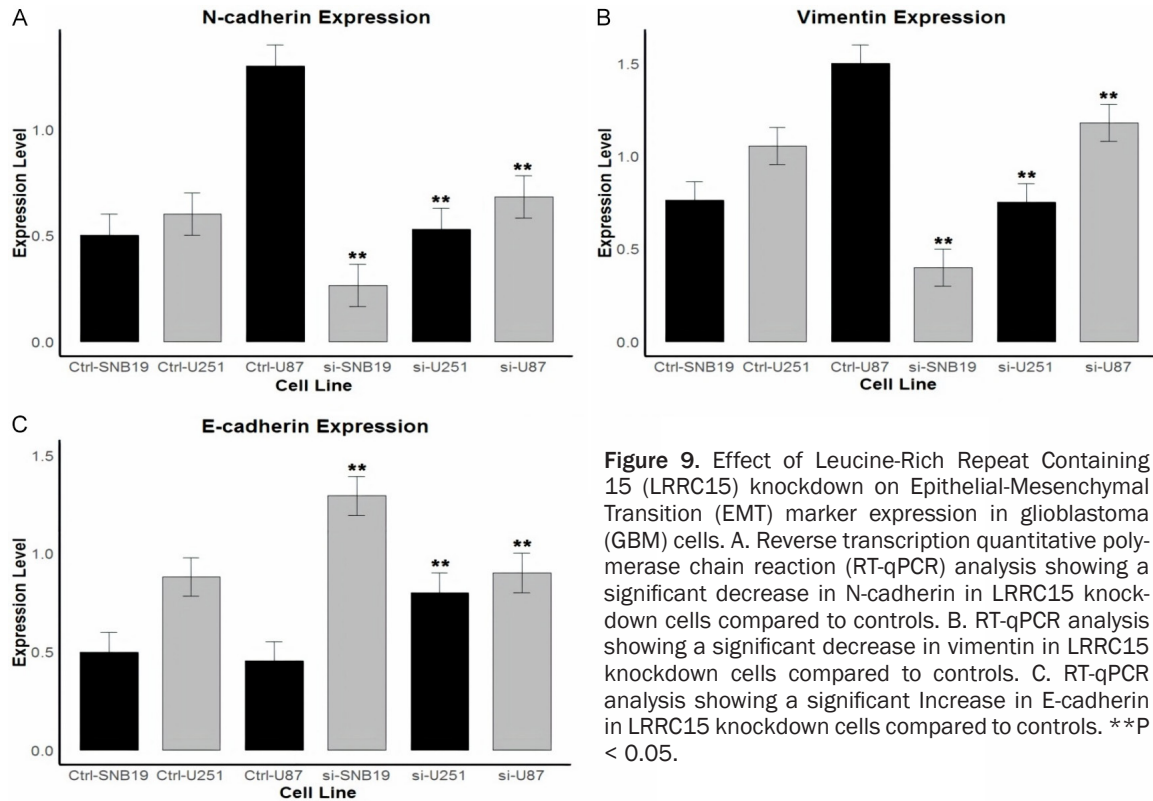


Figure 9. Effect of Leucine-Rich Repeat Containing 15 (LRRC15) knockdown on Epithelial-Mesenchymal Transition (EMT) marker expression in glioblastoma (GBM) cells. A. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis showing a significant decrease in N-cadherin in LRRC15 knockdown cells compared to controls. B. RT-qPCR analysis showing a significant decrease in vimentin in LRRC15 knockdown cells compared to controls. C. RT-qPCR analysis showing a significant increase in E-cadherin in LRRC15 knockdown cells compared to controls. **P < 0.05.

motherapy, and radiotherapy, GBM patients face a poor prognosis, with a median survival of less than two years [46, 47]. The highly invasive nature of GBM and its complex molecular landscape necessitate the identification of new biomarkers and therapeutic targets to improve patient outcomes [48-50]. In this study, we investigated the role of LRRC15, a leucine-rich repeat-containing protein, in GBM progression and its potential as a biomarker and therapeutic target.

Our study revealed elevated LRRC15 expression in GBM, supporting a role for this protein in brain tumor pathophysiology. This observation is consistent with prior studies reporting high LRRC15 expression in various solid tumors, including breast, ovarian, and lung cancers, where it is associated with tumor invasiveness and metastasis [24, 51, 52]. While earlier studies focused on single-dataset analyses, our work integrates multiple sources, providing robust evidence of LRRC15 upregulation specifically in GBM. Our findings also demonstrate that elevated LRRC15 expression correlates with poor prognosis of GBM, showing associations with reduced OS, DFS, and PFS.

