

HTR2C Expression in Relation to Survival and Myeloid Cell Signatures in Lower-Grade Glioma

Dandan Li

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Yuge Li

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Bo Wang

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Yin Lian

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Zhixin Chen

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Lina Gao

Shenzhen Pingle Orthopedic Hospital (Shenzhen Pingshan Traditional Chinese Medicine Hospital)

Shaofeng Jiang

Guilin Medical University

Wei Zhang

w-z12@tsinghua.org.cn

Peking University Shenzhen Hospital

Research Article

Keywords: HTR2C, Glioma, Monocytic lineage, Tumor microenvironment, Tumor purity, Overall survival

Posted Date: October 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11147622/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

We evaluated associations of HTR2C expression with survival and microenvironment scores across glioma cohorts after adjustment for clinical and molecular characteristics.

Methods

Adult primary gliomas historically classified as grades II–III were analyzed in The Cancer Genome Atlas (TCGA) and two Chinese Glioma Genome Atlas (CGGA) cohorts. Cox models assessed overall survival. Partial rank correlations assessed microenvironment scores, adjusting for age, sex, grade, and molecular subtype, with additional ABSOLUTE purity adjustment in TCGA. Benjamini–Hochberg correction controlled false discovery rates. A separate tissue microarray comprised 39 evaluable cores from normal brain and grade I–II tumors.

Results

Survival analyses included 505, 201, and 132 patients. Adjusted hazard ratios per standard-deviation increase in expression were 0.87 (95% confidence interval 0.72–1.05), 0.67 (0.52–0.87), and 0.67 (0.49–0.91) in TCGA, CGGA693, and CGGA325, respectively; sensitivity analyses showed variation across models. In TCGA, adjusted correlations were -0.405 for immune and -0.275 for stromal scores ($n = 409$; both BH-adjusted $P < 0.001$). Monocytic-lineage correlations were -0.188 , -0.210 , and -0.394 across the three cohorts ($n = 411$, 208 , and 139 ; all BH-adjusted $P < 0.01$). Associations differed among myeloid scores and algorithms. Cytoplasmic staining was higher in normal brain than grade II tumors (Holm-adjusted $P = 0.025$).

Conclusions

HTR2C expression was inversely associated with monocytic-lineage scores across cohorts. Its survival association varied with covariate adjustment and analysis population, supporting interpretation within the clinical and molecular context.

1 Introduction

Adult diffuse gliomas are molecularly and clinically heterogeneous. Among tumors historically classified as histological grades II and III, IDH mutation and 1p/19q codeletion status distinguish patient groups with different genomic features and survival outcomes^[1–4]. Integrated genomic analyses showed that these molecular features captured biological differences more accurately than histological classification

alone^[1–3]. Evaluating the association between individual gene expression and survival therefore requires consideration of molecular subtype, age, and histological grade, together with assessment of consistency across cohorts.

HTR2C encodes the serotonin 2C receptor (5-HT2C), a G protein-coupled receptor whose ligand recognition and activation properties have been characterized through structural and functional studies^[5]. In U251 human glioma cells, activation of 5-HT2C increased neprilysin expression^[6]. This cellular finding provides a basis for investigating 5-HT2C biology in glioma. At the patient level, further characterization is needed to establish how tissue HTR2C expression relates to survival and tumor microenvironment features, and whether these associations persist after adjustment for clinical and molecular factors.

Bulk glioma transcriptomes contain expression contributions from malignant and nonmalignant cells, and tumor purity can confound associations derived from tissue-level expression data^[7, 8]. Single-cell studies have further resolved malignant-cell states and nonmalignant populations in diffuse gliomas and distinguished microglia-derived from blood-derived tumor-associated macrophages^[9–11]. These observations support examining broad immune and stromal features alongside specific myeloid-related expression signatures. Incorporating molecular subtype and tumor purity allows assessment of whether associations between HTR2C and these scores persist after accounting for these factors.

In this study, we analyzed TCGA and two CGGA transcriptomic cohorts to examine the association between HTR2C expression and overall survival in patients with primary gliomas historically classified as grades II and III, comparing estimates before and after clinical and molecular adjustment. In TCGA, we additionally assessed associations with immune, stromal, and myeloid-related scores while adjusting for ABSOLUTE tumor purity. External analyses of monocytic lineage and myeloid dendritic cell scores were conducted in CGGA. We also compared HTR2C immunohistochemical scores in a tissue microarray containing normal brain tissue and historically classified grade I and II tumors. The study aimed to determine the consistency of the HTR2C survival association across cohorts and adjustment models and to characterize its associated tumor microenvironment expression features.

2 Methods

2.1 Data sources and cohort selection

Transcriptomic and clinical data were obtained from The Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA). TCGA-LGG HTR2C expression was queried from the UCSC Xena HiSeqV2 dataset (TCGA.LGG.sampleMap/HiSeqV2), and clinical information was retrieved from TCGA.LGG.sampleMap/LGG_clinicalMatrix [12]. Survival data were obtained from TCGA-LGG.survival.tsv in the UCSC Xena GDC hub. TCGA molecular subtype annotations were obtained from worksheet S1A of the pan-glioma supplementary file TableS1.PatientData.20151020.v3.xlsx [3]. The CGGA datasets

comprised the processed FPKM expression and clinical data from the mRNAseq_693 (PRJCA001747) and mRNAseq_325 (PRJCA001746) cohorts [13].

Eligible patients were adults aged ≥ 18 years with primary glioma recorded as histological grade II or III and complete data on age, sex, grade, molecular subtype and HTR2C expression. One primary tumor record per patient was retained in TCGA, and expression, clinical and molecular data were matched using patient identifiers. Histological grades were retained as recorded in the source datasets. Tumors were classified as IDH-mutant and 1p/19q-codeleted, IDH-mutant and non-codeleted, or IDH-wildtype according to IDH and 1p/19q status. CGGA cases with missing molecular annotations or concurrent annotations of IDH-wildtype status and 1p/19q codeletion were excluded. The primary TCGA analyses used the subtype annotations from the pan-glioma study, with one case with discordant molecular information excluded in sensitivity analyses.

TCGA HTR2C expression was analyzed using the $\log_2(\text{RSEM} + 1)$ values retrieved from the Xena dataset, whereas CGGA FPKM values were transformed as $\log_2(\text{FPKM} + 1)$. Expression distributions and subsequent associations were analyzed separately within each cohort. Survival analyses required a survival time greater than zero and a valid event indicator. TCGA microenvironment analyses required complete data on the corresponding scores and ABSOLUTE tumor purity. Analysis populations were selected separately from each base cohort according to the required variables. Complete-case analyses were performed without imputation of missing values. Cohort selection is shown in Fig. 1a, and data sources and analysis populations are summarized in Supplementary Table S1.

2.2 Tissue microarray and immunohistochemistry

The tissue microarray was commercially obtained from Shanghai Outdo Biotech (Shanghai, China) and contained normal cerebral cortex and tumors recorded as grade I or II, with one tissue core per patient. Tissue types and grades were obtained from the pathology records supplied with the microarray. Cores with complete or partial tissue loss were excluded.

HTR2C immunostaining was performed using horseradish peroxidase (HRP) detection and 3,3'-diaminobenzidine (DAB) chromogen. A recombinant rabbit monoclonal antibody against HTR2C (Thermo Fisher Scientific, catalog no. MA5-32717) was used at a dilution of 1:2000, with the EnVision FLEX/HRP detection system (DAKO). Brown staining was considered positive.

Cytoplasmic staining scores were calculated from the original records by multiplying staining intensity by the positive-cell fraction, expressed on a scale of 0–1. Intensity ranges recorded as 0.5–1 and 1–2 were represented by their midpoints, 0.75 and 1.5, respectively. Cytoplasmic scores from individual cores were used for comparisons among tissue groups. Representative images were selected from cores with cytoplasmic scores equal to the median of their respective groups. The tissue microarray composition is provided in Table 2.

2.3 Overall survival analysis

Overall survival (OS) times and event status were obtained from the respective databases. Survival time was measured in days, death was treated as the event, and patients recorded as alive were censored according to the database records. TCGA and the two CGGA cohorts were analyzed separately. Log₂-transformed HTR2C expression was standardized within the primary survival analysis population of each cohort and entered into Cox proportional hazards models ^[14] as a continuous variable. Hazard ratios (HRs) and 95% confidence intervals were reported per one-standard-deviation increase in HTR2C expression.

Three models with sequential covariate adjustment were fitted. M0 included HTR2C expression alone; M1 additionally included age, sex, and histological grade; and M2 further included molecular subtype. Age was modeled per 10-year increase. Sex, histological grade, and molecular subtype were treated as categorical variables, with female sex, grade II, and IDH-mutant, 1p/19q-codeleted tumors as the respective reference categories. All three models used the same complete-case population within each cohort. Tied event times were handled using the Efron method, and regression coefficients were assessed using two-sided Wald tests. A clinical and molecular model without HTR2C was also fitted and compared with M2 using a likelihood ratio test to assess the change in model fit after adding HTR2C.

The proportional hazards assumption was assessed using tests based on scaled Schoenfeld residuals ^[15, 16], with the rank transformation of time as the primary approach and the Kaplan–Meier transformation as a supplementary assessment. In TCGA, HTR2C expression and age were modeled separately using restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentiles of the respective distributions. Each spline model was compared with its corresponding linear model using a likelihood ratio test for nonlinearity. M2 was also refitted after excluding TCGA-CS-5394, a case with discordant molecular annotations.

Post hoc sensitivity analyses were conducted in both CGGA cohorts following the proportional hazards diagnostics. Cox models were stratified jointly by histological grade and molecular subtype, allowing a separate baseline hazard function for each stratum, and adjusted for age and sex. Additional analyses were restricted to patients with IDH-mutant tumors and adjusted for age, sex, histological grade, and 1p/19q codeletion status. These restricted analyses retained the HTR2C expression mean and standard deviation from the primary survival analysis population of the corresponding cohort.

To assess the influence of requiring complete molecular data, supplementary available-case analyses were performed in both CGGA cohorts. These analyses retained the eligibility requirements for age, primary disease status, histological grade, sex, HTR2C expression, and survival data, without requiring complete molecular subtype information. M0 and M1 were then refitted, with HTR2C expression restandardized within the corresponding available-case population of each cohort.

