Research Square

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Single-cell and spatial transcriptomics dissect the prognosis and vascular association of a disulfidptosis gene-set score in glioblastoma

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Abstract

Purpose.

Disulfidptosis is a glucose-starvation-induced regulated cell death, but its prognostic and microenvironmental relevance in glioblastoma (GBM) is unknown. We therefore enumerated the complete disulfidptosis gene pool, defined a prognostic gene-set score (DSscore), and resolved its molecular, immune and cell-type/spatial associations in public bulk, single-cell and spatial data.

Methods.

From all publicly accessible, non-lncRNA disulfidptosis genes (38-gene pool, 100% measurable), univariate Cox regression in the CGGA discovery cohort (n = 693) identified 27 prognostic genes, and each sample was scored by single-sample gene-set enrichment analysis against the full transcriptome (DSscore). Prognostic value was validated in four independent cohorts and adjusted for clinical and molecular covariates. Molecular-class, immune-infiltration and hallmark associations were assessed, and the spatial cell-type association was examined by single-cell and RCTD-deconvolved spatial transcriptomics, with a discriminant-validity analysis.

Results.

High DSscore predicted worse survival in CGGA (median OS 470 versus 2982 days; log-rank $P < 0.001$) and remained independent after adjustment for age, WHO grade, recurrence and IDH status (HR 2.31, 95% CI 1.75–3.04). The direction was consistent across four independent cohorts (pooled HR 1.50, 95% CI 1.19–1.90; $I^2 = 4\%$). DSscore was higher in IDH-wildtype, non-1p19q-codeleted and grade-IV tumors and correlated with immune infiltration and with interferon/inflammatory/EMT hallmark programs. Across four Visium slices (11,126 spots), DSscore was enriched in the vascular-lymphangioblast (endothelial) niche, and single-cell analysis showed the highest DSscore in vascular lymphangioblasts, which expressed the disulfidptosis gate SLC7A11/SLC3A2.

Conclusions.

DSscore marks an immune-infiltrated, high-grade GBM state with independent prognostic value and a vascular-associated spatial profile.

1 Introduction

Glioblastoma is the most common and lethal primary brain tumor in adults, with a median overall survival of approximately 14 months despite surgery, radiotherapy, and temozolomide [1–3]. Its aggressive phenotype is driven by marked intratumoral heterogeneity and a deeply immunosuppressive

tumor microenvironment [4–6], both of which limit the durability of current therapies. There is therefore an urgent need to understand the molecular programs that distinguish the most aggressive, immune-evasive tumor states and to identify quantifiable markers of these states.

Disulfidptosis is a recently defined form of regulated cell death triggered by glucose starvation, entered through the cystine transporter SLC7A11 and executed through disulfide stress on the actin cytoskeleton [7]. In contrast to the better-studied death programs such as ferroptosis and cuproptosis [8–10], disulfidptosis is defined by a small, actin-cytoskeleton- and metabolic-centric gene set, and no MSigDB pathway currently captures it [11]. Crucially, many disulfidptosis genes are simultaneously expressed by both malignant cells and by the abundant immune and stromal cells of the GBM microenvironment, and the same actin-cytoskeleton machinery that executes disulfidptosis is also central to immune-cell motility and endothelial biology. This raises a specific, unresolved question: when a disulfidptosis score correlates with immune infiltration or with outcome in bulk tissue, is that association carried by tumor cells, by non-tumor cells, or by a mixture [12,13]? Neither the identity of the gene pool nor the cell-type origin of the score has been established in GBM, and no spatial resolution of a disulfidptosis score has been reported for this tumor [14,15].

Here we quantify disulfidptosis status by ssGSEA of a Cox-selected disulfidptosis gene set, then dissect its meaning in GBM. We first enumerate the complete disulfidptosis gene pool, build a survival-linked score, and validate it in four independent cohorts with formal meta-analysis. We then characterize its molecular-class and immune associations, its hallmark-pathway signatures, and its cell-type origin at single-cell and spatial resolution, including sensitivity analyses for the score's module composition and scoring implementation. Finally, we localize the score across four deconvolved Visium slices to resolve which cell types carry it in tissue.

2 Methods

2.1 Data collection and processing

Transcriptome and clinical data for glioblastoma were obtained from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn) using the mRNAseq_693 cohort, 2020-05-06 release [16]. The cohort contained 693 samples with expression data; 657 samples with overall survival greater than 0 and complete survival annotation were used for survival analyses, 609 samples with non-missing IDH genotype were used for molecular-class analyses, and 608 complete cases entered the IDH-adjusted multivariable model. Four independent cohorts were used for external validation: TCGA-GBM (n = 152) [17], GSE4412 (n = 85), GSE4271 (n = 77) and GSE7696 (n = 80), obtained from the GDC data portal and the Gene Expression Omnibus. Microarray cohorts were annotated from probe identifiers with the hgu133plus2.db and hgu133a.db packages, probes were collapsed to gene symbols by the mean, and expression was log2-transformed before scoring. Kaplan-Meier time units were used as provided by each source. Processed single-cell transcriptome data for GBM were obtained from CELLxGENE and comprised 3,589 cells from 4 donors profiled by Smart-seq2. Spatial transcriptome data comprised four

10x Genomics Visium slices: Parent (3,468 spots), MGH258 (2,393 spots), ZH8811Abulk (2,485 spots) and ZH916T1 (2,780 spots, GEO GSE237183), for a total of 11,126 spots [18]. Genetic-dependency data were obtained from DepMap 26Q1 Public, comprising the Post-Chronos gene-effect matrix and CCLE expression [19,20], and drug-sensitivity data were obtained from CTRP v2 and GDSC release 8.5 [21,22]. All datasets were publicly available and de-identified, and no ethics approval was required.

2.2 Construction of the disulfidptosis gene-set score (DSscore) model

Six publicly accessible, non-lncRNA disulfidptosis gene sets were curated from the literature: canonical actin 15 genes, actin-ML 9 genes, metabolic 9 genes, metabolic subset 5 genes, no-overlap 11 genes and lysyl-oxidase 3 genes [7,11,23–26]. LncRNA signatures and sets whose complete gene lists were inaccessible were excluded, and the six sets were unioned to 38 unique genes, all of which were measurable in every expression matrix. Table 1 lists each gene symbol, its functional annotation, its univariate hazard ratio and direction, whether it entered the score, and its source PMID. Univariate Cox regression of overall survival was performed for the 38 genes in the CGGA cohort with the R package survival version 3.8.6, which identified 27 prognosis-associated genes, comprising 23 risk and 4 protective genes; the remaining 11 genes were not significant and were excluded. DSscore was defined as the single-sample gene-set enrichment score of the 27 genes computed against the full transcriptome background with the ssgseaParam implementation of the R package GSVA version 2.6.3, using the Barbie single-sample method and $\alpha = 0.25$ [27,28]. SLC7A11 was not prognosis-associated and did not enter the score. Because data-driven cutpoint search can inflate effect estimates and produce cohort-specific thresholds, samples were grouped by a pre-specified median split within each cohort rather than by surv_cutpoint, and no optimal-cutpoint search was performed [29]. Single-cell and spatial scores were computed with a per-gene counts-per-million log1p Z-mean implementation; agreement with the bulk ssGSEA implementation was assessed in CGGA and within Visium slices, and the spatial result was re-derived with per-slice full-background ssGSEA as a robustness check.

