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Original Article

Increased expression of SMAD5-AS1 in glioma is associated with patient prognosis and immunity

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Abstract: The aim of this study was to examine the expression profile, clinical significance, and potential diagnostic/prognostic value of the long non-coding RNA (lncRNA) SMAD5-AS1 in glioma. Methods: Expression of SMAD5-AS1 and its associations with survival were analyzed in patients with lower-grade glioma (LGG) and glioblastoma (GBM) using GEPIA2, R, UALCAN, and TIMER3 platforms. Results: SMAD5-AS1 was significantly upregulated in multiple cancers, particularly gliomas (LGG and GBM; $P < 0.05$), demonstrating strong diagnostic performance for GBM (AUC = 0.87) and moderate performance for LGG (AUC = 0.71). In LGG, high SMAD5-AS1 expression was associated with poorer overall survival (HR = 25.53; $P < 0.001$) and enrichment in higher-grade tumors and astrocytic subtypes ($P < 0.01$), suggesting a link to tumor aggressiveness. Furthermore, SMAD5-AS1 expression was negatively correlated with infiltration by immunosuppressive cells, implicating a role in modulating the tumor immune microenvironment. Conclusion: SMAD5-AS1 represents a promising diagnostic and prognostic biomarker in glioma, with elevated expression linked to increased tumor aggressiveness, immunosuppression, and adverse patient outcomes.

Keywords: SMAD5-AS1, glioma, prognosis, immune infiltration

Introduction

Glioma remains the most prevalent and aggressive primary malignant tumor of the central nervous system, constituting approximately 80% of all malignant brain tumors [1-4]. Despite advances in surgical resection, radiotherapy, and chemotherapy, the prognosis for patients with glioma - particularly glioblastoma (GBM) - remains poor, with a median survival of less than 15 months [5]. Increasing evidence highlights the roles of molecular heterogeneity and an immunosuppressive tumor microenvironment in driving therapeutic resistance and disease progression. Consequently, identifying novel molecular biomarkers for prognosis and immunomodulation holds substantial clinical value.

In recent years, long non-coding RNAs (lncRNAs) have emerged as critical regulators of gene expression, influencing key cancer hallmarks such as proliferation, apoptosis, invasion, and metastasis [6]. SMAD5 antisense RNA 1 (SMAD5-AS1) is a lncRNA located on human chromosome 5, transcribed in the antisense direction relative to SMAD5, with overlapping transcripts that may regulate SMAD5 expression. SMAD5-AS1 has been detected in fetal tissues and certain tumors [7]; however, its expression pattern, clinical relevance, and immunological role in glioma remain poorly characterized.

Previous studies in other malignancies offer functional insights. For instance, in diffuse large B-cell lymphoma (DLBCL), SMAD5-AS1 functions as a competing endogenous RNA (ceRNA) by sponging miR-135b-5p, upregulating APC, and inhibiting the Wnt/β-catenin pathway, thereby suppressing proliferation [8]. In nasopharyngeal carcinoma, SMAD5-AS1 promotes epithelial-mesenchymal transition (EMT) via miR-106a-5p or miR-195-mediated regulation of SMAD5 [9, 10]. These findings suggest context-dependent roles for SMAD5-AS1 in tumor progression, yet its significance in glioma is largely unexplored.

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Given the limited data on SMAD5-AS1 in glioma, we performed a comprehensive bioinformatics analysis using public transcriptomic datasets to evaluate its associations with clinicopathological features, patient survival, and immune infiltration. Our results indicate that SMAD5-AS1 may serve as a novel prognostic biomarker and regulator of the tumor immune microenvironment in glioma.

Materials and methods

Data acquisition and expression analysis

SMAD5-AS1 expression in glioma and normal brain tissues was retrieved from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases via the GEPIA2 platform (http://gepia2.cancer-pku.cn/) [11]. Differential expression in GBM, LGG, and normal brain tissue was assessed using the "Expression DIY" module, with significance defined as $|\log_2 \text{fold change}| \geq 1$ and $P < 0.05$. Results were visualized as box plots.

