

Non-Coding RNA Regulators of the Immunosuppressive Tumour Microenvironment in Glioma: a Cross-Cohort TCGA Analysis Identifying Delivery-Aware Therapeutic Targets

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Abstract

Gliomas are the most common malignant primary brain tumours, and their dismal prognosis is driven in large part by an immunosuppressive tumour microenvironment (TME) dominated by tumour-associated macrophages and microglia, hypoxia and mesenchymal transition. The non-coding RNA (ncRNA) circuitry that couples these programmes remains incompletely defined, and few candidate regulators have been evaluated for therapeutic tractability given the blood-brain barrier (BBB). Here we integrated transcriptomic and clinical data from TCGA-GBM and TCGA-LGG to map ncRNA regulators of the glioma TME and to prioritise delivery-aware targets. Tumours were scored along four TME axes (hypoxia, myeloid/TAM, mesenchymal and lymphoid), and long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) were tested for differential expression, axis correlation, survival association and competing-endogenous-RNA (ceRNA) connectivity. Across 304 GBM and 533 LGG scored tumours, the myeloid, mesenchymal and hypoxia axes were strongly co-activated, defining a TME-high state. A replicated module of lncRNAs (including LINC02381, PRKAR1B-AS1 and LBX2-AS1) and the miRNAs miR-155 and miR-23a was up-regulated in TME-high tumours, with Spearman correlations reaching $\rho = 0.70$ and concordant effects between cohorts. High expression of these ncRNAs was associated with

shorter overall survival (median hazard ratios 1.5-2.2; best $P < 1e-20$). Expression-supported ceRNA reconstruction placed LINC02381 as a hub linking miR-155 and miR-23a and de-repressing target genes enriched for Notch signalling (DLL1). Integrating replication, effect size, survival and network evidence yielded a 50-target, BBB-aware, delivery-aware shortlist mapped to the TAM/microglia and hypoxic-necrotic compartments. These candidates provide a prioritised, experimentally testable basis for antisense, RNA-interference and antagomir strategies against the glioma TME.

Keywords: glioma; tumour microenvironment; long non-coding RNA; tumour-associated macrophages; competing endogenous RNA; blood-brain barrier delivery

Introduction

Diffuse gliomas account for the majority of malignant primary central nervous system tumours, and glioblastoma (GBM, WHO grade 4) remains almost uniformly lethal despite maximal resection, radiotherapy and temozolomide (1,2). The 2021 WHO classification formalised the molecular stratification of gliomas, yet molecular taxonomy alone has not translated into durable therapies, and the immunosuppressive tumour microenvironment (TME) is increasingly recognised as a central barrier to treatment (1,3). Unlike many solid tumours, the glioma TME is dominated by brain-resident microg-

lia and recruited bone-marrow-derived macrophages, which can constitute a substantial fraction of the tumour mass and orchestrate immune evasion, angiogenesis and invasion (4,5).

Single-cell and bulk transcriptomic studies have shown that malignant glioma cells occupy plastic cellular states, and that a mesenchymal-like state is tightly coupled to tumour-associated macrophage (TAM) infiltration, hypoxia and poor outcome (3,6,7). Proneural-to-mesenchymal transition is accompanied by NF1 loss, recruitment of monocyte-derived TAMs and reprogramming of myeloid cells toward an immunosuppressive phenotype, while hypoxic niches further polarise macrophages toward pro-tumorigenic, treatment-resistant states (7,8,9,10). These observations argue that the hypoxia-myeloid-mesenchymal triad behaves as a coordinated programme rather than as independent features, but the upstream regulators that lock tumours into this state remain only partially defined.

Non-coding RNAs have emerged as pervasive regulators of this circuitry. Long non-coding RNAs (lncRNAs) control glioma proliferation, invasion and immune signalling through diverse mechanisms, including chromatin remodelling, transcriptional activation and microRNA sponging (11,12,13). Specific lncRNAs shape the immunosuppressive GBM TME directly: H19-derived products recruit myeloid-derived suppressor cells and TAMs, SPI1-driven MIR222HG promotes proneural-to-mesenchymal transition and macrophage polarisation, and HOXC-AS3 reprogrammes lipid metabolism in the glioma stem-cell niche (13,14,15). lncRNAs also act through hypoxia-linked axes such as OR7E156P/miR-143/HIF1A, and HOTAIR is a consistently elevated, prognostically adverse lncRNA in GBM (16,17). In parallel, machine-learning and signature-based studies have repeatedly extracted TME- and epithelial-mesenchymal-transition-related lncRNA signatures that stratify lower-grade glioma prognosis (18,19). According to the competing-endogenous-RNA (ceRNA) hypothesis,

lncRNAs and other transcripts cross-regulate through shared microRNA response elements, providing a mechanistic framework linking these molecules to coding-gene programmes (20,21,22,23).