2.4 Analysis of immune and stromal scores

Precomputed ESTIMATE immune and stromal scores [7] and ABSOLUTE tumor purity estimates [17] were obtained from worksheet S1A of the TCGA pan-glioma supplementary file

TableS1.PatientData.20151020.v3.xlsx and matched to HTR2C expression and clinical data by patient identifier. This cross-sectional analysis included patients with complete data on HTR2C expression, both ESTIMATE scores, age, sex, histological grade, molecular subtype, and ABSOLUTE tumor purity.

Associations between HTR2C expression and the two scores were assessed sequentially in the same analysis population. Unadjusted Spearman rank correlations were first estimated, followed by partial rank correlations adjusted for age, sex, histological grade, and molecular subtype. The primary analysis additionally adjusted for ABSOLUTE tumor purity. HTR2C expression, each score, and continuous covariates were rank-transformed, with average ranks assigned to tied values; categorical covariates were dummy-coded. The ranks of HTR2C expression and each score were separately regressed on the covariates, and the Pearson correlation between the resulting residuals was calculated. For visualization, residuals from the fully adjusted analysis were divided by their standard deviation.

For the unadjusted, clinical and molecular adjusted, and fully adjusted analyses, 95% confidence intervals were estimated using 2,000 patient-level bootstrap samples drawn with replacement. Ranking and covariate adjustment were repeated in each sample, and the 2.5th and 97.5th percentiles of the correlation estimates defined the confidence limits. Two-sided P values were calculated using a t-distribution approximation, with degrees of freedom determined by the sample size and the rank of the covariate design matrix. The Benjamini–Hochberg procedure^[18] was applied to the two score tests within each adjustment set, and BH-adjusted P values were reported. The two fully adjusted tests constituted the primary testing family.

Sensitivity analyses excluded TCGA-CS-5394, a case with discordant molecular annotations; restricted the analysis to patients also included in the primary survival analysis; and modeled tumor purity using a restricted cubic spline. The spline was constructed from tumor purity ranks, with knots at the 5th, 35th, 65th, and 95th percentiles. Unadjusted analyses and analyses adjusted for clinical and molecular covariates were also repeated in all eligible patients with both scores available to assess the influence of requiring complete tumor purity data. Confidence intervals for sensitivity analyses were estimated using the Fisher z approximation, and Benjamini–Hochberg correction was applied to the two tests within each sensitivity analysis. Patients included in the primary analysis and those excluded because of missing data were also compared descriptively with respect to age, sex, histological grade, molecular subtype, and HTR2C expression.

2.5 Analysis of myeloid-related scores

Precomputed TCGA infiltration estimates were downloaded from the TIMER2.0 resource “Infiltration Estimation for all TCGA tumors” (infiltration_estimation_for_tcga.csv.gz) on September 17, 2026 [19], and matched to expression, clinical, and molecular data by patient identifier. The primary analysis included seven xCell scores [20]: macrophages, monocytes, myeloid dendritic cells, activated myeloid dendritic cells, M1 macrophages, M2 macrophages, and plasmacytoid dendritic cells. Cell labels followed the algorithm annotations in the source matrix. Auxiliary analyses included TIMER [21] macrophage and myeloid dendritic cell scores and MCP-counter [22] monocytic lineage and myeloid

dendritic cell scores. Two MCP-counter monocyte-related fields with identical values in the source matrix were treated as a single score.

Patients with complete data on the selected scores, HTR2C expression, clinical and molecular covariates, and ABSOLUTE tumor purity were included. Using the rank correlation approach described in Section 2.4, associations were estimated without adjustment, after adjustment for age, sex, histological grade, and molecular subtype, and after additional adjustment for tumor purity. For the fully adjusted analyses, 95% confidence intervals were estimated using 2,000 patient-level bootstrap samples, with ranking and covariate adjustment repeated in each sample. Confidence intervals for the other adjustment sets were estimated using the Fisher z approximation. Two-sided P values were calculated using the residual-correlation t approximation. Within each adjustment set, Benjamini–Hochberg correction was applied separately to the seven xCell tests and the four auxiliary tests. The seven fully adjusted xCell tests constituted the primary testing family. The fully adjusted analyses were repeated after excluding TCGA-CS-5394, with Fisher z confidence intervals and separate correction within the same two testing families. The proportion of zero scores and the number of distinct values were also summarized for each score.

Following the TCGA analyses, monocytic lineage and myeloid dendritic cell scores were examined in the CGGA mRNAseq_693 and mRNAseq_325 cohorts. Scores were calculated in Python using the official MCP-counter HUGO marker sets and scoring rule ^[22], as the arithmetic mean of $\log_2(\text{FPKM} + 1)$ expression across available marker genes within each sample. All seven monocytic lineage markers were available in both cohorts. Of the six myeloid dendritic cell markers, only CD1E, CLEC10A, and CLIC2 were available. These three genes were therefore used to calculate an exploratory score; the unavailable genes CD1A, CD1B, and WFDC21P were not imputed as zero. Marker coverage is provided in Supplementary Table S4.

The two CGGA cohorts were analyzed separately. Within each cohort, unadjusted Spearman correlations and partial rank correlations adjusted for age, sex, histological grade, and molecular subtype were calculated in the same complete-case population. Tumor purity was not included in the CGGA models. Confidence intervals for adjusted correlations were estimated using 2,000 patient-level bootstrap samples, with ranking and covariate adjustment repeated in each sample. P values were calculated using a two-sided t approximation, and Benjamini–Hochberg correction was applied jointly to the two adjusted tests within each cohort. The monocytic lineage score based on the complete marker set was the main reported score; the dendritic cell score based on three available genes was reported as an exploratory analysis.

2.6 Statistical analysis

Continuous variables were summarized as medians with the 25th and 75th percentiles, and categorical variables as counts and percentages. Clinical and molecular characteristics were summarized separately for each cohort. The patient was the unit of analysis. Complete-case populations were defined according to the variables required for each analysis, without imputation of missing values.

Cytoplasmic immunohistochemical scores were compared among normal brain tissue, grade I tumors, and grade II tumors using a permutation Kruskal–Wallis test. The test statistic was corrected for tied ranks, and the P value was estimated from 999,999 random permutations of group labels with a plus-one correction. Pairwise comparisons used exact permutation Mann–Whitney tests, enumerating all label allocations that preserved the group sizes. Two-sided P values were calculated as twice the smaller inclusive permutation-tail probability, capped at 1. Holm correction was applied jointly to the three pairwise comparisons.

Methods for survival and correlation analyses are described in Sections 2.3–2.5. Tests of regression coefficients, correlation coefficients, and pairwise comparisons were two-sided. Statistical significance was defined as $P < 0.05$ for tests without multiplicity adjustment, Holm-adjusted $P < 0.05$ for pairwise immunohistochemical comparisons, and BH-adjusted $P < 0.05$ within each testing family for score correlation analyses. Effect estimates were reported with 95% confidence intervals.

Data processing and statistical analyses were performed in Python using pandas, NumPy, SciPy, and lifelines. Statistical figures were generated using Matplotlib.

3 Results

3.1 Cohort characteristics and HTR2C expression across molecular subtypes

The TCGA, CGGA693 and CGGA325 cohorts included 509, 208 and 139 adults with primary glioma, respectively (Fig. 1a; Supplementary Table S1). Median ages were 41, 40 and 39 years, respectively. According to the original histological records, grade II tumors accounted for 48.1%, 49.5% and 66.9% of the respective cohorts; the remaining tumors were grade III. The IDH-mutant, non-codeleted subtype was the most frequent in TCGA and CGGA693, accounting for 48.5% and 46.2%, respectively. In CGGA325, the IDH-mutant, 1p/19q-codeleted subtype was the most frequent, accounting for 38.1%. Clinical and molecular characteristics are summarized in Table 1.

Overall survival analyses included 505, 201 and 132 patients from the respective cohorts, with 124, 77 and 64 recorded deaths. The TCGA analyses of ESTIMATE scores and myeloid-related scores included 409 and 411 patients, respectively. MCP-counter analyses included 208 patients from CGGA693 and 139 from CGGA325 (Fig. 1a; Table 1).

In all three cohorts, median HTR2C expression was lower in IDH-wildtype tumors than in either IDH-mutant subgroup. In TCGA, median expression was higher in the IDH-mutant, 1p/19q-codeleted subgroup than in the non-codeleted subgroup. The two IDH-mutant subgroups had similar median expression levels in CGGA693, whereas the non-codeleted subgroup had a higher median in CGGA325. Expression distributions overlapped across molecular subtypes within each cohort (Fig. 1b–d).

Table 1
Clinical and molecular characteristics

Characteristic	TCGA	CGGA mRNAseq_693	CGGA mRNAseq_325
Patients	509	208	139
Age, years, median (Q1, Q3)	41.0 (33.0, 53.0)	40.0 (33.0, 45.3)	39.0 (35.0, 46.0)
Sex, n (%)			
Female	229 (45.0)	90 (43.3)	55 (39.6)
Male	280 (55.0)	118 (56.7)	84 (60.4)
Historical histological grade, n (%)			
Grade II	245 (48.1)	103 (49.5)	93 (66.9)
Grade III	264 (51.9)	105 (50.5)	46 (33.1)
IDH and 1p/19q molecular subtype, n (%)			
IDH-mutant, 1p/19q-codeleted	168 (33.0)	65 (31.3)	53 (38.1)
IDH-mutant, non-codeleted	247 (48.5)	96 (46.2)	50 (36.0)
IDH-wildtype	94 (18.5)	47 (22.6)	36 (25.9)
Analysis populations			
OS analysis, n	505	201	132
Excluded from OS analysis, n	4	7	7
Deaths, n (% of OS analysis)	124 (24.6)	77 (38.3)	64 (48.5)
Censored, n (% of OS analysis)	381 (75.4)	124 (61.7)	68 (51.5)
Myeloid analysis, n	411	208	139
ESTIMATE analysis with purity, n	409	Not assessed	Not assessed

(a) Selection of patients for the TCGA and two CGGA RNA-sequencing cohorts. Base cohorts included adults with primary tumors of historical histological grades II or III and complete age, sex, grade, molecular-subtype and HTR2C expression data. The TCGA source cohort contained 516 primary patients. The clinical and molecular complete cohorts comprised 509, 208 and 139 patients, respectively. Analysis subsets overlap and were selected separately for overall survival (OS) and

microenvironment analyses. Discordant CGGA annotations refer to IDH-wildtype tumors annotated as 1p/19q-codeleted. (b–d) HTR2C expression in the three molecular subtypes in the corresponding base cohorts. Points represent individual patients. Boxes show the 25th and 75th percentiles, with the median marked inside; whiskers extend to the most extreme values within 1.5 interquartile ranges. All patient points are displayed. TCGA values are $\log_2(\text{RSEM} + 1)$, whereas CGGA values are $\log_2(\text{FPKM} + 1)$. Panels are descriptive; expression values were not pooled or directly compared across datasets. Molecular labels and historical grades do not constitute complete WHO 2021 integrated diagnoses. Data sources, eligibility and analysis membership are provided in Supplementary Table S1

3.2 HTR2C immunostaining in normal brain tissue and grade I and II tumors

The tissue microarray contained samples from 42 patients. After exclusion of three samples with complete or partial tissue loss, 39 were included in the cytoplasmic staining analysis: three normal brain samples, five grade I tumors and 31 grade II tumors, with grades based on the original pathology records (Table 2). Representative staining images are shown in Fig. 2a–c. Median cytoplasmic HTR2C staining scores (interquartile ranges) were 1.00 (0.88–1.18) in normal brain tissue, 0.75 (0.60–0.80) in grade I tumors and 0.45 (0.30–0.68) in grade II tumors (Fig. 2d).