Survival analysis

Prognostic significance was evaluated using R for overall survival (OS) and disease-free survival (DFS). Patients were stratified into high- and low-expression groups based on the median SMAD5-AS1 expression. Kaplan-Meier curves were generated, and differences were tested using the log-rank test.

Clinical correlation analysis

Associations between SMAD5-AS1 expression and clinicopathological features (including WHO grade, histological type (astrocytoma, oligodendroglioma, oligoastrocytoma), molecular subtype (IDH mutation status, 1p/19q co-deletion), age, race, and sex) were analyzed in TCGA glioma cohorts via the UALCAN database [12]. The clinical correlation analysis investigated the association between SMAD5-AS1 expression and the following patient and tumor characteristics (Supplementary Table 1). Differences between subgroups were compared using t-tests, with $P < 0.05$ considered significant.

Immune infiltration analysis

Correlations between SMAD5-AS1 expression and immune cell infiltration were assessed using the TIMER3 platform (https://compbio.cn/timer3/) [13]. Within the "Gene" module, SMAD5-AS1 was selected as the target gene. The "Correlation" module was then used to analyze its association with the infiltration levels of various immune cell types, including CD8+ T cells, macrophages, dendritic cells, neutrophils, and B cells, across multiple cancer types. Results were displayed in a heatmap showing purity-adjusted Spearman's correlation coefficients (ρ). Scatter plots depicting the relationship between immune infiltration scores and SMAD5-AS1 expression were generated for specific cell types. The "Purity Adjustment" option was selected to calculate partial Spearman's correlations and correct for potential bias introduced by tumor purity. For algorithms like EPIC and quanTIseq that inherently estimate cell fractions, purity adjustment was not applied.

Statistical analysis

All statistical analyses were performed using R software (version 4.3.1). Continuous data are presented as median with interquartile range (IQR) or mean ± standard deviation (SD), as appropriate. Categorical variables are summarized as frequencies and percentages. For comparisons between two groups, the Wilcoxon rank-sum test (non-parametric) or Student's t-test (parametric) was applied based on data distribution. Multiple group comparisons were conducted using the Kruskal-Wallis test or one-way ANOVA, followed by post-hoc tests where applicable. Survival analyses utilized Kaplan-Meier curves with the log-rank test for significance, and hazard ratios (HRs) were derived from univariate Cox proportional hazards models. Correlations between variables were assessed using Spearman's rank correlation. Diagnostic performance was evaluated by receiver operating characteristic (ROC) curves, with area under the curve (AUC) reported. A two-sided *P*-value < 0.05 was considered statistically significant for all tests.

Results

Pan-cancer expression profile and diagnostic value of SMAD5-AS1 in glioma

To investigate the oncogenic profile of SMAD5-AS1, we analyzed its expression across multi-