Two gaps limit the translational value of this literature. First, most candidates are reported from single cohorts or single axes, so it is unclear which ncRNAs are robust, cross-cohort regulators of the integrated TME rather than incidental correlates. Second, even validated regulators are rarely evaluated for therapeutic deliverability, despite the fact that the BBB is the dominant obstacle to nucleic-acid therapeutics in glioma (24,25,26,27). Here we address both gaps by integrating TCGA-GBM and TCGA-LGG to score four glioma TME axes, systematically associate lncRNAs and miRNAs with these axes, test survival and ceRNA evidence, and prioritise a delivery-aware shortlist of targets. We show that a compact, cross-cohort-replicated ncRNA module marks and predicts the immunosuppressive TME-high state and converges on actionable, BBB-relevant therapeutic concepts.

Materials and Methods

Data acquisition and cohorts

Harmonised RNA-sequencing, microRNA-sequencing and clinical data for TCGA-GBM and TCGA-LGG were obtained from the NCI Genomic Data Commons (GDC). After chunked archive validation, the analysis included 391 GBM and 534 LGG RNA-seq tumours, 278 GBM and 530 LGG miRNA-seq tumours, and clinical records for 617 GBM and 516 LGG cases. Clinical metadata (sex, vital status, survival time and event) were extracted from the GDC cases API. Cohort characteristics are summarised in Table 1.

Expression preprocessing

STAR-aligned gene-level counts were used as the primary quantification (28). Counts were converted to transcripts-per-million (TPM) for mRNAs and lncRNAs and to reads-per-mil-

lion (RPM) for miRNAs, and transcripts were partitioned into protein-coding mRNA, lncRNA and miRNA classes using GDC gene annotation. Low-expression features were removed prior to association testing. The bioinformatic preprocessing, annotation and downstream computational analyses followed established transcriptomic and variant-analysis pipelines used for high-throughput sequencing data (29,30,31).

Glioma TME scoring

Each tumour was scored along four glioma-relevant TME axes using curated signature gene sets: hypoxia/necrosis, myeloid/TAM-microglia, mesenchymal transition and lymphoid infiltration. Per-axis scores were computed as standardised (z-scored) signature averages within each cohort, and tumours were dichotomised into TME-high and TME-low groups per axis using the cohort-specific score distribution. Inter-axis relationships were quantified by Pearson correlation of the axis scores.

Differential expression and correlation analysis

For every TME axis, lncRNAs and miRNAs were tested for differential expression between TME-high and TME-low tumours, yielding log2 fold-changes and Benjamini-Hochberg false-discovery-rate (FDR) adjusted P-values. In parallel, each ncRNA was correlated with each continuous axis score using Spearman's rank correlation with BH-FDR correction. Analyses were performed independently in GBM and LGG, and cross-cohort concordance was assessed by comparing per-feature, per-axis correlation coefficients between cohorts.

Survival analysis

Associations between ncRNA expression and overall survival were evaluated using Cox proportional-hazards regression and Kaplan-Meier analysis, dichotomising tumours into high- and low-expression groups. Hazard ratios (high vs low) and the best per-feature P-value across cohorts were retained. Candidates with-

out evaluable survival data were recorded separately.

ceRNA network reconstruction

Expression-supported ceRNA edges were reconstructed under the competing-endogenous-RNA model (23), requiring lncRNA-miRNA Spearman $\rho \leq -0.1$, miRNA-mRNA $\rho \leq -0.1$ and lncRNA-mRNA $\rho \geq 0.1$ using glioma-appropriate relaxed thresholds. Reconstructed modules were tested for over-representation of glioma TME and signalling signatures (myeloid/TAM, mesenchymal, hypoxia, angiogenesis, TGF- β , NF- κ B/JAK-STAT, Notch and immune-checkpoint sets) using the hypergeometric test with BH-FDR correction.

Cross-cohort prioritisation and delivery-aware shortlist

Candidate ncRNAs were scored by integrating cross-cohort replication, differential-expression and correlation effect sizes, survival significance and ceRNA-network evidence into a composite priority score. The highest-ranked candidates were then annotated with a therapeutic-direction call (knockdown for up-regulated lncRNAs and inhibition for up-regulated miRNAs; restoration for down-regulated lncRNAs), a likely source compartment, and a BBB-compatible delivery concept (for example, hypoxia-responsive carriers for necrotic/hypoxic regions or myeloid/TAM-directed exosome and nanoparticle strategies). All summary statistics, figures and tables were generated directly from the analysis result tables.

Results and Discussion

The glioma TME is organised around a co-activated myeloid-mesenchymal-hypoxia programme

Across 304 GBM and 533 LGG tumours with complete TME scores, the four axes were not independent (Fig. 2a-d). The myeloid/TAM, mesenchymal and hypoxia axes were strongly positively correlated, with the myeloid-mesenchymal relationship the strongest pairwise as-

sociation, whereas the lymphoid axis tracked most closely with myeloid and mesenchymal activity (Fig. 2a-c). Tumours therefore segregated along a single dominant gradient of joint axis activation, which we refer to as the TME-high state, observed consistently in both cohorts (Fig. 2d).