Table 1
Disulfidptosis-related genes used in this study.

Symbol	Function	HR	Direction	In_DSscore	Source_PMID
INF2	Actin-cytoskeleton	0.94 (0.89–1.00)	Protective**	Yes	36747082
ACTN4	Actin-cytoskeleton	1.63 (1.46–1.82)	Risk**	Yes	36747082
CAPZB	Actin-cytoskeleton	1.20 (1.08–1.33)	Risk**	Yes	36747082
CD2AP	Actin-cytoskeleton	1.57 (1.34–1.83)	Risk**	Yes	36747082
FLNA	Actin-cytoskeleton	1.18 (1.12–1.25)	Risk**	Yes	36747082
FLNB	Actin-cytoskeleton	1.27 (1.11–1.45)	Risk**	Yes	36747082
IQGAP1	Actin-cytoskeleton	1.70 (1.55–1.87)	Risk**	Yes	36747082
MYL6	Actin-cytoskeleton	1.12 (1.04–1.22)	Risk**	Yes	36747082
MYH9	Actin-cytoskeleton	1.57 (1.43–1.73)	Risk**	Yes	36747082
PDLIM1	Actin-cytoskeleton	1.36 (1.28–1.44)	Risk**	Yes	36747082
TLN1	Actin-cytoskeleton	1.58 (1.41–1.78)	Risk**	Yes	36747082
ACTB	Actin-cytoskeleton	1.05 (0.99–1.12)	n.s.	No	36747082
DSTN	Actin-cytoskeleton	0.99 (0.93–1.05)	n.s.	No	36747082
MYH10	Actin-cytoskeleton	1.07 (0.97–1.19)	n.s.	No	36747082
SLC7A11	Actin-cytoskeleton	0.94 (0.84–1.04)	n.s.	No	36747082
LOXL1	Lysyl-oxidase	1.38 (1.30–1.46)	Risk**	Yes	41969489
LOXL4	Lysyl-oxidase	1.32 (1.21–1.44)	Risk**	Yes	41969489
SP6	Lysyl-oxidase	1.46 (1.28–1.66)	Risk**	Yes	41969489

Symbol	Function	HR	Direction	In_DSscore	Source_PMID
NDUFA11	Metabolic-mitochondrial	0.94 (0.89–0.99)	Protective**	Yes	38162649
RPN1	Metabolic-mitochondrial	1.70 (1.44–2.00)	Risk**	Yes	38162649
GYS1	Metabolic-mitochondrial	1.75 (1.51–2.02)	Risk**	Yes	38162649
SLC3A2	Metabolic-mitochondrial	1.05 (0.92–1.19)	n.s.	No	38162649
NCKAP1	Metabolic-mitochondrial	1.05 (0.96–1.15)	n.s.	No	38162649
LRPPRC	Metabolic-mitochondrial	0.94 (0.84–1.06)	n.s.	No	38162649
OXSM	Metabolic-mitochondrial	1.12 (0.98–1.28)	n.s.	No	38162649
NDUFS1	Metabolic-mitochondrial	1.05 (0.94–1.18)	n.s.	No	38162649
NUBPL	Metabolic-mitochondrial	0.97 (0.80–1.18)	n.s.	No	39993959
SLC25A23	Other	0.77 (0.71–0.84)	Protective**	Yes	39692962
SGCD	Other	0.78 (0.66–0.92)	Protective**	Yes	39692962
CENPH	Other	1.44 (1.30–1.59)	Risk**	Yes	39692962
FNDC3B	Other	1.65 (1.50–1.81)	Risk**	Yes	39692962
MGAT2	Other	1.40 (1.26–1.55)	Risk**	Yes	39692962
FAM126A	Other	1.54 (1.41–1.68)	Risk**	Yes	39692962
EVI2B	Other	1.29 (1.18–1.42)	Risk**	Yes	39692962
SNX10	Other	1.21 (1.13–1.29)	Risk**	Yes	39692962
CPM	Other	1.34 (1.24–1.44)	Risk**	Yes	39692962
ARC	Other	1.28 (1.21–1.36)	Risk**	Yes	39692962

Symbol	Function	HR	Direction	In_DSscore	Source_PMID
UBE2D2	Other	1.08 (0.95–1.23)	n.s.	No	39692962

2.3 Survival analysis and multivariable Cox regression analysis

The R package survival version 3.8.6 was used for all survival analyses, and Kaplan-Meier curves were drawn with the R package survminer version 0.5.2 and compared by the log-rank test. Cox proportional-hazards models were fitted with DSscore as a continuous variable and reported per standard deviation (SD), and the pre-specified median split was used for Kaplan-Meier displays. Multivariable models adjusted for age, WHO grade and primary versus recurrent status, and were then additionally adjusted for IDH and 1p19q codeletion status. Nested models were compared by likelihood-ratio test and the concordance index. Hazard ratios were pooled across cohorts by DerSimonian-Laird random effects on the log-hazard-ratio scale [30], with standard errors derived from the reported 95% confidence intervals; heterogeneity was summarized by I^2 and a 95% prediction interval. The four-cohort external estimate was prespecified as the primary pooled result, and the five-cohort estimate including the discovery cohort was reported separately because within-cohort gene selection inflates the discovery effect.

2.4 Sensitivity and discriminant-validity analyses

To test whether the prognostic signal depended on gene selection, full-background ssGSEA scores were recomputed for the unselected 38-gene pool, the 11-gene actin module, a 16-gene non-actin module and a 15-gene variant excluding ARC, and each score was tested by Cox regression in CGGA under the same pre-specified median split. To test whether DSscore reads generic vascular, stromal or EMT content rather than a disulfidptosis-specific program, endothelial, stromal and EMT marker scores were derived [31] and a generic first principal component of the transcriptome was computed; DSscore was re-tested per SD in Cox models that included each covariate, and multicollinearity was assessed by the variance inflation factor. The 27-gene core score was tested univariately and after adjustment for the same covariates. Partial Spearman correlations between DSscore and immune infiltration were computed after conditioning on generic vascular and stromal content, and partial correlations between DSscore and vascular-lymphangioblast fraction were computed per spatial slice after conditioning on a minimal endothelial marker set of PECAM1, VWF and KDR or on glial markers. Mediation of the survival effect by vascular, immune and EMT covariates was assessed by the product method with 500 bootstrap resamples, reporting total, direct and indirect log-hazard contributions [32].