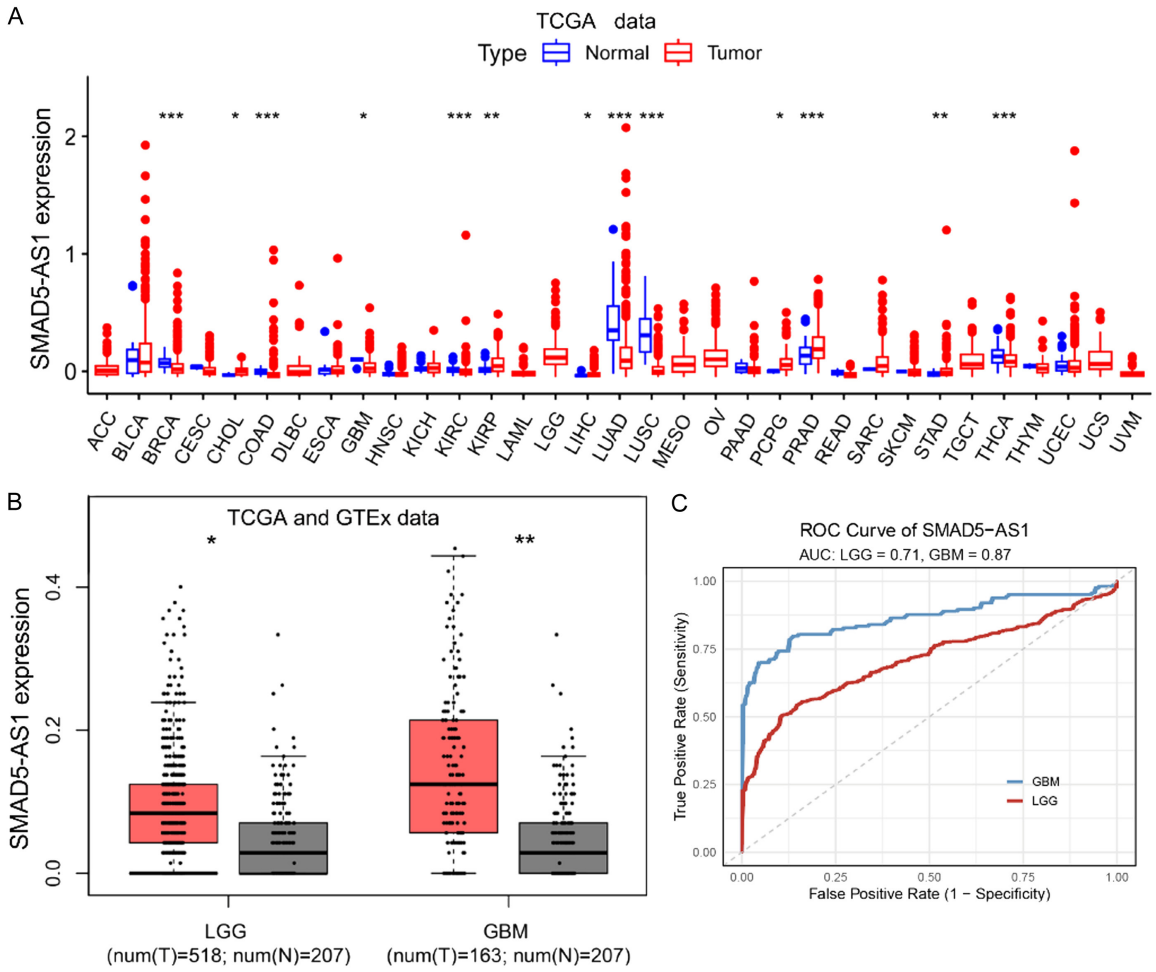

Figure 1. Pan-cancer expression and diagnostic performance of SMAD5-AS1 in glioma. A. Comparison of SMAD5-AS1 transcript levels across 33 tumor types versus matched normal tissues in The Cancer Genome Atlas (TCGA). RNA-seq expression (log_2 TPM) for each cancer type (red) and corresponding normal samples (blue) are shown as boxplots with overlaid individual data points. Statistical significance was assessed by Wilcoxon rank-sum test: P < 0.05; *P < 0.01; **P < 0.001. B. Integrated analysis of SMAD5-AS1 expression in lower-grade glioma (LGG; WHO grades II-III) and glioblastoma multiforme (GBM; WHO grade IV) using combined TCGA and GTEx datasets. Boxplots (with jittered points) compare tumor (red) versus normal brain cortex (gray); sample sizes are indicated below. Significance was determined by Wilcoxon test (P < 0.05; *P < 0.01). C. Receiver operating characteristic (ROC) curves evaluating the ability of SMAD5-AS1 expression to distinguish LGG (red curve) and GBM (blue curve) from normal brain. Area under the curve (AUC) values are reported for each subgroup.

ple cancer types using public datasets. Our analysis revealed that SMAD5-AS1 is significantly dysregulated in several malignancies, including glioma (both LGG and GBM), breast cancer, and lung cancer, when compared to their normal tissue counterparts (all P < 0.05, **Figure 1A**). In glioma, SMAD5-AS1 was markedly upregulated in both LGG (518 tumors vs. 207 normal samples) and GBM (163 tumors vs. 207 normal), with GBM exhibiting the highest expression levels (LGG: P < 0.05; GBM: P < 0.01; **Figure 1B**).