A compact ncRNA module marks the TME-high state and replicates across cohorts

Differential-expression and correlation testing across all four axes converged on a small set of ncRNAs that were consistently up-regulated in TME-high tumours (Fig. 3a-c). The leading lncRNAs (including LBX2-AS1 [ENSG00000257702], PRKAR1B-AS1 [ENSG00000237181], LINC02381 [ENSG00000250742], AC145098.1 [ENSG00000248996] and AL354919.2 [ENSG00000254545]) and the miRNAs miR-155 and miR-23a showed their strongest associations with the mesenchymal and myeloid axes, where Spearman correlations reached $\rho = 0.69$ - 0.70 in GBM and LGG, respectively (Fig. 3b; Table S1). Effect sizes were systematically larger for the mesenchymal and myeloid axes than for the hypoxia or lymphoid axes (Fig. 3b).

These associations replicated between cohorts. Per-feature, per-axis correlation coefficients in GBM and LGG were highly concordant across the 60 ncRNA-axis pairs examined, falling close to the identity line (Fig. 4 and Fig. S2), and the ranking of leading correlates per axis was largely preserved (Fig. S1). All retained associations were positive, indicating uniform up-regulation of the module in TME-high tumours (Fig. 4; Table S1).

TME-high ncRNAs are associated with poor overall survival

High expression of the leading ncRNAs was associated with shorter overall survival (Fig. 5b; Table S2). Median hazard ratios for high- versus low-expression tumours ranged from approximately 1.5 to 2.2, with highly significant best P-values (for example,

MIR155HG [ENSG00000234883] hazard ratio 2.15; AC131097.3 [ENSG00000235151] 2.11; miR-155 2.05, $P = 1.5 \times 10^{-21}$; LINC01842 [ENSG00000267147] $P = 1.9 \times 10^{-25}$). Every leading feature had a hazard ratio greater than one, indicating that elevation of the TME-high module uniformly accompanied worse prognosis (Fig. 5b; Table S2).

A ceRNA hub links the module to Notch signalling

Expression-supported ceRNA reconstruction organised the module around a single hub (Fig. 5a; Table S3). The top-ranked lncRNA, LINC02381 (ENSG00000250742), was negatively correlated with miR-155 and miR-23a (lncRNA-miRNA $\rho = -0.10$ and -0.17 , respectively) and positively correlated with approximately 39 candidate target mRNAs that were themselves negatively correlated with the two miRNAs, the pattern expected of a competing endogenous RNA (Fig. 5a; Table S3). Over-representation analysis of the module's target genes identified Notch signalling, through the ligand DLL1, as the only signature approaching significance (FDR ~ 0.06 ; Fig. S3).

An integrative, delivery-aware target shortlist

Integrating cross-cohort replication, effect size, survival and network evidence into a composite priority score ranked 18,770 candidate ncRNAs and yielded a 50-target shortlist (Fig. 1; Tables 2 and 3). The shortlist was dominated by lncRNAs up-regulated in TME-high tumours, annotated for antisense-oligonucleotide or siRNA knockdown, together with miR-155 and miR-23a as antagomir/inhibitor candidates and a small number of down-regulated lncRNAs flagged for restoration (Table 3). Candidates mapped predominantly to the TAM/microglia and hypoxic-necrotic compartments and were assigned compartment-matched BBB-compatible delivery concepts, most frequently hypoxia-responsive carriers and, in selected cases, myeloid/TAM-directed exosome or nanoparticle strategies (Table 3).