Previous research has linked high LRRC15 expression to worse outcomes in other cancer types, including breast and ovarian cancers [24, 53]. Unlike the well-known targets in GBM such as EGFR, PD-L1, and VEGFA [54, 55], which have been extensively studied for their roles in tumor progression and treatment resistance, LRRC15 presents a unique and underexplored target. Our findings show that LRRC15 was significantly overexpressed in GBM tissues and cell lines, highlighting its potential as a novel biomarker for this aggressive tumor. The elevated expression of LRRC15 correlates with poor prognosis, shorter OS, and DFS, particularly in specific subgroups like African American and female patients. Moreover, LRRC15's expression pattern and its association with immune-related genes suggest that it may play a role in modulating the tumor microenvironment, particularly in immune suppression, which distinguishes it from other biomarkers like VEGFA or EGFR that are more directly involved in tumor angiogenesis and growth [56, 57]. The novelty of LRRC15, therefore, lies not only in its expression patterns and prognostic value but also in its potential for therapeutic targeting, especially in immune-related pathways, making

it a promising candidate for further research and clinical validation.

The low mutational frequency of LRRC15 observed in GBM aligns with findings in other cancers, where LRRC15 mutations are infrequent [58]. Although limited research exists on LRRC15 promoter methylation, studies on similar tumor-promoting genes suggest that hypomethylation often correlates with elevated expression [59]. Our study supports this hypothesis, showing that the LRRC15 promoter is hypomethylated in GBM, potentially contributing to its overexpression. Interestingly, while promoter methylation is a prognostic marker for many oncogenes, we found no significant correlation between LRRC15 methylation and survival outcomes in GBM. This contrast suggests that, in the context of GBM, promoter methylation may regulate LRRC15 expression without significantly impacting patient prognosis. Further investigation is warranted to understand the epigenetic regulation of LRRC15 and its functional implications in GBM.

Our pathway analysis identified significant associations between LRRC15 and processes related to extracellular matrix (ECM) organization, cell adhesion, and focal adhesion pathways in GBM. These findings are consistent with studies in other cancers that link LRRC15 to ECM remodeling and cellular adhesion, processes that facilitate tumor invasion and metastasis [52, 60]. Additionally, our work connects LRRC15 with key GBM-related pathways, such as the cell cycle and focal adhesion kinase (FAK) signaling pathways, highlighting its potential role in GBM proliferation and invasion. Prior research has emphasized the involvement of LRRC15 in promoting invasive phenotypes in various cancers [61-63], yet our study expands on this by demonstrating its specific association with molecular pathways crucial to GBM pathogenesis.

In GBM, we found that LRRC15 expression is associated with the C4 immune subtype, known for a lymphocyte-depleted and immunosuppressive microenvironment. This aligns with studies in other cancers, where high LRRC15 expression is linked to immune suppression and poor immune cell infiltration [64-66]. Furthermore, we observed correlations between LRRC15 and immune inhibitory genes such as TGFB1, TGFRB1, and IL10, suggesting that LR-

RC15 may promote immune evasion in GBM by enhancing immunosuppressive signaling. While previous research has associated LRRC15 with immune suppression in solid tumors, our study uniquely explores its immunomodulatory role in brain tumors. These findings suggest that LRRC15 could serve as a target for counteracting immune evasion mechanisms in GBM, potentially enhancing the efficacy of immunotherapies in a tumor type that traditionally exhibits low responsiveness to immune-based treatments.

To elucidate the functional role of LRRC15 in GBM, we performed knockdown experiments in GBM cell lines, revealing that LRRC15 depletion significantly reduces colony formation, proliferation, and migration. These effects are consistent with studies in breast and lung cancers, where LRRC15 knockdown similarly impaired cell proliferation and invasive potential [51]. Our findings extend these observations to GBM, demonstrating that LRRC15 plays a crucial role in promoting aggressive cellular behaviors that are hallmarks of GBM progression. This suggests that targeting LRRC15 could inhibit tumor growth and invasion, providing a novel therapeutic approach to limit GBM progression. However, to deepen our understanding of the underlying mechanisms, further functional studies are warranted. For instance, generating LRRC15 knockout models using CRISPR/Cas9 technology could provide more definitive evidence of its role in GBM biology. Additionally, investigating downstream signaling pathways activated by LRRC15, such as PI3K/AKT, MAPK, or TGF- β , could elucidate how it supports cell proliferation and invasion. Exploring interactions with ECM components and integrins might also clarify how LRRC15 enhances GBM cell adhesion and migration. These insights could open new therapeutic avenues, particularly in targeting LRRC15 to disrupt tumor-stroma interactions and inhibit GBM progression.