Cytoplasmic staining scores differed across the three groups (permutation $P = 0.0119$). Pairwise comparisons showed lower scores in grade II tumors than in normal brain tissue (Holm-adjusted $P = 0.0251$). The comparisons between normal brain tissue and grade I tumors and between grade I and grade II tumors did not reach statistical significance (both Holm-adjusted $P = 0.2955$).

Representative tissue cores are shown for (a) normal cerebral cortex (A01), (b) pilocytic astrocytoma, historical grade I (A07), and (c) diffuse astrocytoma, historical grade II (B03). The selected cores had cytoplasmic staining scores equal to the median of their respective groups. Scale bars, 500 μm . (d) Cytoplasmic staining scores in normal brain tissue ($n = 3$), grade I tumors ($n = 5$), and grade II tumors ($n = 31$). Each point represents one patient, with one core per patient. Horizontal offsets separate overlapping observations. The bars beside each group indicate the median and interquartile range. Scores were calculated as staining intensity multiplied by the positive-cell fraction (0–1); intensity ranges of 0.5–1 and 1–2 were represented by their midpoints, 0.75 and 1.5, respectively. Cores C11 and C12 were excluded because of tissue loss, and C14 was excluded because of partial tissue loss. Histological diagnoses and grades follow the original tissue records. The omnibus P value was obtained by permuting group labels 999,999 times using the tie-corrected Kruskal–Wallis statistic. Pairwise comparisons used two-sided exact permutation Mann–Whitney tests with Holm correction across all three comparisons

Table 2
Composition of the tissue microarray and analyzed cores

Tissue or recorded histology	Historical grade	Available cores	Excluded cores	Analyzed cores
Cerebral cortex	Not applicable	3	0	3
Ganglioglioma	I	1	0	1
Pilocytic astrocytoma	I	4	0	4
Oligodendroglioma	II	3	0	3
Pleomorphic xanthoastrocytoma	II	2	0	2
Diffuse astrocytoma	II	17	0	17
Oligodendrocyte astrocytoma	II	12	3	9

3.3 Association between HTR2C expression and overall survival

In TCGA, each standard deviation increase in HTR2C expression was associated with a hazard ratio for death of 0.65 in the unadjusted model (95% CI, 0.54–0.77; $P = 1.72 \times 10^{-6}$). The association was attenuated after adjustment for age, sex, historical histological grade and molecular subtype (HR, 0.87; 95% CI, 0.72–1.05; $P = 0.145$; Fig. 3a).

In both CGGA cohorts, higher HTR2C expression was associated with a lower hazard of death after adjustment for the same covariates. The HR per standard deviation increase was 0.67 in CGGA693 (95% CI, 0.52–0.87; $P = 0.002$) and 0.67 in CGGA325 (95% CI, 0.49–0.91; $P = 0.009$; Fig. 3a).

Likelihood-ratio tests comparing the clinical and molecular model with and without HTR2C indicated improved fit in CGGA693 ($\chi^2 = 11.566$; $P = 0.0007$) and CGGA325 ($\chi^2 = 7.855$; $P = 0.0051$), but not in TCGA ($\chi^2 = 2.152$; $P = 0.1424$). In TCGA, restricted cubic spline comparisons did not provide evidence of nonlinearity for HTR2C ($P = 0.6898$) or age ($P = 0.2340$). Model diagnostics in the CGGA cohorts identified departures from the proportional hazards assumption for grade or molecular subtype covariates. After joint stratification by grade and molecular subtype, with adjustment for age and sex, the HR for HTR2C was 0.73 in CGGA693 (95% CI, 0.56–0.95; $P = 0.017$) and 0.77 in CGGA325 (95% CI, 0.57–1.04; $P = 0.090$). Analyses restricted to patients with IDH-mutant tumors, adjusted for age, sex, grade and 1p/19q codeletion status, yielded HRs of 0.80 in CGGA693 ($n = 154$; 95% CI, 0.56–1.13; $P = 0.197$) and 0.68 in CGGA325 ($n = 99$; 95% CI, 0.45–1.02; $P = 0.063$; Fig. 3b). Primary and sensitivity estimates are provided in Supplementary Table S2.

Squares indicate hazard ratios (HRs), and horizontal lines indicate 95% confidence intervals (CIs). Expression was standardized within each primary survival cohort after $\log_2(\text{RSEM} + 1)$ transformation in

TCGA or $\log_2(\text{FPKM} + 1)$ transformation in CGGA. HRs represent a one-standard-deviation increase in expression. (a) M0 was unadjusted; M1 adjusted for age, sex and historical histological grade; M2 additionally adjusted for IDH/1p/19q-defined molecular subtype. The same complete-case population was used for M0–M2 within each cohort: TCGA, 505 patients and 124 deaths; CGGA693, 201 patients and 77 deaths; CGGA325, 132 patients and 64 deaths. (b) TCGA sensitivity analyses excluded one case with discordant molecular information or modeled age using a restricted cubic spline. CGGA analyses stratified the baseline hazard jointly by grade and molecular subtype, with adjustment for age and sex, or restricted the population to IDH-mutant tumors, with adjustment for age, sex, grade and 1p/19q-defined subtype. Sensitivity analyses retained the expression standardization of their primary survival cohort. The CGGA sensitivity models were fitted after proportional-hazards diagnostics identified departures for other covariates. P values are two-sided Wald P values without multiplicity adjustment. The dashed line indicates HR = 1. Primary and sensitivity estimates are provided in Supplementary Table S2

3.4 Associations of HTR2C expression with immune and stromal scores in TCGA

Among the 409 TCGA patients included in the analysis, HTR2C expression was inversely correlated with both ESTIMATE immune and stromal scores. The unadjusted Spearman correlation coefficient was -0.456 for the immune score (95% CI, -0.535 to -0.373) and -0.338 for the stromal score (95% CI, -0.425 to -0.242 ; Fig. 4a).

The inverse associations persisted after adjustment for age, sex, historical histological grade, molecular subtype and ABSOLUTE tumor purity. The partial correlation coefficient was -0.405 for the immune score (95% CI, -0.486 to -0.318 ; $P = 2.33 \times 10^{-17}$; BH-adjusted $P = 4.66 \times 10^{-17}$) and -0.275 for the stromal score (95% CI, -0.365 to -0.178 ; $P = 1.89 \times 10^{-8}$; BH-adjusted $P = 1.89 \times 10^{-8}$; Fig. 4a–c). Both inverse associations persisted in sensitivity analyses excluding the case with discordant molecular annotations, restricting the population to patients also included in the survival analysis, and allowing for a nonlinear association with tumor purity (Supplementary Table S3).

All panels include the same 409 patients with complete clinical, molecular, ESTIMATE and ABSOLUTE purity data. (a) Estimates are shown without adjustment, after adjustment for age, sex, historical grade and molecular subtype, and after additional adjustment for ABSOLUTE tumor purity. Circles indicate the first two adjustment sets, and squares indicate the primary fully adjusted estimates. Horizontal bars show percentile 95% confidence intervals from 2,000 patient-level bootstrap samples, with ranking and covariate adjustment repeated in each sample. Two-sided P values were calculated using a t approximation for the residual correlations. The displayed BH-adjusted P values use Benjamini–Hochberg correction across the two scores within each adjustment set; the two fully adjusted tests constitute the primary testing family. (b, c) Fully adjusted rank residuals for HTR2C expression and the immune or stromal score, respectively, scaled to unit standard deviation for display. Each point represents one patient. Colors identify the molecular subtypes; the line summarizes the overall residual association. Subtypes comprise IDH-mutant, 1p/19q-codeleted tumors ($n = 140$), IDH-mutant, non-

codeleted tumors (n = 193), and IDH-wildtype tumors (n = 76). Fully adjusted results and sensitivity analyses are provided in Supplementary Table S3

3.5 Associations of HTR2C expression with myeloid-related scores in TCGA

Among 411 TCGA patients, HTR2C expression was inversely associated with the xCell macrophage score (partial $\rho = -0.363$; 95% CI, -0.445 to -0.276 ; BH-adjusted $P = 1.44 \times 10^{-13}$), M1 macrophage score (partial $\rho = -0.447$; 95% CI, -0.524 to -0.362 ; BH-adjusted $P = 1.84 \times 10^{-20}$) and monocyte score (partial $\rho = -0.289$; 95% CI, -0.379 to -0.195 ; BH-adjusted $P = 5.47 \times 10^{-9}$) after adjustment for age, sex, historical histological grade, molecular subtype and ABSOLUTE tumor purity. The association with the M2 macrophage score was not statistically significant (partial $\rho = -0.002$; 95% CI, -0.106 to 0.100 ; BH-adjusted $P = 0.962$; Fig. 5a).

Among the xCell dendritic cell scores, the activated myeloid dendritic cell score was inversely associated with HTR2C expression (partial $\rho = -0.361$; 95% CI, -0.447 to -0.264 ; BH-adjusted $P = 1.44 \times 10^{-13}$). A weaker inverse association was observed for the plasmacytoid dendritic cell score (partial $\rho = -0.112$; 95% CI, -0.204 to -0.015 ; BH-adjusted $P = 0.035$). The association with the myeloid dendritic cell score was not statistically significant (partial $\rho = -0.014$; 95% CI, -0.117 to 0.083 ; BH-adjusted $P = 0.916$; Fig. 5a).