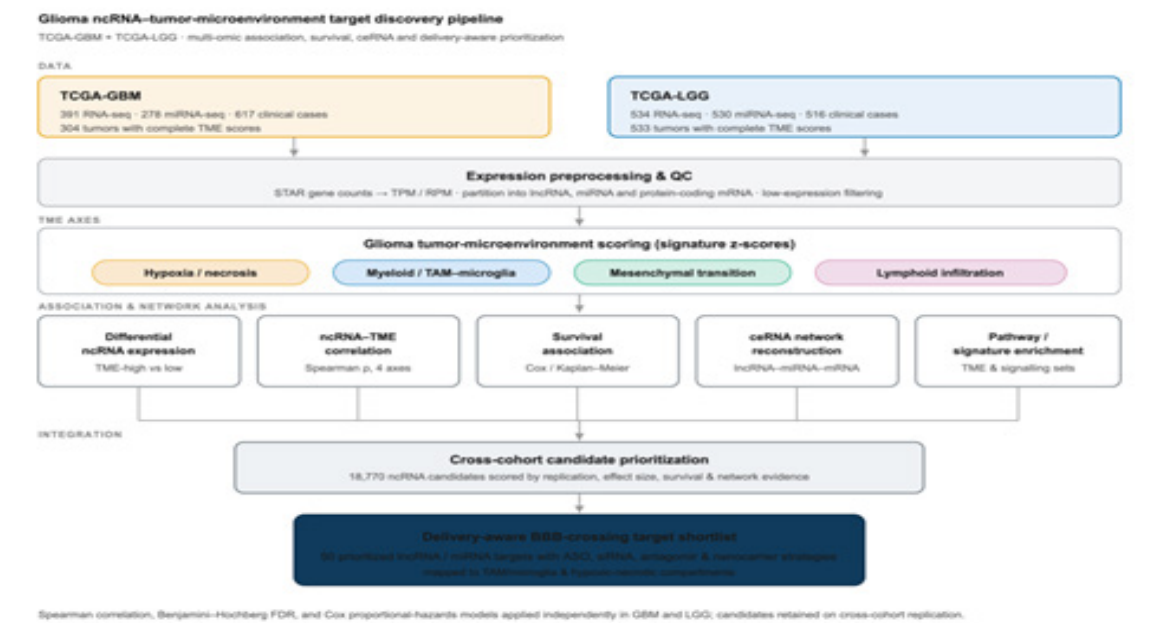


Figure 1. Study design and integrated analysis workflow. Overview of the analytical workflow, including TCGA GBM and LGG cohort selection, TME profiling, ncRNA identification, cross-cohort correlation and replication analyses, ceRNA network construction, survival analysis, and delivery-aware target prioritisation.

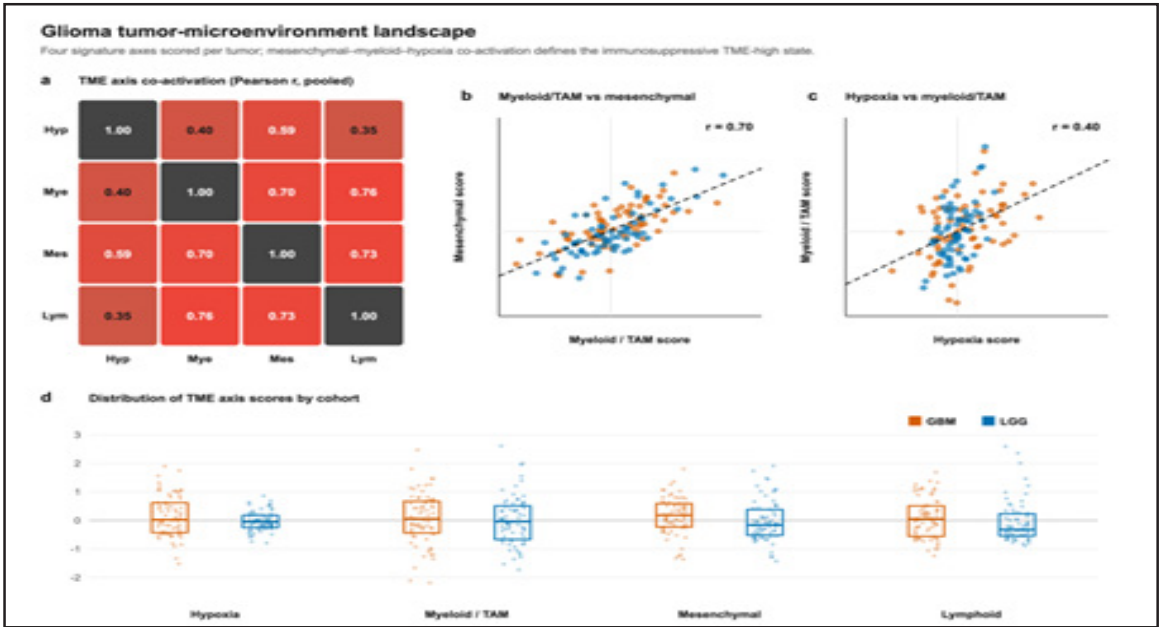


Figure 2. Coordinated myeloid, mesenchymal and hypoxia programmes in the glioma TME. Overview of the major TME-associated transcriptional programmes and their co-activation patterns across the analysed glioma cohorts.