2.5 Analysis of immune, pathway and molecular-class features

Immune infiltration was estimated as the mean expression of MCPcounter marker genes per sample [33,34]. Immune abundances were compared between DSscore groups by the Wilcoxon rank-sum test and correlated with DSscore by Spearman correlation. Cytolytic activity was defined as the geometric

mean of GZMA and PRF1 expression. Eleven immune-checkpoint and interferon genes were correlated with DSscore by Spearman correlation with Benjamini-Hochberg adjustment across the 11-gene family [35]. Pathway enrichment used prerank gene-set enrichment analysis implemented in gseapy version 1.3.0, ranking genes by their Spearman correlation with DSscore and testing against MSigDB Hallmark gene sets [36]; these results are descriptive. IDH mutation, 1p19q codeletion and MGMT promoter methylation status were taken from CGGA clinical annotation; DSscore was compared between molecular classes by the Mann-Whitney test for continuous scores and between DSscore groups by the chi-square test for categorical frequencies, with samples of missing genotype excluded.

2.6 Single-cell and spatial transcriptome analysis

For single-cell data, per-cell DSscore and actin and metabolic module scores were computed by the per-gene counts-per-million log1p Z-mean method and aggregated by annotated cell type; per-type cell numbers and per-donor consistency were reported. SLC7A11 and SLC3A2 expression was summarized per cell type as median log-normalized expression and percentage of positive cells, with Kruskal-Wallis and Mann-Whitney tests. For spatial data, cell-type fractions were estimated by robust cell-type decomposition with the R package spacexr version 1.4.0 [37–39], and DSscore was computed per spot as described above. Because spatial spots are not independent, primary inference used per-slice Spearman correlations combined by the Stouffer method and a linear mixed model with a random slice intercept fitted with statsmodels version 0.14.6 over all 11,126 spots; pooled spot-level tests are descriptive only. Cell-type fractions were centered-log-ratio transformed before correlation analysis to correct for compositional effects, and raw-fraction analyses were retained as robustness checks.

2.7 Spatial ecotype, core-margin gradient and ligand-receptor analysis

All 11,126 spots were clustered jointly by k-means with k = 4, n_init = 20 and a fixed random seed, using six deconvolved cell-type fractions and eight marker-module scores for fibroblast, T cell, macrophage, B cell, hypoxia, proliferation, interferon-gamma and endothelium [40,41], each computed as a per-gene counts-per-million log1p Z-mean. Features were z-scored within each slice to remove batch effects. Ecotype marker genes were defined by the per-spot log2 mean difference versus all other spots, averaged across slices, and bulk samples were projected onto ecotype signatures by the mean Z score of the top 40 markers after excluding ribosomal and mitochondrial genes; each patient was assigned its dominant ecotype. Ecotype association with survival was assessed by Cox models unadjusted, adjusted for age, grade and IDH, and further adjusted for DSscore. The core-margin gradient was defined per slice as the Euclidean distance from each spot to the nearest white-matter ecotype spot, with spots banded into the white-matter ecotype plus distance quintiles and tested by per-slice Spearman correlation with Stouffer combination; SLC7A11 and hypoxia were scored per spot from the same matrices. Cell-cell communication was assessed with a curated 18-pair ligand-receptor panel covering VEGFA, CXCL12, CXCL9/10, CCL2, SPP1, COL1A1, COL4A1, FN1, PDGFB, ANGPT1, DLL4, CD274, LGALS9 and TGFB1 ligands. The interaction score was the mean log-normalized ligand expression in the source cell type

multiplied by the mean receptor expression in the target cell type, with the percentage of positive cells reported.

2.8 Genetic-dependency and drug-sensitivity analysis

Genetic dependency was assessed with the DepMap 26Q1 Public Post-Chronos gene-effect matrix joined to model metadata [19,42]. SLC7A11 dependency in 71 glioma cell lines was compared with all 1,208 lines [43] by median gene effect and the Wilcoxon test; the correlation between SLC7A11 expression and dependency was computed on the 66 lines with matched CCLE expression, and co-dependency of SLC7A11 with SLC2A1 and SLC3A2 was assessed by Spearman correlation. The dependency spectrum of the 27-gene program was summarized across glioma lines and compared with the all-line median. Drug sensitivity was assessed from two independent resources. CTRP v2 area-under-the-curve data for 545 compounds across 596 lines [21] were joined to SLC7A11 gene effect by ACH model identifier, testing the genetic-dependency axis; GDSC release 8.5 fitted log half-maximal inhibitory concentrations [22] for 272 compounds in GDSC2 and 373 compounds in GDSC1 were joined to SLC7A11 CCLE expression by Sanger model identifier, testing the expression axis. Spearman correlations were computed per compound with Benjamini-Hochberg adjustment within each dataset, and the central-nervous-system and glioma subsets were analyzed descriptively. CTRP contains no glioma lines, which is reported as a limitation.

2.9 Statistical analyses

Spearman correlation was used for associations involving expression or score variables, and the Mann-Whitney and Wilcoxon rank-sum tests were used for group comparisons. Multiple comparisons were adjusted by the Benjamini-Hochberg method where a test family was defined [35]; the nine MCPcounter abundances were presented descriptively without family-wide control, whereas the 11-gene checkpoint family and the drug-sensitivity panels were adjusted. All tests were two-sided and $P < 0.05$ was considered significant. Hazard ratios greater than 1 with $P < 0.05$ indicated risk factors, hazard ratios less than 1 with $P < 0.05$ indicated protective factors, and $P > 0.05$ indicated non-significant factors. The proportional-hazards assumption was not formally tested, which is reported as a limitation. Analyses were performed in R version 4.6.1 with GSVA version 2.6.3, survival version 3.8.6, survminer version 0.5.2 and spacexr version 1.4.0, and in Python version 3.12.10 with statsmodels version 0.14.6, scipy version 1.16.3, scikit-learn version 1.8.0, gseapy version 1.3.0 and lifelines version 0.3.

3 Results

3.1 The disulfidptosis gene pool is small but fully enumerated, and a Cox-selected ssGSEA score (DSscore) is defined

Six publicly accessible, non-lncRNA disulfidptosis gene sets were curated and unioned to 38 unique genes, all of which were measurable in CGGA, TCGA, single-cell, and Visium data (100% coverage). The disulfidptosis gene pool is literature-defined, and no MSigDB disulfidptosis pathway currently exists; every excluded set is documented (Table 1) [7,11,26]. Univariate Cox regression across the 38-gene pool

in CGGA (n = 693) identified 27 genes with a significant effect on overall survival (23 risk, 4 protective; Fig. 1A, B). We defined DSscore = ssGSEA(27 prognosis-associated genes) per sample, computed with the GSVA package against the full transcriptome. We read DSscore as a quantitative measure of curated disulfidptosis gene-set activity; how specifically it isolates the disulfidptosis program is addressed by the module-decomposition and parallel-score analyses below.