Receiver operating characteristic (ROC) curve analysis quantified the diagnostic potential of SMAD5-AS1. It demonstrated excellent discriminatory power for GBM (AUC = 0.87) and moderate power for LGG (AUC = 0.71, **Figure 1C**), suggesting its utility as a diagnostic biomarker, particularly for glioblastoma.

Prognostic significance of SMAD5-AS1 in lower-grade glioma

Univariate Cox analysis indicated a significant association between high SMAD5-AS1 expres-

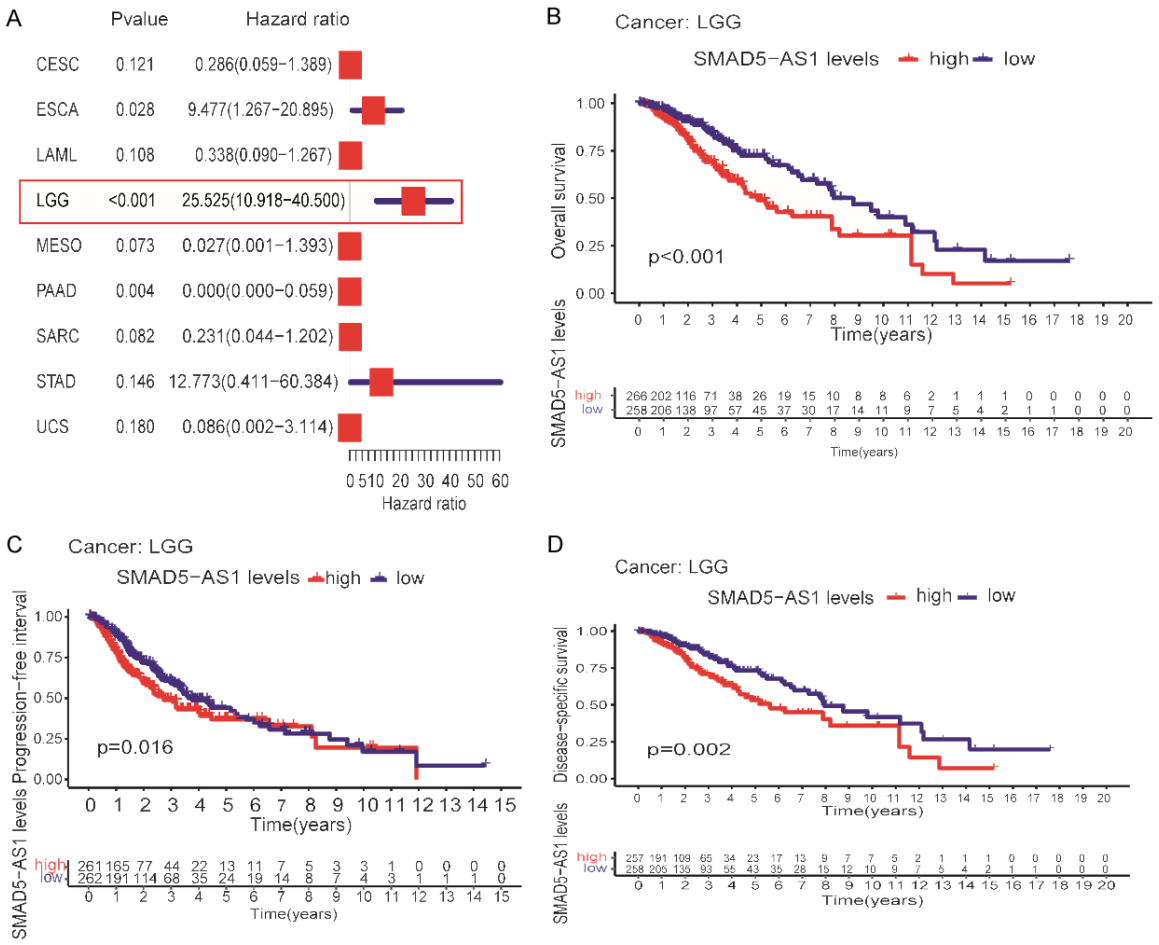

Figure 2. Prognostic significance of SMAD5-AS1 in lower-grade glioma (LGG). (A) Pan-cancer univariate Cox proportional hazards analysis of SMAD5-AS1 expression versus overall survival across TCGA tumor types. (B-D) Kaplan-Meier survival analyses in TCGA LGG cohort stratified into "high" and "low" SMAD5-AS1 expression groups by median cutoff. (B) Overall survival (OS), (C) progression-free interval (PFI), and (D) disease-specific survival (DSS) curves were compared by log-rank test; patient numbers at risk are shown beneath each plot.

sion and adverse overall survival (OS) in several cancers, most notably in LGG (HR = 25.53, 95% CI = 10.92-40.50, P < 0.001; **Figure 2A**). Kaplan-Meier analysis confirmed that LGG patients with high SMAD5-AS1 expression had significantly worse OS (P < 0.001; **Figure 2B**). Furthermore, the high-expression group experienced a significantly shorter progression-free interval (PFI, P = 0.016; **Figure 2C**) and poorer disease-specific survival (DSS, P = 0.002; **Figure 2D**). These results underscore SMAD5-AS1 as a potent negative prognostic biomarker in LGG.

Correlation between SMAD5-AS1 expression and clinicopathological features