We observed that LRRC15 knockdown leads to a reduction in EMT markers (N-cadherin, vimentin) and an increase in epithelial marker E-cadherin in GBM cells, indicating a reversal of EMT. EMT is a key process in cancer metastasis, facilitating tumor cell migration and invasion. Prior studies have linked LRRC15 to EMT in other cancers, where it promotes the expression of mesenchymal markers [61]. Our results demonstrate, for the first time, the role of LR-

RC15 in promoting EMT specifically in GBM. This finding suggests that LRRC15 may contribute to the invasive nature of GBM by facilitating EMT, thereby enhancing the tumor's metastatic potential. Targeting LRRC15 to inhibit EMT processes could thus be a promising strategy to limit GBM invasiveness and improve clinical outcomes.

Despite the established role of LRRC15 in cancer cell survival and proliferation, we found no significant correlation between LRRC15 expression and drug sensitivity in GBM. This contrasts with studies in other cancers, where LRRC15 expression has been linked to chemotherapy resistance. Our results suggest that while LRRC15 may drive tumor progression in GBM, its influence on drug response could be limited or context-specific. Nonetheless, targeting LRRC15 directly or in combination with chemotherapy could still yield therapeutic benefits by inhibiting key processes associated with GBM aggressiveness, such as proliferation, migration, and EMT.

Conclusion

In summary, our study sheds light on the multifaceted role of LRRC15 in GBM, highlighting its potential as a diagnostic, prognostic, and therapeutic target. LRRC15 appears to contribute to GBM progression through mechanisms involving immune modulation, EMT, and ECM remodeling, all of which are essential for tumor growth and invasion. The association between LRRC15 and immune suppression is particularly notable, as it suggests potential avenues for immunotherapy that could counteract GBM's immunosuppressive microenvironment. While our study advances the understanding of LRRC15 in GBM, further research is needed to investigate its molecular interactions and regulatory mechanisms in this context. Additionally, preclinical studies targeting LRRC15 could validate its therapeutic potential and pave the way for clinical trials aimed at improving outcomes for GBM patients.

Disclosure of conflict of interest

None.

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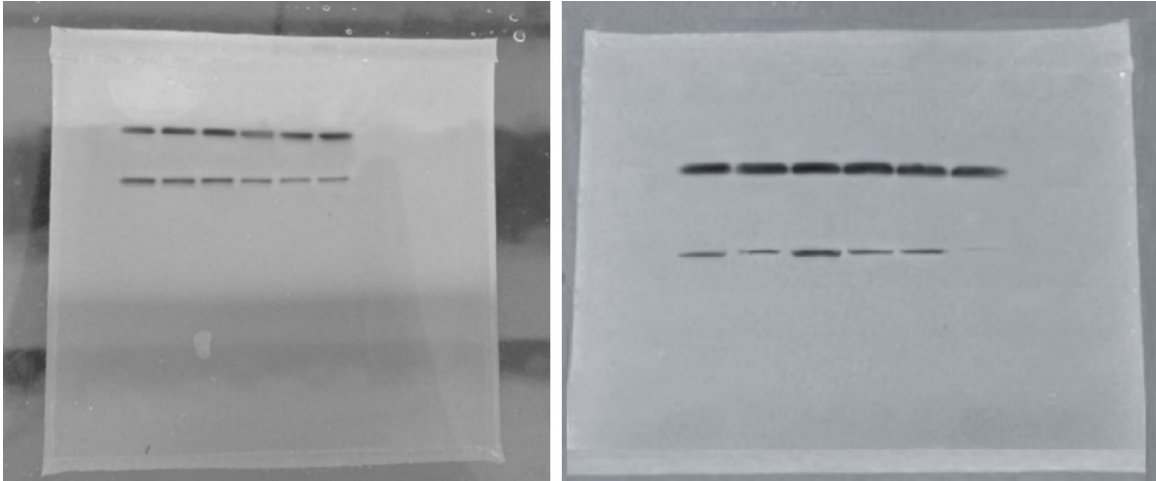
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LRRC15 as a prognostic biomarker in glioblastoma



Supplementary Figure 1. Uncut Western blot bands of GAPDH and LRRC15.