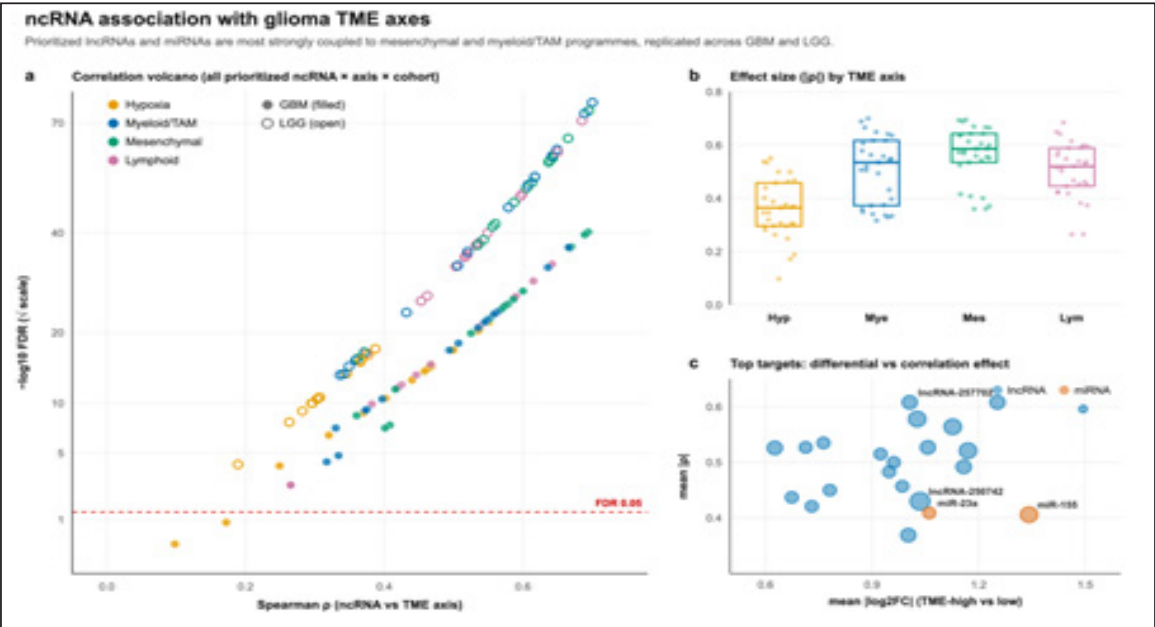


Figure 3. Association of a compact ncRNA module with mesenchymal and myeloid TME axes. Relationships between prioritised ncRNAs and the major mesenchymal- and myeloid-associated TME programmes identified in the analysis.

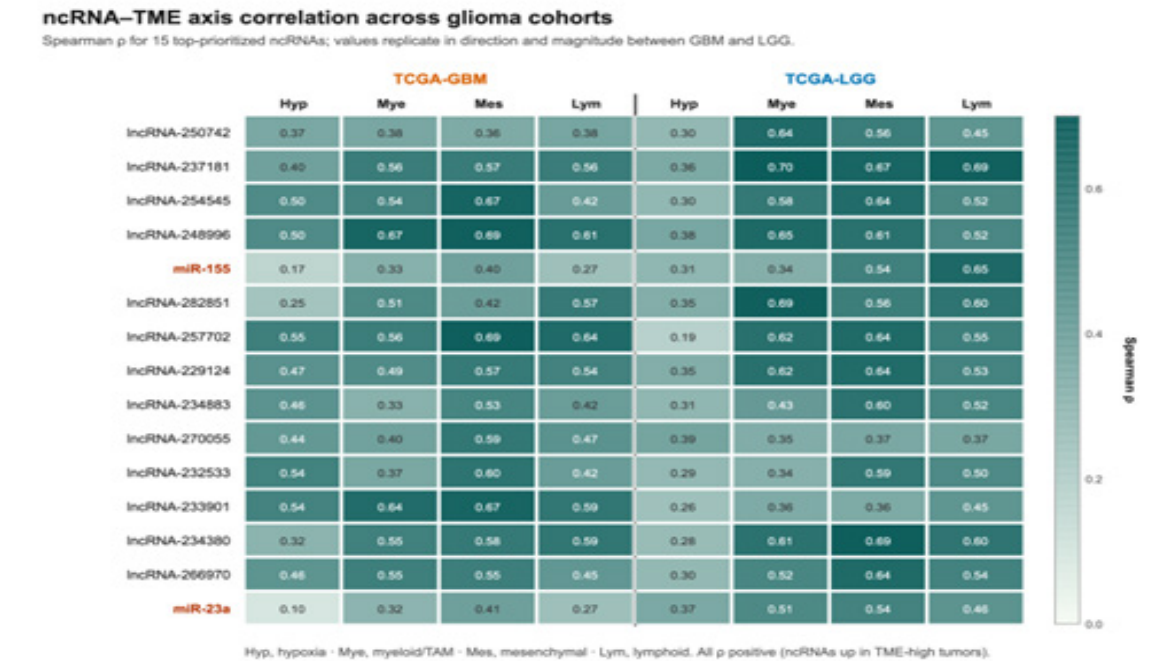


Figure 4. Cross-cohort ncRNA–TME correlation heatmap in GBM and LGG. Correlation patterns between prioritised ncRNAs and TME-associated axes across the TCGA GBM and LGG cohorts, highlighting consistent cross-cohort associations.