Analysis of 4,792 glioma patients, stratified into a high-expression group (n = 2,401) and a low-expression group (n = 2,391) based on SMAD5-AS1 expression levels, demonstrated significant associations between SMAD5-AS1 expression and several key clinicopathological features (**Table 1**).

No significant differences in SMAD5-AS1 expression were observed across racial groups (Caucasian, African American, and Asian; **Figure 3A**) or across age cohorts (21-40, 41-60, 61-80, and 81-100 years; **Figure 3D**), indicating that SMAD5-AS1 expression is independent of race and age. In contrast, SMAD5-AS1 expression was significantly higher in WHO grade 3 gliomas compared with grade 2 tumors (P < 0.01; **Figure 3B**), suggesting an association with increasing tumor malignancy.

Regarding histological subtypes, SMAD5-AS1 expression levels differed markedly, with the

Table 1. SMAD5-AS1 expression associated with clinical pathological characteristics

Variable	high N = 240[1]	low N = 239[1]	*P*-value[2]
Diagnosis.Age			0.6
< 40	108 (45%)	113 (47%)	
≥ 40	132 (55%)	126 (53%)	
Histologic.Grade			0.001
G2	97 (40%)	132 (55%)	
G3	143 (60%)	107 (45%)	
Race.Category			0.6
American Indian or Alaska Native	1 (0.4%)	0 (0%)	
Asian	2 (0.8%)	5 (2.1%)	
Black or African American	10 (4.2%)	11 (4.6%)	
White	227 (95%)	223 (93%)	
Sex			0.8
Female	105 (44%)	107 (45%)	
Male	135 (56%)	132 (55%)	
Tumor.Type			0.001
Astrocytoma	110 (46%)	72 (30%)	
Oligoastrocytoma	58 (24%)	64 (27%)	
Oligodendroglioma	72 (30%)	103 (43%)	
subtype			< 0.001
IDHmut-codel	58 (24%)	100 (42%)	
IDHmut-non-codel	124 (52%)	109 (46%)	
IDHwt	58 (24%)	30 (13%)	

[1]n (%). [2]Pearson's Chi-squared test; Fisher's exact test.

highest expression observed in astrocytomas, intermediate levels in oligoastrocytomas, and the lowest in oligodendrogliomas ($P < 0.01$; **Figure 3C**). This pattern highlights a potential relationship between SMAD5-AS1 expression and specific glial cell lineages.