3.2 DSscore predicts worse survival in the discovery cohort and with a consistent direction across four independent cohorts

The direction was consistent in all four independent cohorts (Fig. 1C; Table 2): significantly worse survival with high DSscore in TCGA-GBM (n = 152; median 360 vs 480 d, HR = 1.63, P = 0.009) [17] and GSE4412 (n = 85; 293 vs 684 d, HR = 2.01, P = 0.011), and the same direction in GSE4271 (HR = 1.41, P = 0.18) and GSE7696 (HR = 1.08, P = 0.75), the latter two being small cohorts (n = 77/80) with limited power (Supplementary Fig. S1). Random-effects (DerSimonian–Laird) pooling of the four independent cohorts [30] gave a pooled HR of 1.50 (95% CI 1.19–1.90, P < 0.001) with low heterogeneity (I^2=4%), the transferable estimate we consider most representative; pooling all five cohorts yields 1.86 with I^2=90%, dominated by the CGGA discovery effect. Cohort characteristics are in Table S1 and per-sample scores in Table S2.

Table 2
Association of DSscore with overall survival. (a) CGGA multivariable Cox (n = 608; adjusted for age, WHO grade, primary/recurrent status and IDH)

Variable	HR (95% CI)	P
DSscore (High vs Low)	2.31 (1.75–3.04)	< 0.001
Age (per yr)	1.01 (1.00–1.02)	0.009
Grade WHO III vs II	2.56 (1.82–3.58)	< 0.001
Grade WHO IV vs II	3.88 (2.71–5.55)	< 0.001
Recurrent vs Primary	1.89 (1.53–2.34)	< 0.001
IDH mutant vs wildtype	0.70 (0.54–0.92)	0.011

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a. Multivariable Cox model in CGGA (n = 608; adjusted for age, WHO grade, primary/recurrent status, and IDH)

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HR, hazard ratio; CI, confidence interval; P values are Wald tests; IDH-mutant HR < 1 = protective (IDH-wildtype is the reference).

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(b) Univariate DSscore (high vs low) per independent cohort and random-effects pooling

Dataset	n	HR (95% CI)	P
Pooled (4 external)	–	1.50 (1.19–1.90)	< 0.001
TCGA-GBM	152	1.63 (1.13–2.35)	0.009
GSE4412	85	2.01 (1.18–3.45)	0.011
GSE4271	77	1.41 (0.85–2.34)	0.178
GSE7696	80	1.08 (0.66–1.77)	0.749

In a multivariable Cox model adjusted for age, WHO grade, and primary/recurrent status, DSscore remained an independent risk factor (HR high vs low 2.68, 95% CI 2.12–3.40, P < 0.001). Because DSscore anchors strongly to IDH status [44–47], we additionally adjusted for IDH: DSscore remained independent (n = 608, HR 2.31, 95% CI 1.75–3.04, P < 0.001; Fig. 1D; IDH-mutant HR 0.70, P = 0.011), and further adjustment for 1p19q codeletion left it significant (HR 1.96, 1.48–2.59). As continuous per-SD variable in the IDH-adjusted model, DSscore gave HR 1.42 (1.24–1.63, P < 0.001). On an equal-n subset (n = 608), the no-IDH model gave HR = 2.69 (95% CI 2.10–3.45; P < 0.001) and the IDH-adjusted model HR = 2.31 (1.75–3.04), so the attenuation after IDH adjustment is an IDH effect rather than a sample-reduction artefact; adding IDH significantly improved fit (likelihood-ratio P = 0.010; C-index 0.766→0.770), and DSscore remained significant in all specifications.

High DSscore was associated with markedly worse survival in CGGA (Fig. 1E). In CGGA (n = 657 of 693 with usable survival data; median split pre-specified), patients with high DSscore had a median OS of 470 d versus 2982 d (log-rank P < 0.001; univariate HR for high score 3.97 [95% CI 3.20–4.92]). Because the 27 genes were selected within CGGA, this within-CGGA estimate is expected to be inflated by selection and we present it as a discovery-cohort effect size. Per-cohort KM curves for the three significant cohorts are shown in Fig. 1E-G.

Two sensitivity analyses addressed the gene-selection step. First, an unselected score over the full 38-gene pool reproduced the CGGA association (median-split HR 3.22, 95% CI 2.61–3.97, P < 0.001), indicating that the signal is not created by the selection step. Second, decomposing the 27 genes into the actin-cytoskeleton module (11 genes) and the remaining non-actin module (16 genes) showed that both modules individually carry the prognostic association (actin module HR 3.73; non-actin module HR 3.78; excluding the actin-binding gene ARC: HR 3.20; all P < 0.001; Spearman ≥ 0.92 with DSscore; Supplementary Fig. S2). Critically, DSscore remained independently prognostic after adjusting for generic endothelial, stromal and EMT scores (continuous per SD, HR 2.12, 95% CI 1.85–2.44, P < 0.001; adjusted for a single composite of the three generic scores, HR 2.02, P < 0.001; variance-inflation factors < 5), indicating that the survival signal does not simply report generic vascular/stromal content. Mediation analysis showed that none of these programs significantly mediated the survival effect (indirect effects near zero, bootstrap 95% CIs including zero). A stripped score of the 11 non-cytoskeletal, non-ECM genes remained prognostic univariately (per SD HR 1.65, 95% CI 1.46–1.87, P < 0.001) but did not retain clear independence after adjustment (HR 1.19, 95% CI 0.96–1.48, P = 0.115), so the survival association is carried predominantly by the cytoskeletal/ECM component. The CGGA signal was also not a grade-composition artifact: within WHO-IV tumors alone, high DSscore remained predictive (HR = 1.55, P = 0.0024), and it stratified survival within both IDH-mutant (HR = 3.12, P < 0.001) and IDH-wildtype (HR = 1.86, P < 0.001) subgroups.

3.3 DSscore marks a high-grade, IDH-wildtype molecular class

DSscore was higher in molecular classes with poorer prognosis (Fig. 2A) [44,46,47]. Categorically, among the 609 tumors with known IDH genotype, the High-DSscore group contained 235 IDH-wildtype versus 94 IDH-mutant tumors, whereas the Low-DSscore group contained 239 IDH-mutant versus 41 IDH-wildtype tumors (chi-square $P < 0.001$; Fig. 2B). IDH-wildtype tumors had a higher DSscore than IDH-mutant tumors (+ 1.22 vs + 0.98, $P < 0.001$; Fig. 2C); WHO grade IV was higher than grade II (+ 1.21 vs + 0.96, Kruskal $P < 0.001$); and non-1p19q-codel tumors were higher than codel tumors (+ 1.11 vs + 0.89, $P < 0.001$). MGMT-methylated and unmethylated groups did not differ significantly ($P = 0.064$; Supplementary Fig. S3C). The four IDH-by-DSscore classes separated survival into a graded series from IDH-mutant/low-DSscore to IDH-wildtype/high-DSscore (worst; Fig. 2D, E). DSscore therefore anchors to the standard glioma molecular axis while carrying prognostic information beyond it.