Auxiliary analyses of the same patients, adjusted for the same covariates, showed an inverse association between HTR2C expression and the MCP-counter monocytic-lineage score (partial $\rho = -0.188$; 95% CI, -0.281 to -0.090 ; BH-adjusted $P = 2.80 \times 10^{-4}$). The association with the TIMER macrophage estimate was not statistically significant (partial $\rho = 0.007$; 95% CI, -0.097 to 0.104 ; BH-adjusted $P = 0.891$). Myeloid dendritic cell scores from TIMER and MCP-counter were both inversely associated with HTR2C expression, with partial correlation coefficients of -0.313 (95% CI, -0.401 to -0.222 ; BH-adjusted $P = 4.72 \times 10^{-10}$) and -0.182 (95% CI, -0.275 to -0.087 ; BH-adjusted $P = 2.99 \times 10^{-4}$), respectively (Fig. 5b).

Exclusion of one patient with discordant molecular annotations yielded similar effect estimates in the remaining 410 patients. The xCell myeloid dendritic cell and plasmacytoid dendritic cell scores contained 190 (46.2%) and 189 (46.0%) zero values, respectively. Fully adjusted results are provided in Supplementary Table S3.

All analyses include the same 411 patients with complete clinical and molecular information, ABSOLUTE purity and the selected cell scores. (a) Seven xCell scores. (b) Four auxiliary scores from TIMER and MCP-counter. Squares show partial rank correlations adjusted for age, sex, historical grade, molecular subtype and ABSOLUTE tumor purity. Horizontal bars indicate percentile 95% confidence intervals from 2,000 patient-level bootstrap samples, with ranks and covariate adjustment recalculated in each sample. Two-sided P values were obtained using a t approximation. Benjamini–Hochberg correction was applied separately to the seven xCell tests and the four auxiliary tests. The dashed line indicates zero correlation.

All selected scores are shown, including nonsignificant results. The MCP-counter monocytic-lineage estimate corresponds to the source column Monocyte_MCPCOUNTER; the identical Macrophage/Monocyte_MCPCOUNTER column was counted once. DC, dendritic cell. M1, M2 and activated are algorithm-defined cell labels. All algorithms were evaluated in the same TCGA cohort; panel b is an algorithm sensitivity analysis. Fully adjusted results are provided in Supplementary Table S3

3.6 Associations of HTR2C expression with monocytic-lineage scores in the CGGA cohorts

The CGGA693 and CGGA325 analyses included 208 and 139 patients, respectively, with all seven markers for the MCP-counter monocytic-lineage score available in both cohorts. After adjustment for age, sex, historical histological grade and molecular subtype, HTR2C expression was inversely associated with the monocytic-lineage score in both cohorts. The partial correlation coefficient was -0.210 in CGGA693 (95% CI, -0.342 to -0.071 ; $P = 0.00265$; BH-adjusted $P = 0.00530$) and -0.394 in CGGA325 (95% CI, -0.534 to -0.235 ; $P = 2.41 \times 10^{-6}$; BH-adjusted $P = 4.83 \times 10^{-6}$; Fig. 6a, b).

Of the six markers in the myeloid dendritic cell signature, CD1E, CLEC10A and CLIC2 were available in both cohorts, and scores were calculated from these three genes. After adjustment for the same covariates, this score was inversely associated with HTR2C expression in CGGA693 (partial $\rho = -0.177$; 95% CI, -0.309 to -0.038 ; BH-adjusted $P = 0.0115$). The association in CGGA325 was not statistically significant (partial $\rho = -0.068$; 95% CI, -0.244 to 0.111 ; BH-adjusted $P = 0.432$). Complete results and marker coverage are provided in Supplementary Table S4.

Adjusted rank residuals are shown for HTR2C expression and the monocytic-lineage score in (a) CGGA693 ($n = 208$) and (b) CGGA325 ($n = 139$). All seven monocytic-lineage markers were available in both cohorts. Scores were calculated as the arithmetic mean of $\log_2(\text{FPKM} + 1)$ expression across these markers. Expression and score ranks were separately adjusted for age, sex, historical grade and molecular subtype, and residuals were scaled to unit standard deviation for display. Each point represents one patient; lines summarize the residual associations. Partial rank correlations and percentile 95% confidence intervals from 2,000 patient-level bootstrap samples are shown. The BH-adjusted P values use Benjamini–Hochberg correction across the two evaluated scores within each cohort, including the incomplete myeloid dendritic-cell score reported in Supplementary Table S4. CGGA models did not include ABSOLUTE purity. The two cohorts were analyzed separately

4 Discussion

This study examined the relationships of HTR2C expression with overall survival and tumor microenvironment-related transcriptional features in three glioma cohorts. After adjustment for available clinical and molecular factors, higher HTR2C expression was consistently associated with lower MCP-counter monocytic-lineage scores in TCGA and both CGGA cohorts. In TCGA, inverse associations were also observed with the ESTIMATE immune and stromal scores and several myeloid-related scores. Higher HTR2C expression was associated with a lower hazard of death in the primary adjusted analyses

of both CGGA cohorts, whereas the corresponding association in TCGA was attenuated after molecular adjustment. Immunohistochemistry further showed lower cytoplasmic HTR2C staining in grade II tumors than in normal brain tissue. These findings indicate a consistent inverse relationship between HTR2C expression and monocytic-lineage-related transcriptional features across the three cohorts.

IDH mutation and 1p/19q codeletion define glioma subgroups with distinct genomic characteristics and survival outcomes and provide greater prognostic resolution than grade alone ^[1–4]. Against this molecular background, the survival analyses showed a consistently inverse direction between HTR2C expression and mortality across all three cohorts. The association was particularly reproducible in the two independent CGGA datasets. After adjustment for age, sex, historical grade, and molecular subtype, each standard-deviation increase in HTR2C expression was associated with a 33% lower hazard of death in both CGGA693 and CGGA325. The close agreement between these estimates, despite differences in cohort size and patient composition, supports the reproducibility of the association. Addition of HTR2C also significantly improved the fit of the clinical and molecular models in both CGGA cohorts, indicating that HTR2C provided prognostic information beyond the variables included in these models. In TCGA, higher HTR2C expression was strongly associated with a lower hazard of death before molecular adjustment and remained significant after adjustment for age, sex, and grade. The estimate moved toward the null after molecular subtype was included, in parallel with the marked differences in HTR2C expression among IDH/1p/19q-defined subgroups. This attenuation places the survival association within the established molecular heterogeneity of glioma and suggests that HTR2C may partly capture biological differences represented by these subtypes.

Bulk glioma transcriptomes contain RNA derived from malignant cells together with signals from immune, stromal, and resident neural populations. Differences in these cellular components contribute to transcriptional variation among glioma subtypes, as demonstrated by single-cell studies of oligodendroglioma and IDH-mutant astrocytoma ^[9, 10]. In the TCGA cohort, higher HTR2C expression was associated with lower ESTIMATE immune and stromal scores ^[7]. These associations remained after adjustment for age, sex, historical grade, molecular subtype and ABSOLUTE tumor purity ^[17], with partial correlation coefficients of – 0.405 for the immune score and – 0.275 for the stromal score. The stronger association with the immune score indicates that the HTR2C-related transcriptional pattern was more closely linked to immune components than to the broader stromal component captured by ESTIMATE. Sequential models were fitted in the same 409 patients, allowing changes in the estimates to be attributed to covariate adjustment rather than differences in the analysis population. The inverse associations were also maintained after exclusion of the case with discordant molecular annotations, restriction to patients included in the survival analysis, and nonlinear adjustment for tumor purity. Persistence of these relationships after adjustment for molecular subtype and tumor purity indicates that the association was not determined solely by the greater nonmalignant-cell contribution of low-purity tumors. HTR2C-high tumors may therefore represent a transcriptional context characterized by reduced immune and stromal expression programs within otherwise comparable molecular and clinical groups.

Analysis of myeloid-related scores further specified the immune context associated with HTR2C expression. After adjustment for clinical factors, molecular subtype, and tumor purity, higher HTR2C expression was inversely associated with the xCell macrophage, monocyte, M1 macrophage, and activated myeloid dendritic-cell scores. Among these associations, the xCell M1 macrophage score showed the largest correlation coefficient. Importantly, the xCell monocyte score and the MCP-counter monocytic-lineage score yielded concordant inverse associations despite differences in their marker genes and scoring procedures^[20, 22]. Myeloid dendritic-cell scores derived from TIMER and MCP-counter were also inversely associated with HTR2C expression, extending the observed pattern to additional myeloid-related transcriptional programs. Experimental and single-cell studies have established that glioma-associated microglia and recruited macrophages have distinct ontogenies and occupy diverse, tumor-instructed transcriptional states^[11, 25, 28–30]. Conventional M1 and M2 markers can overlap within these states; the xCell M1 association should therefore be understood as an M1-related expression signature rather than evidence of a discrete polarization state^[11, 25–27]. The convergence of the monocyte and monocytic-lineage results provides a specific cellular direction for investigating how HTR2C relates to the myeloid compartment of the glioma microenvironment.

The monocytic-lineage association was externally replicated in both CGGA cohorts using the complete seven-gene MCP-counter marker set. MCP-counter was developed to estimate the relative abundance of defined cell populations from bulk transcriptomic data and was validated using RNA mixtures and tissue immunohistochemistry^[22]. After adjustment for age, sex, historical grade, and molecular subtype, HTR2C expression was inversely associated with the monocytic-lineage score in CGGA693 and CGGA325, with partial correlation coefficients of -0.210 and -0.394 , respectively. Both associations remained significant after BH correction. The same direction was observed in the TCGA MCP-counter analysis after additional adjustment for ABSOLUTE tumor purity. Because the three cohorts were processed and analyzed separately, the concordant results were not dependent on pooling expression values or applying a common normalization scale across datasets. Replication across TCGA and two independent CGGA cohorts provides robust transcriptomic evidence linking higher HTR2C expression to a lower monocytic-lineage signature in glioma and identifies this cellular component as a priority for subsequent spatial localization and functional investigation.

Immunohistochemical analysis extended the transcriptomic findings to the protein and tissue levels. HTR2C staining was detectable in normal cerebral cortex and in grade I and II tumors, with median cytoplasmic scores of 1.00, 0.75, and 0.45, respectively. The ordered decrease across the three tissue groups was supported by the overall permutation test, and grade II tumors showed significantly lower cytoplasmic staining than normal brain tissue after correction for multiple comparisons. This pattern was compatible with the transcriptomic observation that lower HTR2C expression occurred in molecularly less favorable tumors and was associated with a higher hazard of death in the survival analyses. Previous work in U251 glioma cells showed that pharmacological activation of 5-HT_{2C} altered downstream neprilysin expression, demonstrating that the receptor can participate in biological responses in a glioma-cell context^[6]. The present immunohistochemical findings provide experimental

evidence that differences in HTR2C are also detectable at the protein level in human brain and glioma tissues.