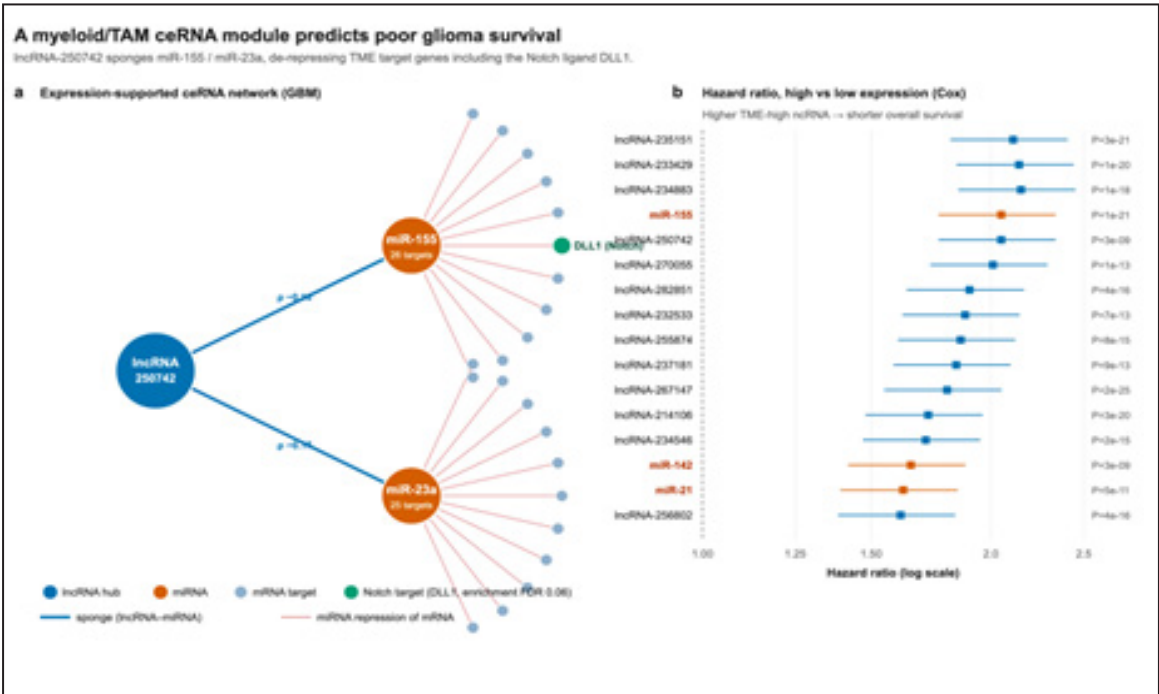


Figure 5. Expression-supported ceRNA network and survival associations. Integrated ceRNA relationships among prioritised ncRNAs and associated RNA targets, together with their observed associations with patient survival.

Table 1. Clinical and molecular characteristics of the analysed glioma cohorts.

Characteristic	TCGA- GBM	TCGA-LGG
Clinical cases (GDC)	617	516
RNA-seq tumors	391	534
miRNA-seq tumors	278	530
Tumors with complete TME scores	304	533
Sex: female	230	230
Sex: male	366	285
Sex: not reported	21	1
Vital status: de-ceased	492	126
Vital status: alive / not reported	125	390
Primary histology	Glioblastoma (WHO grade IV)	Astrocytoma, oligodendroglioma, oligoastrocytoma (WHO grade II-III)
TME axes scored	hypoxia, myeloid/TAM, mesenchymal, lymphoid	hypoxia, myeloid/TAM, mesenchymal, lymphoid

Table 2. Prioritised ncRNA candidates associated with glioma TME programmes.

Rank	Feature	Class	Final score	Direction	Mean log-2FC	Mean rho	Best survival P	Median HR (high vs low)	Therapeutic strategy
1	ENSG00000250742	lncRNA	35	up in TME-high	1.03	0.43	3.5e-09	2.05	ASO/siRNA knock-down
2	ENSG00000237181	lncRNA	34	up in TME-high	1.13	0.56	8.7e-13	1.84	ASO/siRNA knock-down
3	ENSG00000254545	lncRNA	34	up in TME-high	1.17	0.52	2e-12	1.53	ASO/siRNA knock-down
4	ENSG00000248996	lncRNA	34	up in TME-high	1.03	0.58	6.6e-09	1.77	ASO/siRNA knock-down
5	hsa-mir-155	miRNA	34	up in TME-high	1.34	0.41	1.5e-21	2.05	antago-mir/inhibitor
6	ENSG00000282851	lncRNA	33	up in TME-high	1.06	0.53	3.7e-16	1.9	ASO/siRNA knock-down
7	ENSG00000257702	lncRNA	33	up in TME-high	1.01	0.61	5e-11	1.5	ASO/siRNA knock-down
8	ENSG00000276855	lncRNA	33	up in TME-high	1.0	0.37	8.2e-10	1.54	ASO/siRNA knock-down
9	ENSG00000229124	lncRNA	33	up in TME-high	0.63	0.53	2.8e-07	1.92	ASO/siRNA knock-down
10	ENSG00000203644	lncRNA	33	up in TME-high	1.16	0.49	0.0002	1.43	ASO/siRNA knock-down
11	ENSG00000265743	lncRNA	33	up in TME-high	1.25	0.61	0.012	1.33	ASO/siRNA knock-down
12	ENSG00000234883	lncRNA	32	up in TME-high	0.78	0.45	1.1e-18	2.15	ASO/siRNA knock-down
13	ENSG00000234546	lncRNA	32	up in TME-high	0.67	0.44	2.1e-15	1.71	ASO/siRNA knock-down
14	ENSG00000270055	lncRNA	32	up in TME-high	0.73	0.42	1.4e-13	2.01	ASO/siRNA knock-down
15	ENSG00000232533	lncRNA	32	up in TME-high	0.99	0.46	7.1e-13	1.88	ASO/siRNA knock-down