3.4 DSscore is immune-associated and enriched for inflammatory/mesenchymal pathways

DSscore correlated positively with immune infiltration and with immune/inflammatory programs. Hallmark gene-set enrichment on DSscore-correlated genes, run separately per cohort (Fig. 3A) [36], showed consistent enrichment for interferon-γ response (mean NES + 3.02, positive in 5/5 cohorts), TNF-α signaling via NF-κB (+ 2.95, 5/5), IL-6/JAK/STAT3 (+ 2.73, 5/5), inflammatory response (+ 2.72, 5/5), interferon-α response (+ 2.70, 5/5), allograft rejection (+ 2.67, 5/5), and epithelial–mesenchymal transition (+ 2.54, positive in 4/5; the exception was TCGA-GBM). Because this prerank design uses DSscore-correlated genes, the enrichment is a descriptive characterization of the score's covariance structure rather than an independent test. Using MCPcounter, all nine immune or stromal abundances [33] were positively associated with DSscore; fibroblasts ($\rho = 0.75$), monocytes (0.48), endothelial cells (0.48), neutrophils (0.41), T cells (0.37), myeloid dendritic (0.36), cytotoxic lymphocytes (0.35), B cells (0.21), and NK cells (0.13); each was more abundant in the High- than the Low-DSscore group (Wilcoxon $P < 0.001$ for all; Fig. 3B; Supplementary Fig. S4). Across all eleven immune-checkpoint and interferon-associated genes tested (BH-adjusted) [48], CXCL10 ($\rho = 0.58$), CXCL9 (0.52), PDCD1 (0.52), TNFRSF9 (0.51), ICOS (0.46), HAVCR2 (0.45), CD274 (0.45), and CD276 (0.45) correlated strongly; TIGIT ($\rho = 0.18$, $q < 0.001$) and CTLA4 ($\rho = 0.17$, $q < 0.001$) correlated weakly but significantly; only LAG3 was not significant ($\rho = -0.06$, $P = 0.12$; Fig. 3C). A cytolytic CYT score was positively associated ($\rho = 0.45$; Fig. 3D). The immune-program association replicated in every cohort while the survival effect varied in magnitude, so the High-DSscore state is best read as an immune-inflamed, checkpoint-active phenotype; the observation that the following cell-origin analysis sets out to resolve.

3.5 The score is associated with a vascular profile but its survival signal is not explained by generic vascular/stromal/EMT content

We resolved which cells carry the DSscore at single-cell and spatial resolution. In single cells (3,589 GBM cells from 4 donors, Smart-seq2; Fig. 4A; Supplementary Fig. S5) [12,13], the DSscore overlay (Fig. 4B) was highest in vascular lymphangioblasts (VL; n = 51 cells, + 0.23) and neoplastic cells (n = 1,091, + 0.05), intermediate in neurons (n = 21) and myeloid cells (n = 1,847), and lowest in oligodendrocyte

precursor cells (− 0.07), astrocytes (− 0.13), and oligodendrocytes (− 0.20) (Fig. 4C); VL was the highest-scoring cell type within every donor (4/4). Decomposing the score into its constituent modules (Fig. 4D), the actin-cytoskeleton component was vascular/endothelial-enriched (VL + 0.43, versus astrocytes − 0.22 and oligodendrocyte precursor cells − 0.14, with myeloid cells near zero at + 0.02), whereas the metabolic component was tumor/neuronal-intrinsic (neoplastic cells + 0.21, neurons + 0.45, versus myeloid cells − 0.15), based on per-gene Z-scores within single cells.

The defining gate of disulfidptosis was itself expressed in the VL compartment [7,43,49]: SLC7A11 was detectable in 39% of VL cells (versus 28% of neoplastic cells; Mann–Whitney P = 0.018) and SLC3A2, the xCT chaperone of SLC7A11, had the second-highest expression of all seven cell types in VL (median 2.09 versus 2.06 in neoplastic cells). Astrocytes expressed SLC7A11/SLC3A2 most strongly (88 cells) yet carried one of the lowest DSscores, so gate expression alone does not produce a high score; rather, the VL compartment combines gate expression with the highest actin-program activity.

Across four Visium slices (Parent 3,468; MGH258 2,393; ZH8811Abulk 2,485; ZH916T1 2,780 spots; 11,126 total) deconvolved by RCTD and analyzed with composition-corrected (CLR-transformed) cell fractions [37], the DSscore was enriched in the vascular-lymphangioblast (endothelial) niche in all four slices [14,15,50] (ρ=+0.29, + 0.18, + 0.24, + 0.30; Fig. 4E). Because spots are not independent observations, we made stratified inference primary: a mixed-effects model over all 11,126 spots (DSscore ~ VL fraction + random slice intercept) gave β = 0.26, P < 0.001; the Stouffer combination of the four per-slice rank correlations gave Z = 23.3 (P < 0.001); and per-slice Spearman correlations were all highly significant (each P < 0.001), whereas the dichotomized (per-slice median-split) contrast was marginal in ZH916T1 (P = 0.036, not significant after Bonferroni correction for four tests); ZH916T1 nonetheless carried the strongest continuous rank correlation (ρ=+0.30). Using raw (untransformed) RCTD fractions instead of CLR gave the same direction in 4/4 slices (ρ=+0.35, + 0.13, + 0.22, + 0.14). The effect size is modest: VL-high spots (per-slice median split) had median DSscore + 0.058 versus − 0.012 (Fig. 4F). The associations with myeloid cell (ρ=+0.26 to − 0.14) and neoplastic cell (− 0.04 to + 0.20) fractions were directionally inconsistent across slices and were therefore not treated as findings. To test whether the vascular association is specific to a disulfidptosis program or shared with constitutive endothelial biology, we repeated the correlation on CLR-transformed VL fractions controlling for a minimal endothelial-marker baseline (PECAM1, VWF, KDR): the DSscore–VL correlation attenuated from ρ=+0.29/+0.18/+0.24/+0.30 to + 0.17/+0.11/+0.17/+0.02, remaining positive in all four slices, and strengthened when a non-endothelial (glial) control was used instead (+ 0.41/+0.25/+0.33/+0.48). A stripped score of the 11 non-cytoskeletal, non-ECM genes, however, did not localize to the endothelial compartment (ρ=+0.02/+0.16/−0.09/+0.06 versus VL). The vascular enrichment is therefore an endothelial-niche-associated profile that is nevertheless carried by the score's cytoskeletal/ECM component rather than by a metabolically-defined disulfidptosis program, and we re-frame the cell-origin finding as a vascular-associated profile rather than a disulfidptosis-specific origin.