The replicated inverse relationship between HTR2C expression and monocytic-lineage scores raises a testable hypothesis that HTR2C is linked to a glioma state with reduced monocytic-lineage representation. Determining the cellular source of HTR2C is central to evaluating this relationship. Single-cell and spatial transcriptomic analyses can distinguish expression in malignant cells, resident neural cells, microglia, and blood-derived macrophages, while RNA in situ hybridization or multiplex immunofluorescence with OLIG2, SOX2, IBA1, and CD68 can confirm cellular localization in tissue. Once the principal source is established, HTR2C perturbation in the relevant cell model can be combined with measurements of chemokine expression, conditioned-medium migration assays, and glioma–myeloid coculture experiments. These approaches can determine whether variation in HTR2C expression is accompanied by changes in monocyte recruitment or myeloid-cell state and clarify the biological basis of the association observed across the three cohorts.

Study limitations

The retrospective nature of the TCGA and CGGA cohorts meant that complete WHO 2021 integrated diagnoses, treatment information, and several clinical variables were not uniformly available. Molecular subtype was therefore incorporated where possible, and the main findings were examined across three independent datasets to enhance their reliability. In addition, the associations between HTR2C and immune features were derived primarily from bulk transcriptomic data. Transcriptome-based cell-type scoring is informative for cohort-level comparisons, but its estimates depend on marker definitions, data processing, and the populations represented in the tissue ^[23, 24]. Although the inverse association with the MCP-counter monocytic-lineage score was consistently reproduced in both CGGA cohorts and supported by complementary immunohistochemical findings, transcriptomic deconvolution cannot determine the precise cellular source of HTR2C or establish a causal regulatory relationship. Spatial transcriptomic analyses, single-cell profiling, and cell-specific functional experiments may further define the cellular context and biological role of HTR2C in the glioma microenvironment.

5 Conclusions

In cohorts of primary gliomas historically classified as grades II and III, the inverse association between HTR2C expression and monocytic-lineage scores was supported by TCGA and both CGGA cohorts. In TCGA, associations with immune, stromal, and selected myeloid scores persisted after adjustment for clinical factors, molecular subtype, and tumor purity. The survival association varied with covariate adjustment and the population analyzed, requiring interpretation within the clinical and molecular context. This study characterizes an HTR2C-associated microenvironment expression pattern supported across cohorts. Further investigation should prioritize its cellular source and relationship with monocytic-lineage populations.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable. The manuscript contains no identifiable individual participant data.

Competing interests

The authors declare no competing financial or non-financial interests.

Funding

This work was supported by the Open Funds of the Guangxi Key Laboratory of Tumor Immunology and Microenvironmental Regulation (2023KF011 and 2024KF012).

Author Contribution

Dandan Li: Conceptualization, Methodology, Investigation, Visualization, Writing - original draft, Funding acquisition. Yuge Li: Methodology, Visualization, Writing - original draft. Bo Wang: Data curation, Visualization, Writing - review and editing. Yin Lian: Resources, Formal analysis, Writing - review and editing. Zhixin Chen: Data curation, Investigation. Lina Gao: Resources, Visualization, Writing - review and editing. Shaofeng Jiang: Supervision, Writing - review and editing. Wei Zhang: Conceptualization, Project administration, Supervision, Writing - review and editing. All authors read and approved the final manuscript.

Acknowledgements

The authors thank TCGA, CGGA, UCSC Xena and TIMER2.0, together with the investigators and participants who generated and made these resources available.

Data availability

TCGA-LGG HTR2C expression was queried from the UCSC Xena TCGA hub dataset TCGA.LGG.sampleMap/HiSeqV2 (<https://xenabrowser.net/datapages/?dataset=TCGA.LGG.sampleMap%2FHiSeqV2&host=https%3A%2F%2Ftcga.xenahubs.net>); clinical data

were obtained from TCGA.LGG.sampleMap/LGG_clinicalMatrix (https://tcga.xenahubs.net/download/TCGA.LGG.sampleMap/LGG_clinicalMatrix); and overall survival data were obtained from the GDC Xena dataset TCGA-LGG.survival.tsv (<https://gdc.xenahubs.net/download/TCGA-LGG.survival.tsv.gz>). Molecular subtype, ESTIMATE immune and stromal scores, and ABSOLUTE tumor-purity estimates were taken from TableS1.PatientData.20151020.v3.xlsx, worksheet “S1A. TCGA discovery dataset,” in the TCGA pan-glioma study supplementary data (GDC file identifier a0886693-4c6e-40de-9cb1-8bfc4bb30a7; <https://api.gdc.cancer.gov/data/a0886693-4c6e-40de-9cb1-8bfc4bb30a7>).

CGGA processed FPKM expression and clinical data were obtained from the mRNAseq_693 cohort (FPKM: <https://cgga-cns.org.cn/smart/api/file/getFile?fid=155987771754942469&key=d875dadd>; clinical: <https://cgga-cns.org.cn/smart/api/file/getFile?fid=74514420788760581&key=c6d4622a>; raw sequencing accession PRJCA001747) and the mRNAseq_325 cohort (FPKM: <https://cgga-cns.org.cn/smart/api/file/getFile?fid=74513238338965509&key=82c34930>; clinical: <https://cgga-cns.org.cn/smart/api/file/getFile?fid=74508089948246021&key=e9878946>; raw sequencing accession PRJCA001746). The cohort download catalog is <https://cgga-cns.org.cn/download>. Precomputed TCGA infiltration estimates from xCell, TIMER, and MCP-counter were obtained from the TIMER2.0 resource “Infiltration Estimation for all TCGA tumors” (file: [infiltration_estimation_for_tcga.csv.gz](https://compbio.cn/timer2/infiltration_estimation_for_tcga.csv.gz); https://compbio.cn/timer2/infiltration_estimation_for_tcga.csv.gz; accessed September 17, 2026).

Summary data generated in this study are provided in the article and Supplementary Information. Further tissue microarray data are available from the corresponding author on reasonable request, subject to data-use restrictions.

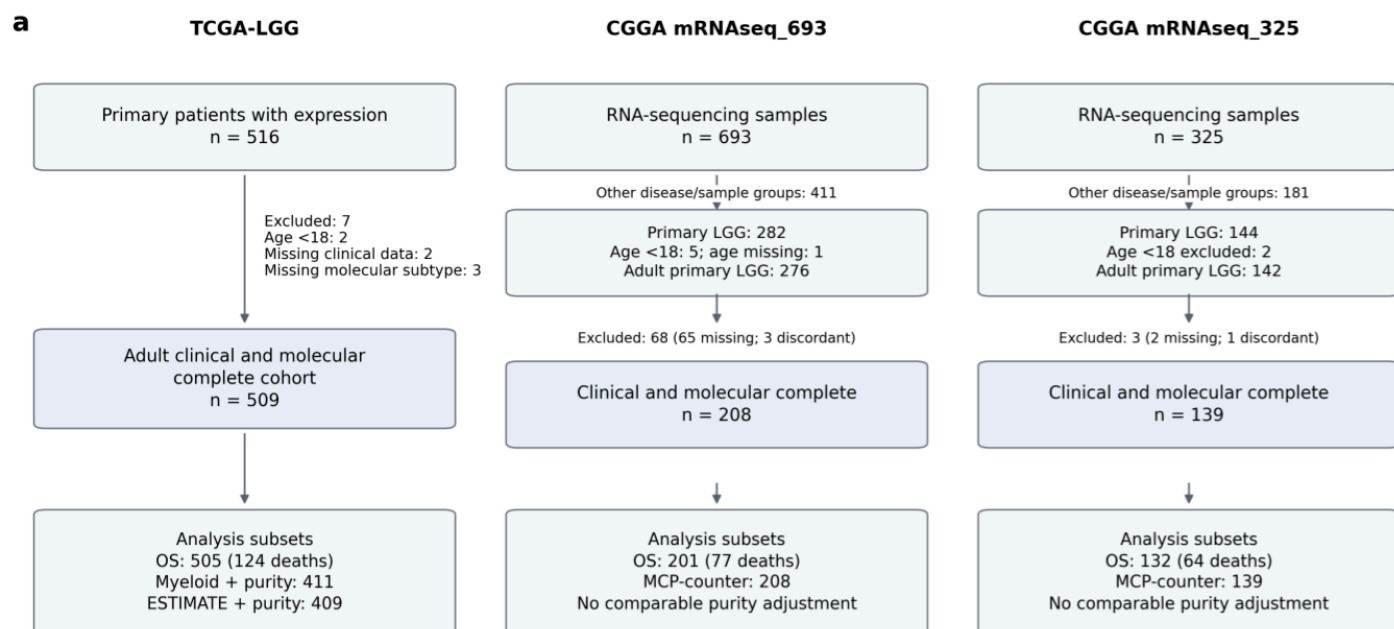
References

1. Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N Engl J Med*. 2015;372(26):2481–98. <https://doi.org/10.1056/NEJMoa1402121>.
2. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. *N Engl J Med*. 2015;372(26):2499–508. <https://doi.org/10.1056/NEJMoa1407279>.
3. Ceccarelli M, Barthel FP, Malta TM, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell*. 2016;164(3):550–63. <https://doi.org/10.1016/j.cell.2015.12.028>.
4. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro Oncol*. 2021;23(8):1231–51. <https://doi.org/10.1093/neuonc/noab106>.
5. Peng Y, McCorvy JD, Harpsøe K, et al. 5-HT_{2C} receptor structures reveal the structural basis of GPCR polypharmacology. *Cell*. 2018;172(4):719–e73014. <https://doi.org/10.1016/j.cell.2018.01.001>.