Furthermore, SMAD5-AS1 expression varied significantly among molecular subtypes, including IDH-mutant with 1p/19q codeletion (IDHmut-codel), IDH-mutant without codeletion (IDHmut-non-codel), and IDH-wildtype (IDHwt) gliomas ($P < 0.001$).

Collectively, these findings indicate that SMAD5-AS1 expression in gliomas is closely correlated with tumor grade, histological subtype, and molecular classification, but not with patient demographics such as race or age. Such associations may provide valuable insights into the biological behavior of gliomas and could have implications for future diagnostic or therapeutic strategies.

Association of SMAD5-AS1 with the tumor immune microenvironment

To investigate the immunological implications of *SMAD5-AS1*, we analyzed its correlation with immune scores and immune cell infiltration (**Figure 4A**). In GBM samples, *SMAD5-AS1* expression showed a significant negative correlation with the immune score (Spearman's $\rho = -0.211$, $P < 0.05$), indicating a potential link with an immunosuppressive tumor microenvironment. However, no significant associations were detected between *SMAD5-AS1* expression and stromal or immune scores in LGG.

Further partial correlation analysis revealed that in GBM, high *SMAD5-AS1* expression was negatively associated with infiltration of regulatory T cells (Tregs), M2 macrophages, and other immunosuppressive immune cell subsets (**Figure 4B**). These trends were less pronounced and often non-significant in LGG, suggesting a context-specific immune regulatory role of *SMAD5-AS1* that may be more prominent in high-grade gliomas.

Discussion

This study provides a comprehensive evaluation of the lncRNA SMAD5-AS1 in glioma, revealing its significant upregulation, diagnostic potential, prognostic value, and immune correlates. The pronounced overexpression of SMAD5-AS1 in gliomas aligns with the established dysregulation of lncRNAs in cancer and underscores their biomarker potential [8, 9]. The diagnostic potential of SMAD5-AS1 for glioma was further supported by receiver operating characteristic (ROC) curve analysis. Marked discriminatory capacity was observed in glioblastoma (GBM), while its utility appeared more moderate in low-grade glioma (LGG). The strong diagnostic performance for GBM (AUC = 0.87)