Module decomposition of the spatial signal showed that neither module alone reproduces it: the actin module correlated with the VL niche in MGH258 (+ 0.18) and ZH8811Abulk (+ 0.14) but not in Parent (+

0.02) or ZH916T1 (+ 0.05), and the non-actin module was inconsistent (+ 0.08, − 0.02, + 0.00, − 0.16). The full 27-gene score, which integrates both modules, gave the stable association. In bulk, both modules were individually prognostic; in space, the endothelial association is a property of the combined program, most plausibly the balance between actin-cytoskeleton and metabolic activity; rather than of either module alone.

Because the bulk score uses full-background ssGSEA while single-cell/spatial scores use per-gene Z-means, we quantified implementation agreement. Within slices, the two implementations correlated tightly (Spearman ρ = 0.79–0.89). A full-background ssGSEA recomputation of the 27-gene score on each slice reproduced significantly higher VL fraction in high-score spots in 4/4 slices (per-slice Mann-Whitney $P < 0.001$ for all), although the continuous rank correlation was positive in 3/4 slices (ρ = 0.09–0.16) and slightly negative in ZH916T1 (− 0.056). In bulk CGGA, ssGSEA and Z-mean implementations correlated at ρ = 0.68. The cell-origin conclusion is therefore robust to the median-split formulation of either implementation, while the continuous spatial gradient is implementation-dependent; we report the Z-mean implementation throughout and flag this as a boundary of interpretation.

3.6 Spatial ecotypes resolve the niche into four recurrent tissue states

To place tumor, immune, stromal, and vascular cells in one spatial framework, we clustered all 11,126 spots across the four slices on joint cell-type composition and marker-module activity [15,51] (fibroblast, T-cell, macrophage, B-cell, hypoxia, proliferation, interferon, endothelium; per-slice z-scoring to remove batch; k = 4, stable across slices; Fig. 5A, B; Supplementary Fig. S6). The four ecotypes were biologically unambiguous and each recurred in all four slices: EC0, white-matter/normal-brain tracts (n = 2,256; PLP1, MBP, OLIG1, BCAN), immune-cold; EC1, hypoxic tumor core (n = 5,475; VEGFA, GAPDH, LDHA, ENO1, VIM; highest neoplastic fraction); EC2, rare immune-vascular aggregates (n = 47; CD19 + B cells, HLA-DRA+ APCs, endothelium; small immune-rich and vascular spot aggregates present in every slice); and EC3, the vascular-mesenchymal niche (n = 3,348; endothelium, mural MYL9 + cells, fibroblast/basement-membrane collagen IV/FN1/DCN). DSscore differed sharply across ecotypes (Kruskal–Wallis $P < 0.001$; Fig. 5C; medians − 0.058, + 0.022, + 0.108, + 0.078 for EC0–EC3): the two vascular ecotypes (EC2, EC3) carried the highest disulfidptosis program, the white matter the lowest.

Using distance from white-matter (EC0) spots as an anatomical axis (Fig. 5D), DSscore increased from white matter (pooled band mean − 0.086) to deep tumor core (+ 0.096), positively in all four slices (per-slice ρ=+0.02 to + 0.57; Stouffer Z = 33.45 over the four DSscore and four VL-fraction tests), in parallel with hypoxia (per-slice ρ=+0.13 to + 0.43) and neoplastic fraction [40]. The vascular-lymphangioblast fraction likewise rose toward the core in 3/4 slices. In contrast, SLC7A11 was not core/margin-graded (direction inconsistent across slices); a niche-localized rather than bulk-graded pattern, which we do not over-interpret. The disulfidptosis program is therefore anchored in the vascularized tumor interior rather than the white-matter invasive front.Projecting each ecotype's top-40 marker signature onto bulk CGGA assigned every patient a dominant ecotype (EC0 n = 321, EC3 n = 184, EC2 n = 95, EC1 n = 93). Dominant

ecotype was strongly prognostic (global log-rank P < 0.001; Fig. 5E) [51]: versus EC0, EC1 gave univariate HR 5.95 (P < 0.001) and EC3 HR 3.01 (P < 0.001). After adjustment for age, grade, and IDH, EC1 (hypoxic core) remained independent (HR 2.63, P < 0.001) and EC3 remained significant (HR 1.58, P = 0.0015). Critically, after further adjustment for DSscore, EC3 and EC2 lost significance (HR 0.92, P = 0.63; HR 1.06, P = 0.74) while EC1 remained independent (HR 1.69, P = 0.0071; Fig. 5F); the vascular-mesenchymal ecotype's prognostic signal is captured by DSscore, whereas the hypoxic tumor core carries risk information beyond it. In TCGA-GBM (all WHO grade IV, n = 152), dominant ecotype was not prognostic (global P = 0.61), consistent with grade compression of ecotype risk within a single-grade cohort. All three tumor-side ecotypes were checkpoint-active in bulk (EC1/EC2/EC3 versus CD274 ρ = 0.45–0.61, GZMA ρ = 0.67–0.77, PRF1 ρ = 0.64–0.77), whereas EC0 was immune-cold (CD274 ρ = 0.05); an internal normal-brain control.

3.7 The vascular niche is a signaling hub: ECM and CXCL12 outputs, VEGF input

A targeted ligand–receptor panel on the single-cell cohort identified the directional wiring of the niche (Fig. 6A). As a source, vascular lymphangioblasts dominated three outputs: (i) extracellular matrix signals to tumor cells; COL1A1–DDR1 (65% of VL cells ligand-positive; 71% of neoplastic cells receptor-positive; top-scoring niche→tumor axis) and FN1–ITGAV/ITGA5 (FN1 expressed in 96% of VL cells); (ii) the canonical perivascular recruitment signal CXCL12 (VL the dominant source by far, 0.89 versus ≤ 0.18 in any other type) to CXCR4 + myeloid cells; and (iii) basal-membrane COL4A1–DDR2 signaling. As a target, the niche received VEGFA from neoplastic and myeloid cells (VEGFA–FLT1/KDR; Fig. 6B), closing the loop with the hypoxic core: the hypoxic tumor state (EC1) produces the angiogenic input [52, 53], and the vascular-mesenchymal state (EC3) both receives it and carries the disulfidptosis program. Myeloid cells formed the other hub (SPP1–CD44 to neoplastic cells, the strongest pair overall; PDGFB–PDGFRB to the niche; CCL2–CCR2 and LGALS9–HAVCR2 autocrine; Fig. 6C), whereas classical checkpoint pairs (CD274–PDCD1, CXCL9/10–CXCR3) were below single-cell detection in this cohort; the checkpoint activity of Fig. 3C is a bulk-level property. The full ligand–receptor expression pattern across cell types is shown in Fig. 6D and Supplementary Fig. S7.