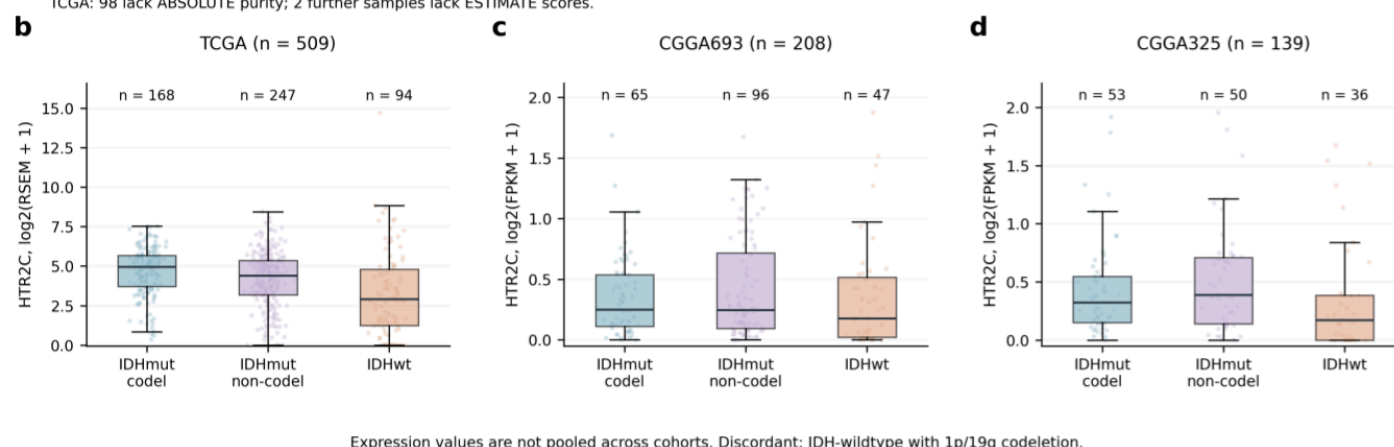
6. Tian XL, Yu LH, Li WQ, Hu Y, Yin M, Wang ZJ. Activation of 5-HT_{2C} receptor promotes the expression of neprilysin in U251 human glioma cells. *Cell Mol Neurobiol*. 2015;35(3):425–32. <https://doi.org/10.1007/s10571-014-0138-6>.
7. Yoshihara K, Shahmoradgoli M, Martínez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 2013;4:2612. <https://doi.org/10.1038/ncomms3612>.
8. Aran D, Sirota M, Butte AJ. Systematic pan-cancer analysis of tumour purity. *Nat Commun*. 2015;6:8971. <https://doi.org/10.1038/ncomms9971>.
9. Tirosh I, Venteicher AS, Hebert C, et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature*. 2016;539(7628):309–13. <https://doi.org/10.1038/nature20123>.
10. Venteicher AS, Tirosh I, Hebert C, et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. *Science*. 2017;355(6332):eaai8478. <https://doi.org/10.1126/science.aai8478>.
11. Müller S, Kohanbash G, Liu SJ, et al. Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis for regional differences in macrophage activation in the tumor microenvironment. *Genome Biol*. 2017;18(1):234. <https://doi.org/10.1186/s13059-017-1362-4>.
12. Goldman MJ, Craft B, Hastie M, et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol*. 2020;38(6):675–8. <https://doi.org/10.1038/s41587-020-0546-8>.
13. Zhao Z, Zhang KN, Wang Q, et al. Chinese Glioma Genome Atlas (CGGA): a comprehensive resource with functional genomic data from Chinese glioma patients. *Genomics Proteom Bioinf*. 2021;19(1):1–12. <https://doi.org/10.1016/j.gpb.2020.10.005>.
14. Cox DR. Regression models and life-tables. *J R, Stat Soc Series B. Stat Methodol*. 1972;34(2):187–220. <https://doi.org/10.1111/j.2517-6161.1972.tb00899.x>
15. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982;69(1):239–41. <https://doi.org/10.1093/biomet/69.1.239>.
16. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81(3):515–26. <https://doi.org/10.1093/biomet/81.3.515>.
17. Carter SL, Cibulskis K, Helman E, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol*. 2012;30(5):413–21. <https://doi.org/10.1038/nbt.2203>.
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Stat Methodol*. 1995;57(1):289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
19. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res*. 2020;48(W1):W509–14. <https://doi.org/10.1093/nar/gkaa407>.
20. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol*. 2017;18(1):220. <https://doi.org/10.1186/s13059-017-1349-1>.

21. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* 2017;77(21):e108–10. <https://doi.org/10.1158/0008-5472.CAN-17-0307>.
22. Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol.* 2016;17(1):218. <https://doi.org/10.1186/s13059-016-1070-5>.
23. Sturm G, Finotello F, Petitprez F, et al. Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. *Bioinformatics.* 2019;35(14):i436–45. <https://doi.org/10.1093/bioinformatics/btz363>.
24. Avila Cobos F, Alquicira-Hernandez J, Powell JE, Mestdagh P, De Preter K. Benchmarking of cell type deconvolution pipelines for transcriptomics data. *Nat Commun.* 2020;11(1):5650. <https://doi.org/10.1038/s41467-020-19015-1>.
25. Bowman RL, Klemm F, Akkari L, et al. Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. *Cell Rep.* 2016;17(9):2445–59. <https://doi.org/10.1016/j.celrep.2016.10.052>.
26. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nat Neurosci.* 2016;19(1):20–7. <https://doi.org/10.1038/nn.4185>.
27. Quail DF, Joyce JA. The microenvironmental landscape of brain tumors. *Cancer Cell.* 2017;31(3):326–41. <https://doi.org/10.1016/j.ccell.2017.02.009>.
28. Klemm F, Maas RR, Bowman RL, et al. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. *Cell.* 2020;181(7):1643–e166017. <https://doi.org/10.1016/j.cell.2020.05.007>.
29. Friebel E, Kapolou K, Unger S, et al. Single-cell mapping of human brain cancer reveals tumor-specific instruction of tissue-invading leukocytes. *Cell.* 2020;181(7):1626–e164220. <https://doi.org/10.1016/j.cell.2020.04.055>.
30. Pombo Antunes AR, Scheyltjens I, Lodi F, et al. Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization. *Nat Neurosci.* 2021;24(4):595–610. <https://doi.org/10.1038/s41593-020-00789-y>.

Figures



Analysis subsets overlap. OS exclusions: TCGA 4; CGGA693 7; CGGA325 7.
TCGA: 98 lack ABSOLUTE purity; 2 further samples lack ESTIMATE scores.



Expression values are not pooled across cohorts. Discordant: IDH-wildtype with 1p/19q codeletion.

Figure 1

Cohort selection and HTR2C expression across molecular subtypes

(a) Selection of patients for the TCGA and two CGGA RNA-sequencing cohorts. Base cohorts included adults with primary tumors of historical histological grades II or III and complete age, sex, grade, molecular-subtype and HTR2C expression data. The TCGA source cohort contained 516 primary patients. The clinical and molecular complete cohorts comprised 509, 208 and 139 patients, respectively. Analysis subsets overlap and were selected separately for overall survival (OS) and microenvironment analyses. Discordant CGGA annotations refer to IDH-wildtype tumors annotated as 1p/19q-codeleted. (b–d) HTR2C expression in the three molecular subtypes in the corresponding base cohorts. Points represent individual patients. Boxes show the 25th and 75th percentiles, with the median marked inside; whiskers extend to the most extreme values within 1.5 interquartile ranges. All patient points are displayed. TCGA values are $\log_2(\text{RSEM} + 1)$, whereas CGGA values are $\log_2(\text{FPKM} + 1)$. Panels

are descriptive; expression values were not pooled or directly compared across datasets. Molecular labels and historical grades do not constitute complete WHO 2021 integrated diagnoses. Data sources, eligibility and analysis membership are provided in Supplementary Table S1