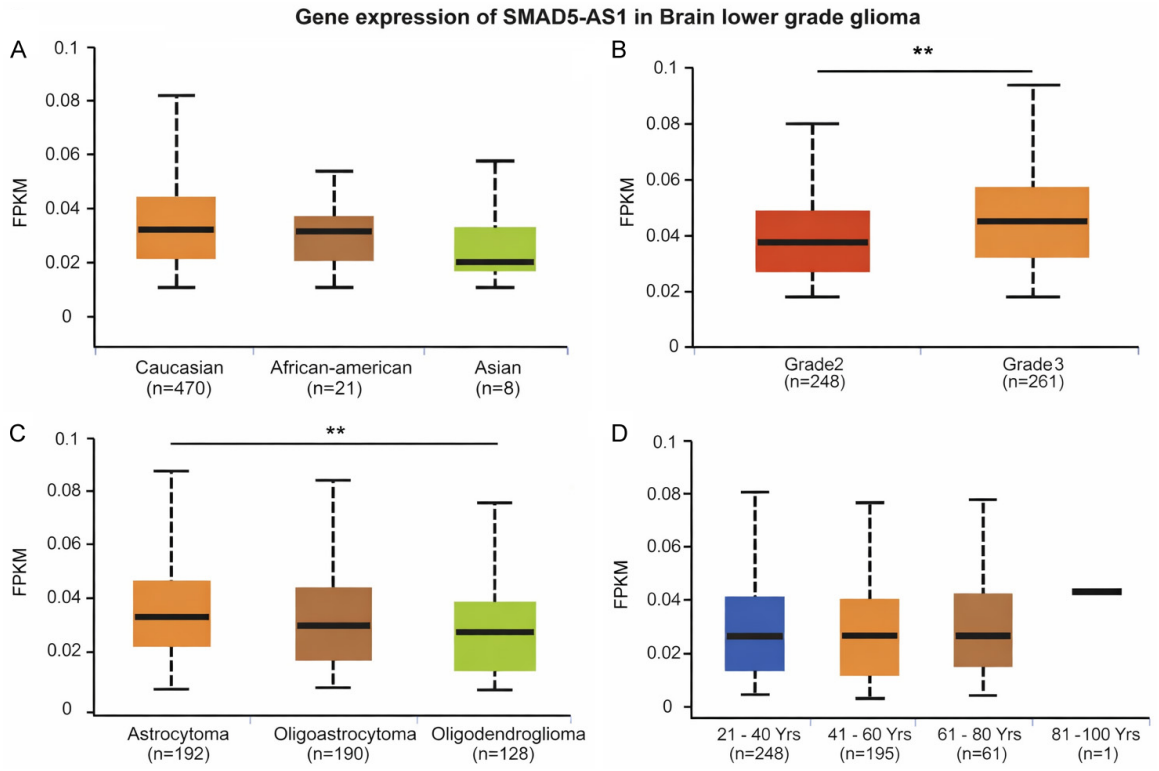

Figure 3. SMAD5-AS1 expression stratified by clinical and demographic variables. (A) Racial groups, (B) WHO grade, (C) Histological subtype, (D) Patient age. Group comparisons were performed by two-sided Wilcoxon rank-sum test; $P < 0.01$ is indicated by "**".

is consistent with reports on other glioma-associated lncRNAs like MALAT1 and HOTAIR [14-18].

The robust prognostic relevance of SMAD5-AS1 in LGG, evidenced by high hazard ratios for poor OS, PFI, and DSS, positions it as a potent independent predictor of adverse outcomes. This finding parallels the reported significance of lncRNAs such as HOTAIR and UCA1 in glioma progression [19, 20]. The association of high SMAD5-AS1 expression with advanced tumor grade and astrocytic histology further highlights its relevance to tumor aggressiveness and stratification, independent of demographic factors like age or race.

The novel immunological association identified here suggests that SMAD5-AS1 may contribute to shaping an immunosuppressive microenvironment in GBM, characterized by lower overall immune infiltration and reduced presence of specific immunosuppressive cells. This aligns with emerging evidence on lncRNAs modulating tumor immunity [21, 22] and may be particularly relevant in GBM, where immune evasion is a major therapeutic hurdle [23]. The weaker immune correlations in LGG may reflect inherent differences in the immune landscape between low-grade and high-grade gliomas.

This study has several limitations. First, the conclusions are primarily derived from bioinformatic analyses and require experimental validation in glioma models to confirm the functional impact of SMAD5-AS1 on tumor progression and immune regulation. Second, while correlations with immune infiltration are evident, causal relationships need to be established through mechanistic studies. Third, the focus on transcriptional data leaves post-transcriptional regulation and protein-level interactions unexplored. Future research should aim to elucidate the precise mechanisms by which SMAD5-AS1 influences immune suppression, disease progression, and therapeutic resistance, particularly in treatment-refractory GBM.

Conclusions

This study identifies SMAD5-AS1 as a previously under-characterized oncogenic lncRNA that