3.8 SLC7A11 is a modest genetic dependency in glioma; its interpretation is bounded by the assay's glucose-replete conditions

In the univariate Cox screen across the 38-gene pool, SLC7A11 expression was not prognostic in CGGA (HR = 0.94, P = 0.22) and was therefore not part of the DSscore: at the bulk-expression level, SLC7A11 marks a state of susceptibility rather than the aggressive executing program selected by the Cox screen. At the perturbation level, SLC7A11 was a significant but modest genetic dependency in glioma cell lines [19,42,43] (median CERES − 0.80, n = 71, versus − 0.74 across all 1,208 DepMap lines; Wilcoxon P = 0.020; Fig. 7A; Supplementary Fig. S8), and its expression correlated weakly negatively with dependency across the 66 lines with matched expression (ρ=−0.15, P = 0.227; Fig. 7B); expression level and dependency are dissociable quantities. SLC7A11 dependency did not correlate positively with either

glucose transport (SLC2A1, ρ=−0.15, P = 0.20; Fig. 7C) or its own transporter partner SLC3A2 (ρ=−0.06, P = 0.64). Because the analysis fails to detect even the known SLC7A11–SLC3A2 partnership, we interpret co-dependency in this panel as uninformative for combination hypotheses, not as evidence against them. Finally, disulfidptosis executes only under glucose deprivation, whereas DepMap lines are cultured in glucose-replete conditions; the observed dependency therefore reflects SLC7A11's baseline cystine-transport and antioxidant function rather than disulfidptosis execution, which raises the question the next section addresses: is the gate's dependency pharmacologically tractable at all?

3.9 SLC7A11-dependent and SLC7A11-high lines are associated with sensitivity to xCT/GPX4 and Hsp90/ER-stress drugs

We then asked whether the gate's dependency is reflected in drug response. In the CTRP pan-cancer matrix (545 compounds, 596 cell lines), the two probe compounds of the xCT/ferroptosis axis behaved exactly as the dependency data predict: erastin (direct SLC7A11/xCT inhibitor) and 1S,3R-RSL-3 (GPX4 inhibitor, downstream of xCT) [8] were both preferentially active in lines with stronger SLC7A11 dependency (AUC versus CERES, ρ=+0.157, P < 0.001, q = 0.008; ρ=+0.124, P = 0.003, q = 0.031; Fig. 7D). In GDSC (release 8.5; 272 GDSC2 and 373 GDSC1 compounds tested, pan-cancer) [22], SLC7A11-high lines were most sensitive to the Hsp90 inhibitor tanespimycin/17-AAG [54] (GDSC2 ρ=−0.148, q = 0.001; GDSC1 ρ=−0.269, q < 0.001), followed by the MEK inhibitor trametinib (GDSC2 ρ=−0.093, q = 0.028) and the ER-stress inducer thapsigargin [55] (GDSC1 ρ=−0.126, q = 0.006; top sensitive hits in Fig. 7E; full ranked tables in Table S3 (drug-sensitivity block)). Within the 38 CNS/glioma lines of GDSC, no single compound reached significance (temozolomide ρ=−0.02, P = 0.90; all |ρ|≤0.18), a power-limited negative we report as such. Across the 27-gene program, the most essential genes in glioma lines were CAPZB, RPN1, TLN1, CENPH and MYH9, but each showed dependency close to the all-lines median, indicating common-essential rather than glioma-selective vulnerability (Fig. 7F). These pharmacological results describe the gate as associated with sensitivity to xCT/GPX4-axis and proteostasis (Hsp90/ER-stress) drugs in pan-cancer screens (hypothesis-generating); while the interpretation boundary remains: these screens measure the cystine-economy and stress axes, not disulfidptosis execution, which requires glucose deprivation. Pathway-level analysis of the GDSC hits sharpened this picture in two directions (hypergeometric test over the annotated compound pathways): the sensitive side showed no pathway-level enrichment; SLC7A11-high vulnerability is compound-specific (the xCT/GPX4 probes and the replicated Hsp90/ER-stress hits), not a class effect of generic ROS-inducing or hypoxia-mimetic compounds, none of which were significant; conversely, the resistant side was significantly enriched for chromatin/HDAC (GDSC1 P = 0.0006), genome-integrity, PI3K/MTOR, and apoptosis-regulation classes; SLC7A11-high lines are broadly stress-adapted and resistant to standard targeted classes, and only compounds that attack their metabolic entry (xCT/GPX4) or proteostasis (Hsp90/ER stress) expose a vulnerability.

4 Discussion

High DSscore in GBM defines an immune-infiltrated, inflammation- and EMT-associated, high-grade molecular state whose prognostic signal survives adjustment for IDH status and replicates directionally in four independent cohorts with a pooled HR of 1.50. At single-cell and four-slice spatial scale the score tracks a vascular-associated profile that persists after controlling for minimal endothelial markers, although this enrichment is carried by the score's cytoskeletal/ECM component rather than by a metabolically-defined disulfidptosis program. Spatially, the score resolves into recurrent tissue states: the vascular-mesenchymal ecotype carries the disulfidptosis program, while the hypoxic tumor core forms a second, independent axis of risk, comprising two spatially coupled compartments connected by VEGF and ECM signaling.

The immune association of the DSscore, and its enrichment for IFN-γ/NF-κB/inflammatory programs, is concordant with an immune-inflammatory high-grade state. The spatial result sharpens this: the score is not merely high wherever there are immune cells but specifically tracks the endothelial-vascular compartment [50], across all four slices, by stratified and mixed-effects inference robust to the non-independence of spatial spots. This argues against a simple tumor-autonomous readout. Discriminant analysis shows that the spatial vascular enrichment is an endothelial-niche association that is nevertheless carried by the score's cytoskeletal/ECM component, whereas the survival signal is independent of generic vascular/stromal/EMT content; the score is therefore best read as an immune- and vascular-associated high-grade program whose prognosis is not reducible to that generic content (Supplementary Fig. S9).

SLC7A11 defines entry into disulfidptosis but is not itself prognostic and is not part of the score [7][43]; the Cox-selected executing program (actin-cytoskeleton plus metabolic genes) carries the outcome association, and both modules are individually prognostic. The single-cell analysis partially reunites the two in the VL compartment: VL cells combine the highest program activity with detectable SLC7A11 (39% of cells) and the second-highest SLC3A2 expression, whereas astrocytes express the gate strongly without carrying the program. Gate expression is therefore necessary-context, not sufficient; the score reads the executing machinery, and the VL niche is where that machinery co-localizes with the gate. The modest SLC7A11 dependency in glioma lines (median CERES − 0.80) should be read as evidence that the gate is rate-limiting for baseline cystine economy in a subset of lines, not as a disulfidptosis vulnerability; disulfidptosis execution requires glucose deprivation, which dependency screens do not model.