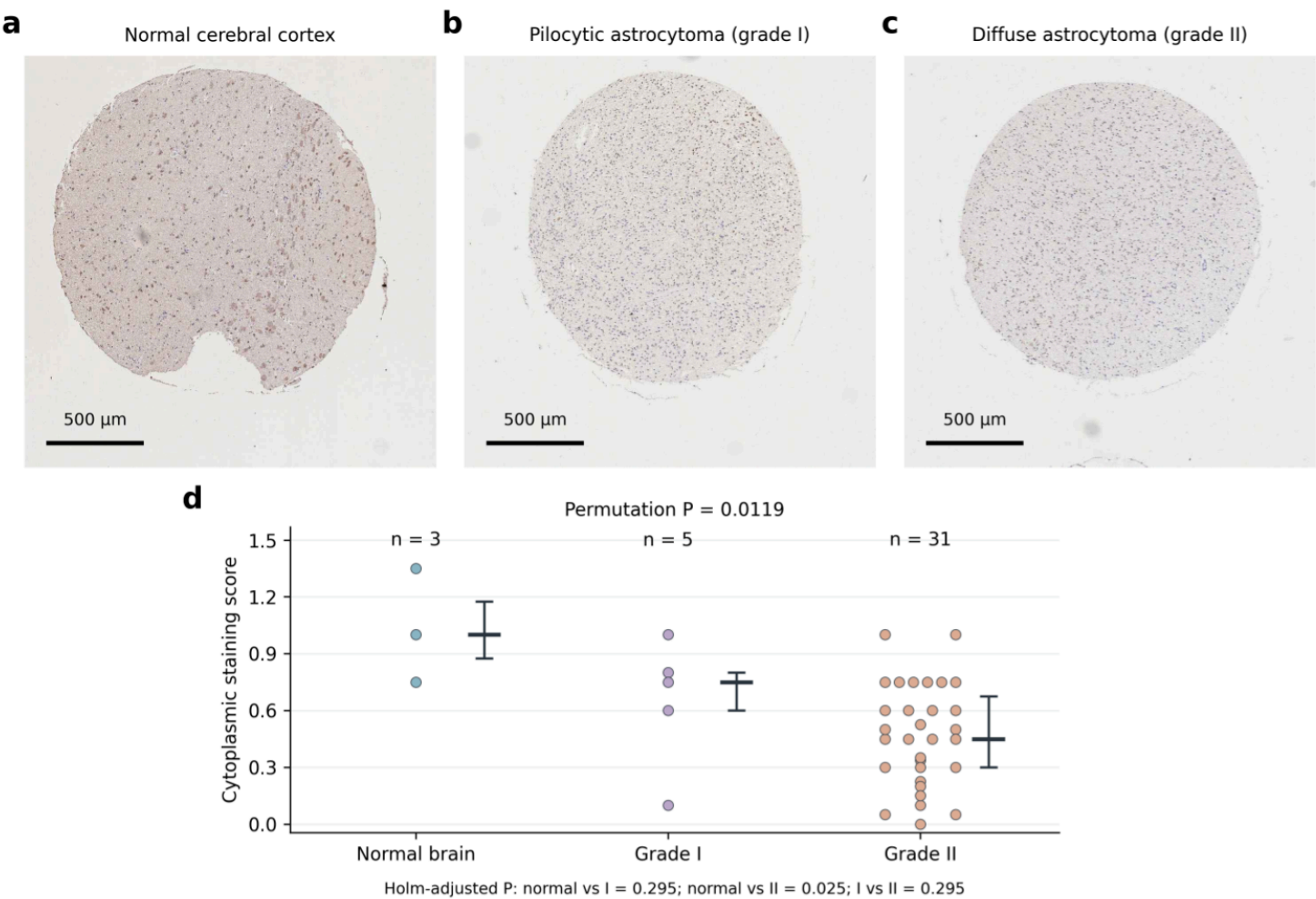


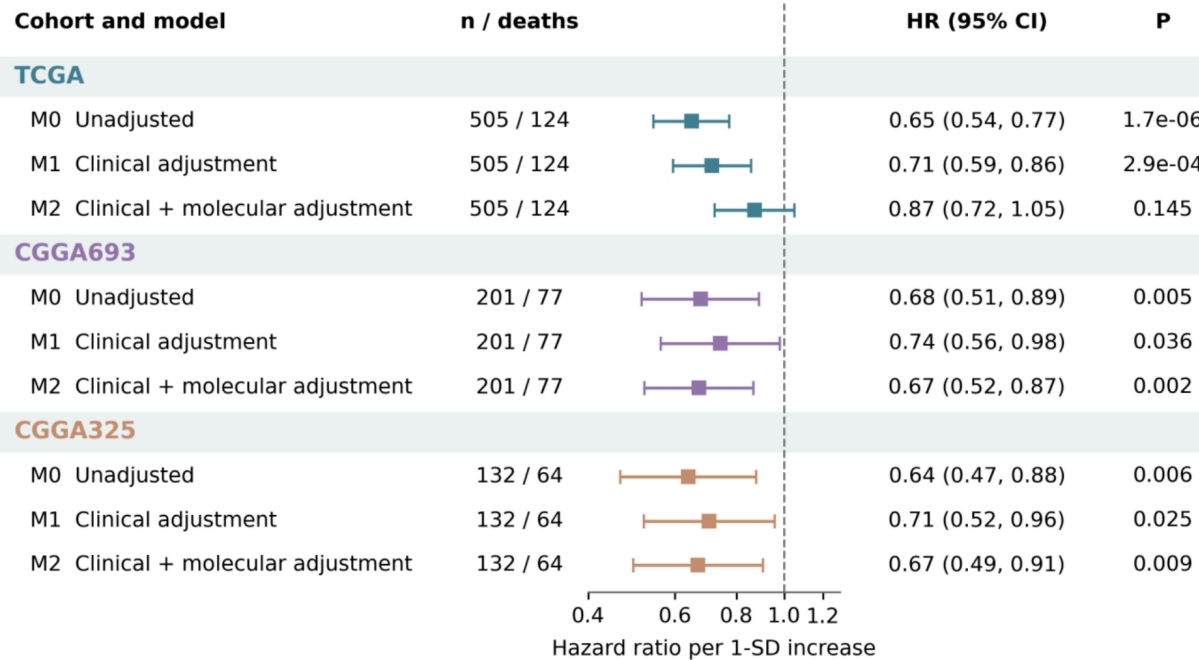
Figure 2

HTR2C immunohistochemistry in normal brain tissue and historically classified grade I and II tumors

Representative tissue cores are shown for (a) normal cerebral cortex (A01), (b) pilocytic astrocytoma, historical grade I (A07), and (c) diffuse astrocytoma, historical grade II (B03). The selected cores had cytoplasmic staining scores equal to the median of their respective groups. Scale bars, 500 µm. (d) Cytoplasmic staining scores in normal brain tissue (n = 3), grade I tumors (n = 5), and grade II tumors (n = 31). Each point represents one patient, with one core per patient. Horizontal offsets separate overlapping observations. The bars beside each group indicate the median and interquartile range. Scores were calculated as staining intensity multiplied by the positive-cell fraction (0–1); intensity ranges of 0.5–1 and 1–2 were represented by their midpoints, 0.75 and 1.5, respectively. Cores C11 and C12 were excluded because of tissue loss, and C14 was excluded because of partial tissue loss. Histological diagnoses and grades follow the original tissue records. The omnibus P value was obtained by permuting group labels 999,999 times using the tie-corrected Kruskal–Wallis statistic. Pairwise

comparisons used two-sided exact permutation Mann–Whitney tests with Holm correction across all three comparisons

a Sequential adjustment models



b Sensitivity analyses

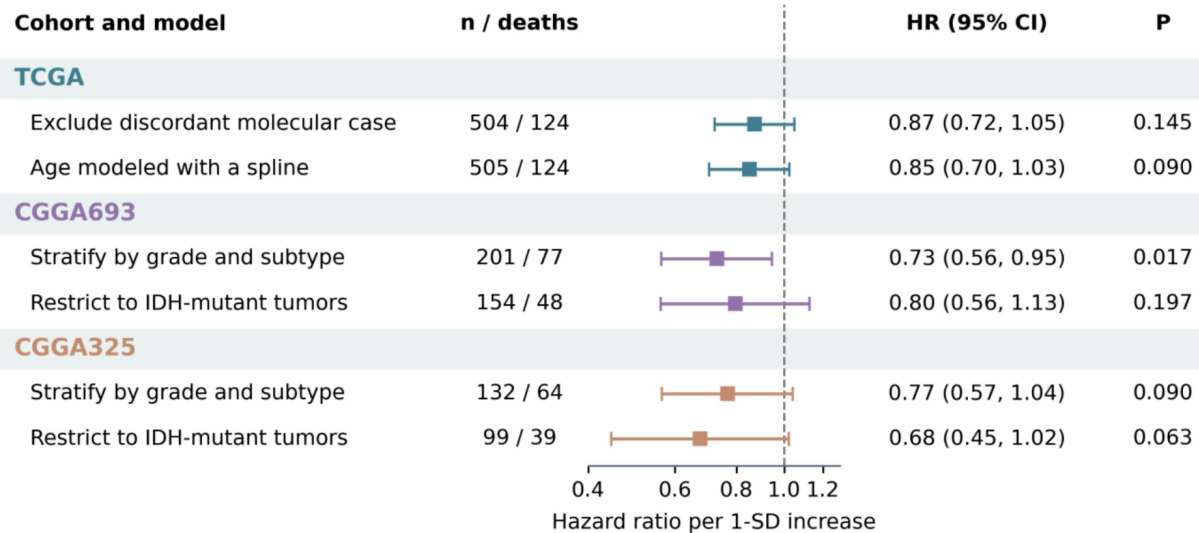


Figure 3

Association between continuous HTR2C expression and overall survival across three transcriptomic cohorts

Squares indicate hazard ratios (HRs), and horizontal lines indicate 95% confidence intervals (CIs). Expression was standardized within each primary survival cohort after $\log_2(\text{RSEM} + 1)$ transformation in TCGA or $\log_2(\text{FPKM} + 1)$ transformation in CGGA. HRs represent a one-standard-deviation increase in expression. (a) M0 was unadjusted; M1 adjusted for age, sex and historical histological grade; M2 additionally adjusted for IDH/1p/19q-defined molecular subtype. The same complete-case population was used for M0–M2 within each cohort: TCGA, 505 patients and 124 deaths; CGGA693, 201 patients and 77 deaths; CGGA325, 132 patients and 64 deaths. (b) TCGA sensitivity analyses excluded one case with discordant molecular information or modeled age using a restricted cubic spline. CGGA analyses stratified the baseline hazard jointly by grade and molecular subtype, with adjustment for age and sex, or restricted the population to IDH-mutant tumors, with adjustment for age, sex, grade and 1p/19q-defined subtype. Sensitivity analyses retained the expression standardization of their primary survival cohort. The CGGA sensitivity models were fitted after proportional-hazards diagnostics identified departures for other covariates. P values are two-sided Wald P values without multiplicity adjustment. The dashed line indicates HR = 1. Primary and sensitivity estimates are provided in Supplementary Table S2

a Sequential adjustment in TCGA (n = 409)