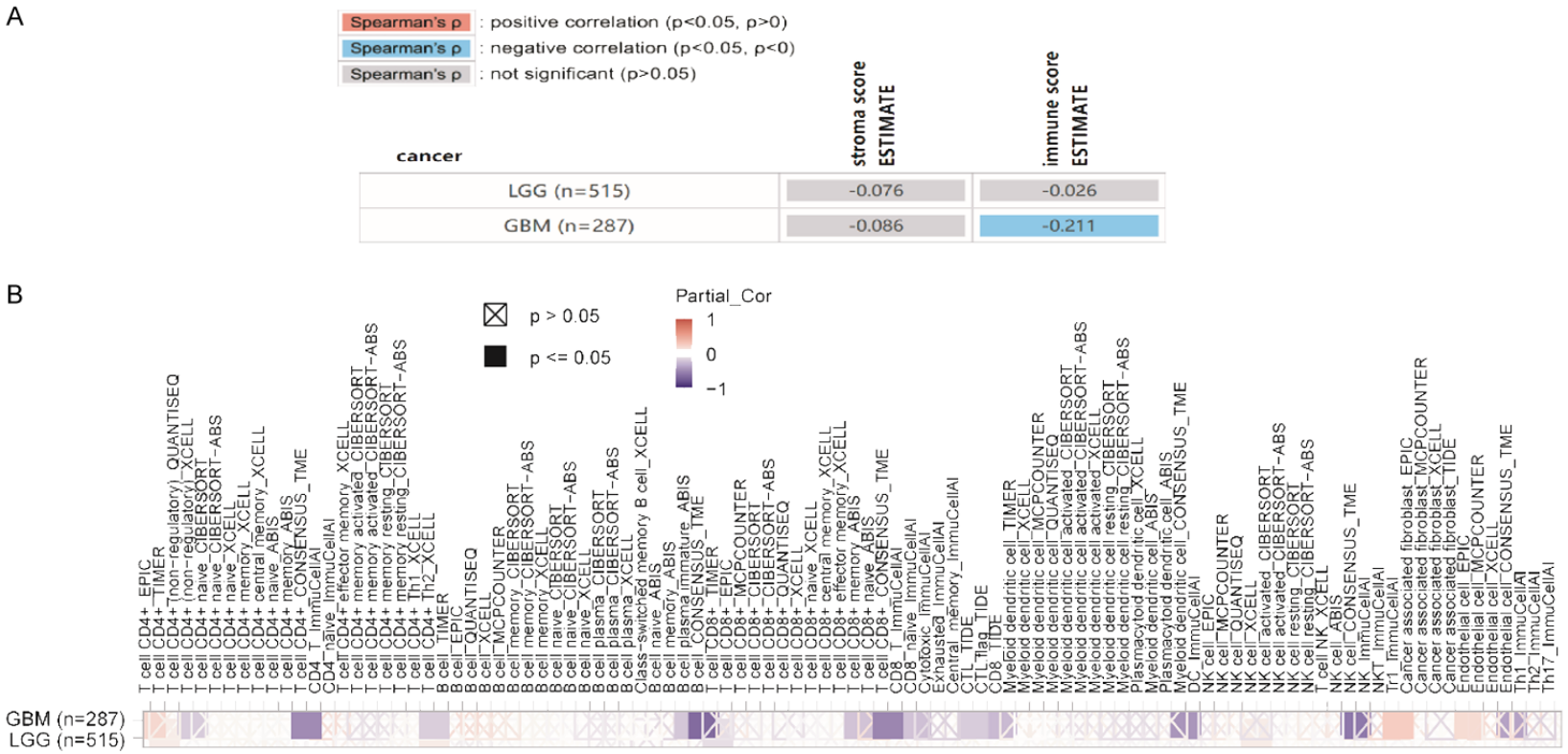

Figure 4. Correlation between SMAD5-AS1 expression and tumor immune microenvironment in gliomas. A. Spearman correlation analysis between SMAD5-AS1 expression and ESTIMATE-derived stromal and immune scores in LGG and GBM. B. Partial correlation heatmap showing the association of SMAD5-AS1 with various immune cell infiltration levels estimated by multiple algorithms.

is markedly overexpressed in glioma. Its upregulation is closely associated with unfavorable clinicopathological features, poor prognosis, and an immunosuppressive microenvironment in GBM. These findings provide a foundation for further functional investigations and suggest that SMAD5-AS1 could represent a novel therapeutic target in glioma management.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Clinical correlation analysis

Analysis Dimension	Specific Variables/Categories
Demographics	Age (< 40 vs. ≥ 40 years), Sex, Race (Caucasian, African American, Asian, etc.)
Pathological Features	WHO Grade (G2 vs. G3), Histological Type (Astrocytoma, Oligodendroglioma, Oligoastrocytoma)
Molecular Subtypes	IDH mutation status combined with 1p/19q co-deletion status (IDHmut-codel, IDHmut-non-codel, IDHwt)
Tumor Microenvironment	ESTIMATE-derived Immune Score and Stromal Score