Single-cell and spatial transcriptomics are increasingly used to resolve cell-type attribution in solid tumours, as illustrated for renal cancer [56]. The finding that a cell-death score can be immune-associated and prognostic is not unique to disulfidptosis: similar score-based analyses of other regulated death programs, such as ferroptosis and cuproptosis in multiple tumours [9,10], have linked death-program scores to immune infiltration and outcome. Our result refines this in two ways specific to GBM [5,57–59]. First, the disulfidptosis score is a risk factor rather than a protective one, so reading a cell-death score without establishing its direction is incomplete [24,60]. Second, the cell-type association of the score, resolved at spatial resolution with stratified inference, is a vascular-associated profile rather

than a malignant- or myeloid-only one, and the survival signal is independent of generic vascular/stromal content. This specificity, in direction and cell type, is what a death-program score must establish before its immune or tumor attribution is interpreted.

Whether the endothelial-vascular disulfidptosis signature reflects a paracrine vulnerability, and whether it predicts response to anti-angiogenic or metabolic therapy [52,61], are testable with matched protein-level, perturbation, or glucose-deprivation culture data. The general requirement; resolve a curated cell-death gene-list score by cell-type origin before interpreting its immune or tumor attribution; applies to any death-program score computed on immune-infiltrated tissue.

5 Limitations

Dataset sizes and validation status are stated explicitly: this is a secondary analysis of public data (CGGA discovery n = 657 with survival; external cohorts n = 152, 85, 77 and 80; single-cell n = 3,589 cells from four donors; Visium n = 11,126 spots across four slices), and no experimental validation study accompanies it. Entirely in-silico; no wet-lab death assay validates that DSscore measures disulfidptosis mechanistically, and the 27-gene set overlaps actin/ECM biology that is also enriched in vascular and mesenchymal states [31]. Our safeguards are partial rather than conclusive: both gene modules are individually prognostic, the 38-gene unselected score is prognostic, the VL compartment expresses the gate, but we cannot exclude that DSscore partly reads generic vascular/mesenchymal content; discriminant-validity analysis showed that the immune association is substantially a proxy for that generic content (partial $\rho = 0.07$ to 0.18 versus raw 0.45, depending on the covariate structure), that the spatial vascular enrichment persists after controlling for minimal endothelial markers but is carried by the score's cytoskeletal/ECM component, and that the survival signal is independent of generic endothelial/stromal/EMT scores (per SD HR 2.12, $P < 0.001$). The CGGA effect size is inflated by within-cohort gene selection (discovery HR 3.97 versus pooled external HR 1.50); TCGA-GBM and GSE4412 are significant while GSE4271 and GSE7696 are directionally consistent but underpowered (n = 77/80). ICB response could not be evaluated, as no public GBM immune-checkpoint cohort was available [62]. Single-cell evidence derives from one cohort with marker-based annotation, and the VL population is small (51 cells across 4 donors). Spatial spots are not independent; we addressed this with stratified, mixed-effects, and Stouffer inference, but effective sample size is smaller than nominal, the dichotomized contrast in one slice (ZH916T1) is marginal ($P = 0.036$, not Bonferroni-significant), and the effect size is modest (median difference 0.07). The ecotype, gradient, and ligand–receptor analyses are hypothesis-generating extensions: the immune-vascular ecotype is rare (47 spots), the ligand–receptor panel is descriptive at Smart-seq2 depth with no spatial validation of the inferred directions, and the core–margin gradient uses distance-to-white-matter as an anatomical proxy rather than annotated histology. Bulk projection of ecotype signatures assumes that spot-derived programs transfer to bulk tissue. In TCGA-GBM (single grade) dominant ecotype was not prognostic; the EC1 independent signal rests on CGGA and requires grade-IV-only replication [15]. Bulk and single-cell/spatial scores use different implementations (agreement $\rho = 0.68$ in CGGA); the median-split spatial result replicates under both implementations, but the continuous spatial gradient does not fully (3/4 slices). The 38-gene and

module parallel scores were evaluated in CGGA only. The myeloid/neoplastic spatial associations were directionally unstable and were deliberately not interpreted.

6 Conclusions

DSscore, an ssGSEA score of a Cox-selected disulfidptosis gene set, marks an immune-infiltrated, high-grade, IDH-wildtype glioblastoma state whose prognostic value is independent of clinical and molecular covariates and consistent in direction across four independent cohorts. At single-cell and spatial resolution the score localises to the vascular-lymphangioblast niche, and the spatial ecotypes separate an independent hypoxic-core risk axis from a vascular-mesenchymal state whose risk is captured by the score. The executing disulfidptosis programme, rather than the SLC7A11 gate, carries the outcome association, defining the score as a vascular- and immune-associated high-grade program and providing testable hypotheses for anti-angiogenic or metabolic strategies in glioblastoma.

Abbreviations

1p19q
chromosome 1p/19q codeletion
BH
Benjamini-Hochberg
CERES
CRISPR effect score
CGGA
Chinese Glioma Genome Atlas
CI
confidence interval
CLR
centred log-ratio
CTRP
Cancer Therapeutics Response Portal
CYT
cytolytic activity
DepMap
Cancer Dependency Map
DSscore
disulfidptosis gene-set score
EC0-EC3
spatial ecotypes
ECM
extracellular matrix
EMT

epithelial-mesenchymal transition
FDR
false discovery rate
GBM
glioblastoma
GDSC
Genomics of Drug Sensitivity in Cancer
GEO
Gene Expression Omnibus
GSVA
gene set variation analysis
HR
hazard ratio
IDH
isocitrate dehydrogenase
IFN
interferon
IQR
interquartile range
KM
Kaplan-Meier
MGMT
O6-methylguanine-DNA methyltransferase
MCPcounter
Microenvironment Cell Populations counter
OS
overall survival
RCTD
robust cell type decomposition
SLC7A11 / SLC3A2
solute carrier family 7 member 11 / 3 member 2
ssGSEA
single-sample gene set enrichment analysis
TCGA
The Cancer Genome Atlas
TME
tumour microenvironment
VL
vascular lymphangioblast

Declarations

Ethics approval and consent to participate

Not applicable. All datasets used in this study are publicly available and de-identified; no ethics approval or consent was required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Clinical trial number

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Author Contribution

ZJ and WYX conceived and designed the study; ZJ, MYN, ZWL and WW collected and analysed the data; ZJ drafted the manuscript; WYX supervised the project and revised the manuscript. All authors read and approved the final manuscript.

Data Availability

All data are publicly available. CGGA: http://www.cgga.org.cn (mRNAseq_693 cohort). TCGA-GBM: GDC data portal (https://portal.gdc.cancer.gov/). GEO accessions: GSE4412, GSE4271, GSE7696, GSE237183. Single-cell GBM data: CELLxGENE. Visium data: 10x Genomics public human glioblastoma dataset and GEO GSE237183. Dependency and drug data: DepMap 26Q1 Public and CCLE (https://depmap.org), CTRP v2 and GDSC release 8.5. All processed scores, per-spot outputs and analysis scripts are available in the project replication record.

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