16	ENSG00000233901	lncRNA	32	up in TME-high	0.95	0.48	9.7e-13	1.65	ASO/siRNA knock-down
17	ENSG00000287255	lncRNA	32	up in TME-high	0.92	0.51	5.7e-12	1.66	ASO/siRNA knock-down
18	ENSG00000234380	lncRNA	32	up in TME-high	0.71	0.53	7.6e-12	1.8	ASO/siRNA knock-down
19	ENSG00000266970	lncRNA	32	up in TME-high	0.96	0.5	8.9e-12	1.56	ASO/siRNA knock-down
20	hsa-mir-23a	miRNA	32	up in TME-high	1.06	0.41	2.2e-06	1.53	antago-mir/inhibitor

Table 3. Delivery-aware shortlist of prioritised ncRNA targets.

Rank	Feature	Class	Final score	Evidence score	Direction	Therapeutic strategy	Delivery concept	Likely source compartment
1	ENSG00000250742	lncRNA	35	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
2	ENSG00000237181	lncRNA	34	26	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
3	ENSG00000254545	lncRNA	34	26	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
4	ENSG00000248996	lncRNA	34	26	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
5	hsa-mir-155	miRNA	34	24	up	antagomir/inhibitor	Hypoxia-responsive BBB carrier	TAM/microglia-rich
6	ENSG00000282851	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
7	ENSG00000257702	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
8	ENSG00000276855	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
9	ENSG00000229124	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
10	ENSG00000203644	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich

11	ENSG00000265743	lncRNA	33	25	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
12	ENSG00000234883	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
13	ENSG00000234546	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
14	ENSG00000270055	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
15	ENSG00000232533	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
16	ENSG00000233901	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
17	ENSG00000287255	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
18	ENSG00000234380	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
19	ENSG00000266970	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
20	ENSG00000233038	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
21	ENSG00000287263	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
22	ENSG00000184809	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
23	ENSG00000226791	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
24	ENSG00000271646	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
25	hsa-mir-23a	miRNA	32	24	up	antagomir/inhibitor	Hypoxia-responsive BBB carrier	TAM/microglia-rich
26	ENSG00000264456	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
27	ENSG00000255521	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich

28	ENSG00000244459	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
29	ENSG00000233621	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
30	ENSG00000270504	lncRNA	32	24	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
31	ENSG00000267147	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
32	ENSG00000235151	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
33	ENSG00000233429	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
34	ENSG00000214106	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
35	ENSG00000256802	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
36	ENSG00000255874	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
37	ENSG00000240875	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
38	ENSG00000245067	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
39	ENSG00000273341	lncRNA	31	23	up	ASO/siRNA knockdown	Myeloid/TAM exosome or nanoparticle	TAM/microglia-rich
40	ENSG00000254389	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
41	ENSG00000253210	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
42	ENSG00000237424	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
43	ENSG00000186594	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
44	ENSG00000272691	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich

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45	ENSG00000265206	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
46	ENSG00000257585	lncRNA	31	23	down	lncRNA restoration / pathway rescue	Hypoxia-responsive BBB carrier	TAM/microglia-rich
47	ENSG00000227039	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
48	ENSG00000259004	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
49	ENSG00000270210	lncRNA	31	23	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich
50	ENSG00000276980	lncRNA	30	26	up	ASO/siRNA knockdown	Hypoxia-responsive BBB carrier	TAM/microglia-rich

Discussion

This integrative analysis of TCGA-GBM and TCGA-LGG shows that the glioma TME behaves as a coordinated programme in which the myeloid, mesenchymal and hypoxia axes are jointly activated. The strength of the myeloid-mesenchymal coupling is consistent with the established association of tumour-associated macrophage infiltration with the mesenchymal cellular state and with the recruitment of immunosuppressive, monocyte-derived macrophages during proneural-to-mesenchymal transition (3,6,7). The convergence of hypoxia onto this axis pair aligns with hypoxia-driven, HIF-1-dependent M2 polarisation of glioma macrophages and its links to angiogenesis and temozolomide resistance (8,9,10). These parallels support the interpretation of our TME-high state as the bulk-transcriptomic correlate of the macrophage-rich, mesenchymal glioma phenotype consistent with a biologically coherent TME state.

The compact ncRNA module that marks this state is biologically plausible and concordant with prior work. miR-155 is a canonical immune- and macrophage-associated microRNA with established roles in glioma immune regulation (24), and several module-as-

sociated lncRNAs echo previously reported TME- and mesenchymal-transition-related signatures in glioma (13,15,18,19). Notably, the gene symbols of the leading features reinforce this interpretation: one of the most strongly survival-associated lncRNAs is MIR155HG, the host transcript of miR-155, directly linking the lncRNA and miRNA arms of the module, while VIM-AS1, the antisense transcript of vimentin, ties the module to the canonical mesenchymal marker. Individual lncRNAs are already known to shape the immunosuppressive GBM microenvironment directly, including H19-derived products, SPI1-driven MIR222HG and HOXC-AS3 (13,14,15), and to act through hypoxia-linked axes such as OR7E156P/miR-143/HIF1A (16). The adverse prognostic value we observed is likewise consistent with the worse outcome long associated with the mesenchymal, macrophage-rich phenotype (6,7) and with the elevated, prognostically unfavourable expression reported for module members such as HOTAIR (17). Crucially, because GBM and LGG differ substantially in grade, IDH status and clinical behaviour, the cross-cohort concordance we observed argues that the module reflects a grade-spanning property of the glioma TME rather than a cohort-specific feature.

Our ceRNA results provide a mechanistic hypothesis linking the module to a specific downstream pathway. Under the competing-endogenous-RNA model (23), the LINC02381-miR-155/miR-23a hub is expected to de-repress shared target genes, and the enrichment of Notch signalling through DLL1 nominates a testable axis connecting ncRNA dysregulation to a pathway with well-described roles in glioma stemness and the perivascular niche. miR-155 and miR-23a are credible nodes for such a circuit given their roles in glioma immune modulation and macrophage biology (24), and the broader relevance of extracellular-vesicle-associated miRNAs, including miR-21, to glioma intercellular communication and BBB-permeable signalling (26). We emphasise that these edges are correlation-supported and use relaxed thresholds; they are prioritisation hypotheses rather than validated physical interactions.

A distinguishing feature of this work is the explicit coupling of target discovery to therapeutic deliverability. Because the prioritised ncRNAs localise to the TAM/microglia and hypoxic-necrotic compartments, and because the BBB is the dominant obstacle to nucleic-acid therapeutics in glioma, each candidate was annotated with a compartment-matched delivery concept. This framing reflects the central lesson of the glioma nucleic-acid-therapeutics literature, that therapeutic efficacy is inseparable from the sophistication of the delivery platform, where hypoxia-responsive carriers, lipid nanoparticles and engineered exosomes are leading strategies (25,26,27). By pairing robust, cross-cohort target prioritisation with such concepts, the shortlist is intended to be directly actionable for preclinical testing of antisense, RNA-interference and antagomir approaches.

Several limitations should be acknowledged. The TME axes and ceRNA edges are inferred from bulk expression, so cell-of-origin assignment and direct molecular interactions require single-cell and experimental validation; IDH/MGMT-aware modelling and independent cohorts (for example, CGGA) were not incor-

porated in the present build; and the delivery concepts are conceptual annotations rather than validated formulations. These constraints define a clear validation pathway: single-cell deconvolution of the candidate compartments, experimental perturbation of the ceRNA and Notch axes, and replication in independent glioma cohorts. The analytical framework used here, integrating standardised transcriptomic preprocessing, signature scoring, association testing and computational prioritisation, mirrors approaches that have proven robust for multi-omic biomarker discovery and for translational genomics in defined patient populations (12,29,30,31,32,33), and is readily extensible to such validation as glioma molecular taxonomy continues to mature (1,3).

Conclusion

We set out to identify cross-cohort ncRNA regulators of the immunosuppressive glioma TME and to prioritise those with therapeutic deliverability. Integrating TCGA-GBM and TCGA-LGG, we found that the myeloid, mesenchymal and hypoxia axes form a co-activated TME-high state, and that a compact, replicated module of lncRNAs and the miRNAs miR-155 and miR-23a marks this state, correlates with it at rho up to 0.70, and is associated with shorter overall survival (hazard ratios 1.5-2.2). Expression-supported ceRNA analysis nominated LINC02381 as a hub linking miR-155/miR-23a to Notch (DLL1) signalling, and integrative prioritisation produced a 50-target, BBB-aware, delivery-aware shortlist mapped to the TAM/microglia and hypoxic compartments. The principal limitation is that associations are derived from bulk data and remain to be validated experimentally and in independent cohorts. By uniting robust, cross-cohort target discovery with compartment-aware delivery concepts, this work provides a prioritised and testable foundation for ncRNA-directed therapeutics against the glioma microenvironment.

Declarations

Ethics Statement: This study used pub-

licly available, de-identified TCGA data and did not involve direct recruitment of human participants or animal experimentation; therefore, institutional ethical approval was not applicable.

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Conflict of Interest

The author declares no conflict of interest.

Data Availability

All primary data are publicly available through the NCI Genomic Data Commons (TCGA-GBM and TCGA-LGG). Derived result tables and figure-generation code are available from the corresponding author on reasonable request.

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