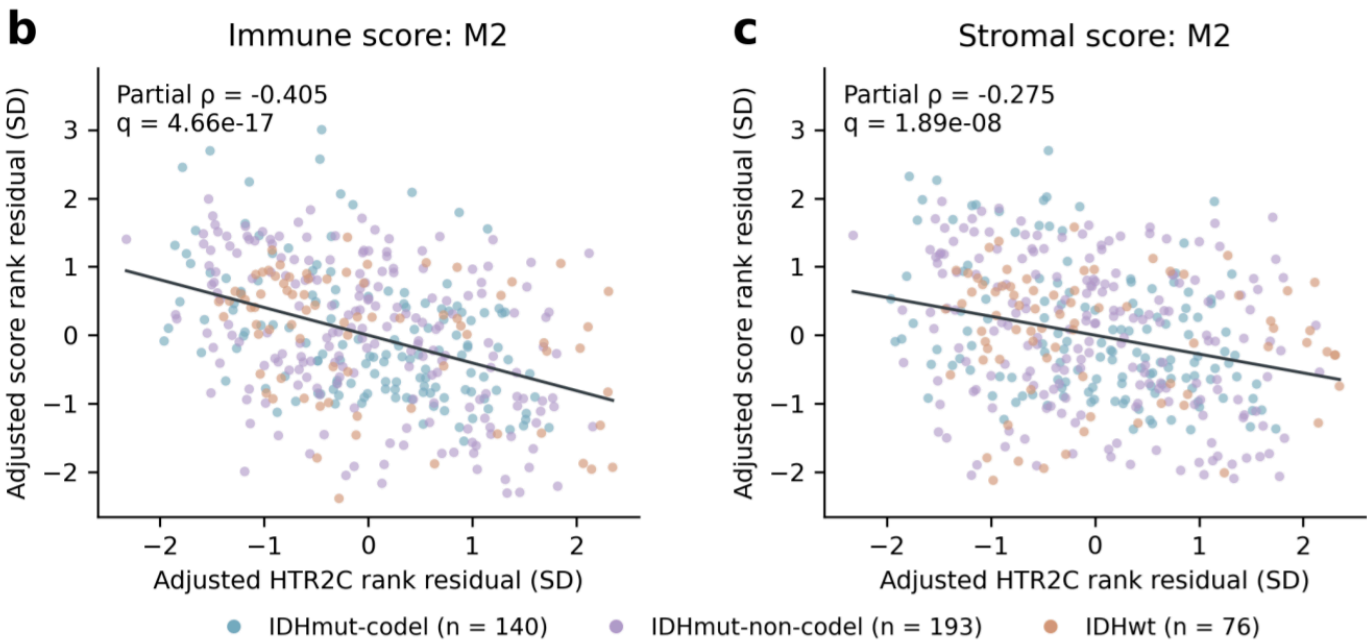
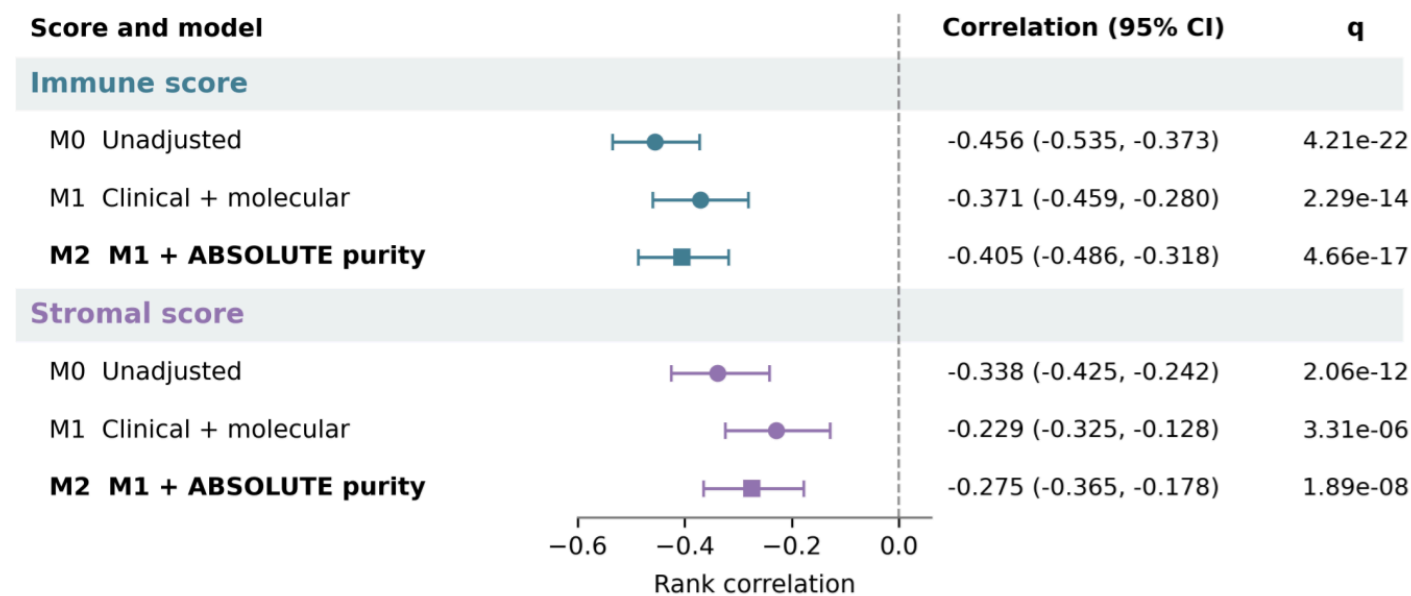


Figure 4

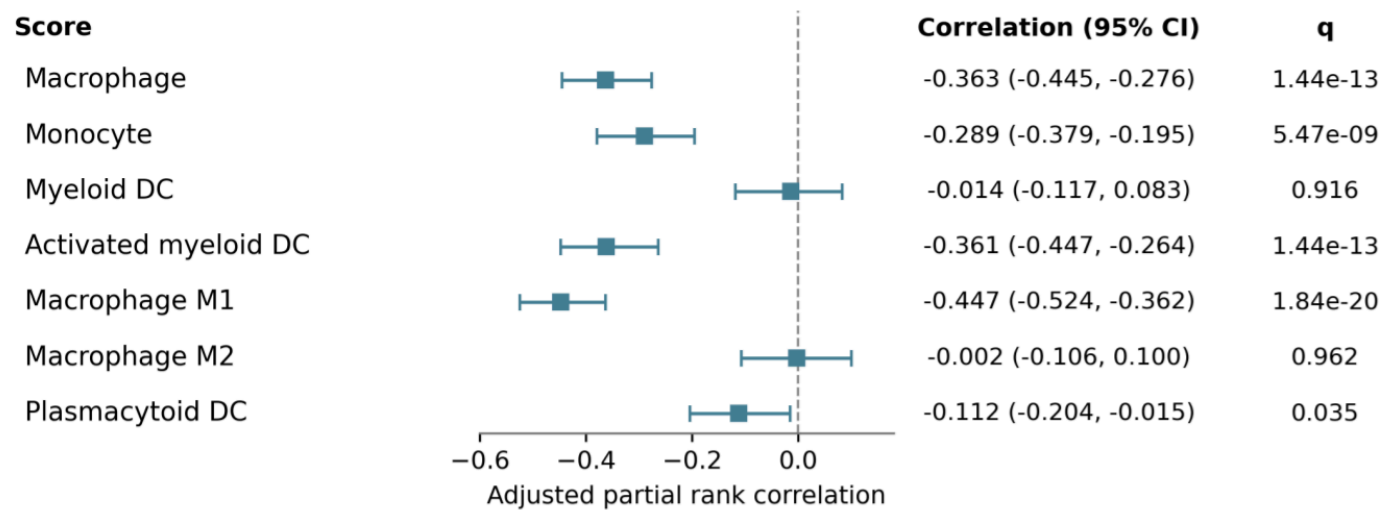
Associations of HTR2C expression with ESTIMATE immune and stromal scores in TCGA

All panels include the same 409 patients with complete clinical, molecular, ESTIMATE and ABSOLUTE purity data. (a) Estimates are shown without adjustment, after adjustment for age, sex, historical grade and molecular subtype, and after additional adjustment for ABSOLUTE tumor purity. Circles indicate the first two adjustment sets, and squares indicate the primary fully adjusted estimates. Horizontal bars show percentile 95% confidence intervals from 2,000 patient-level bootstrap samples, with ranking and covariate adjustment repeated in each sample. Two-sided P values were calculated using a t

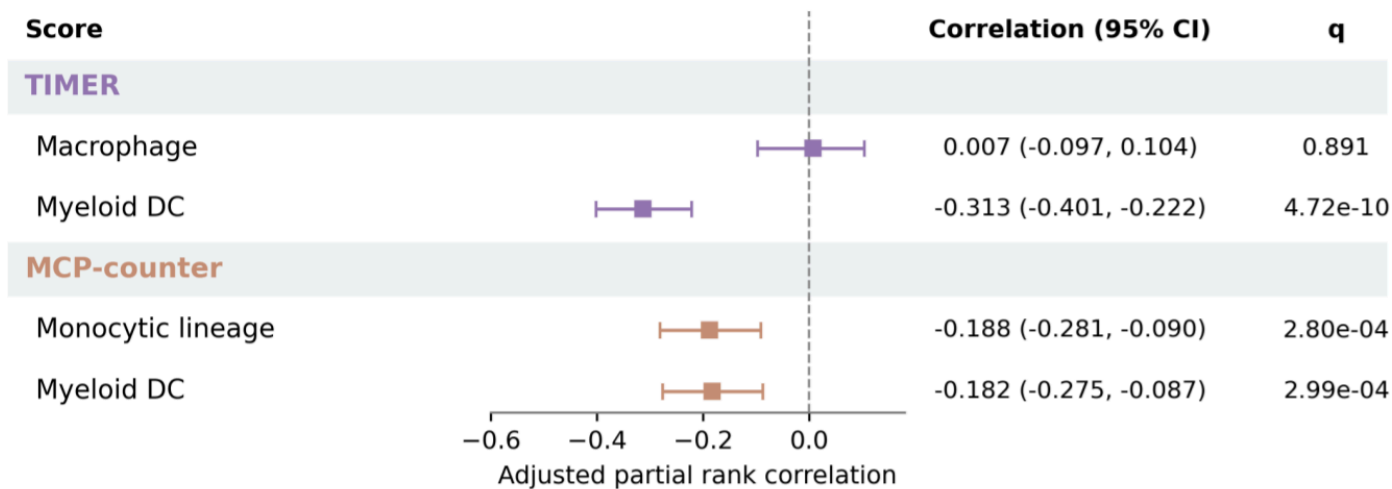
approximation for the residual correlations. The displayed BH-adjusted P values use Benjamini–Hochberg correction across the two scores within each adjustment set; the two fully adjusted tests constitute the primary testing family. (b, c) Fully adjusted rank residuals for HTR2C expression and the immune or stromal score, respectively, scaled to unit standard deviation for display. Each point represents one patient. Colors identify the molecular subtypes; the line summarizes the overall residual association. Subtypes comprise IDH-mutant, 1p/19q-codeleted tumors (n = 140), IDH-mutant, non-codeleted tumors (n = 193), and IDH-wildtype tumors (n = 76). Fully adjusted results and sensitivity analyses are provided in Supplementary Table S3

TCGA (n = 411)

a xCell scores



b Auxiliary analyses with TIMER and MCP-counter



Adjusted for age, sex, grade, molecular subtype and ABSOLUTE purity

Figure 5

Associations of HTR2C expression with myeloid-related scores in TCGA

All analyses include the same 411 patients with complete clinical and molecular information, ABSOLUTE purity and the selected cell scores. (a) Seven xCell scores. (b) Four auxiliary scores from TIMER and MCP-counter. Squares show partial rank correlations adjusted for age, sex, historical grade, molecular subtype and ABSOLUTE tumor purity. Horizontal bars indicate percentile 95% confidence intervals from 2,000 patient-level bootstrap samples, with ranks and covariate adjustment recalculated in each sample. Two-sided P values were obtained using a t approximation. Benjamini–Hochberg correction was applied separately to the seven xCell tests and the four auxiliary tests. The dashed line indicates zero correlation. All selected scores are shown, including nonsignificant results. The MCP-counter monocytic-lineage estimate corresponds to the source column Monocyte_MCPCOUNTER; the identical Macrophage/Monocyte_MCPCOUNTER column was counted once. DC, dendritic cell. M1, M2 and activated are algorithm-defined cell labels. All algorithms were evaluated in the same TCGA cohort; panel b is an algorithm sensitivity analysis. Fully adjusted results are provided in Supplementary Table S3

MCP-counter monocytic lineage (7/7 markers)

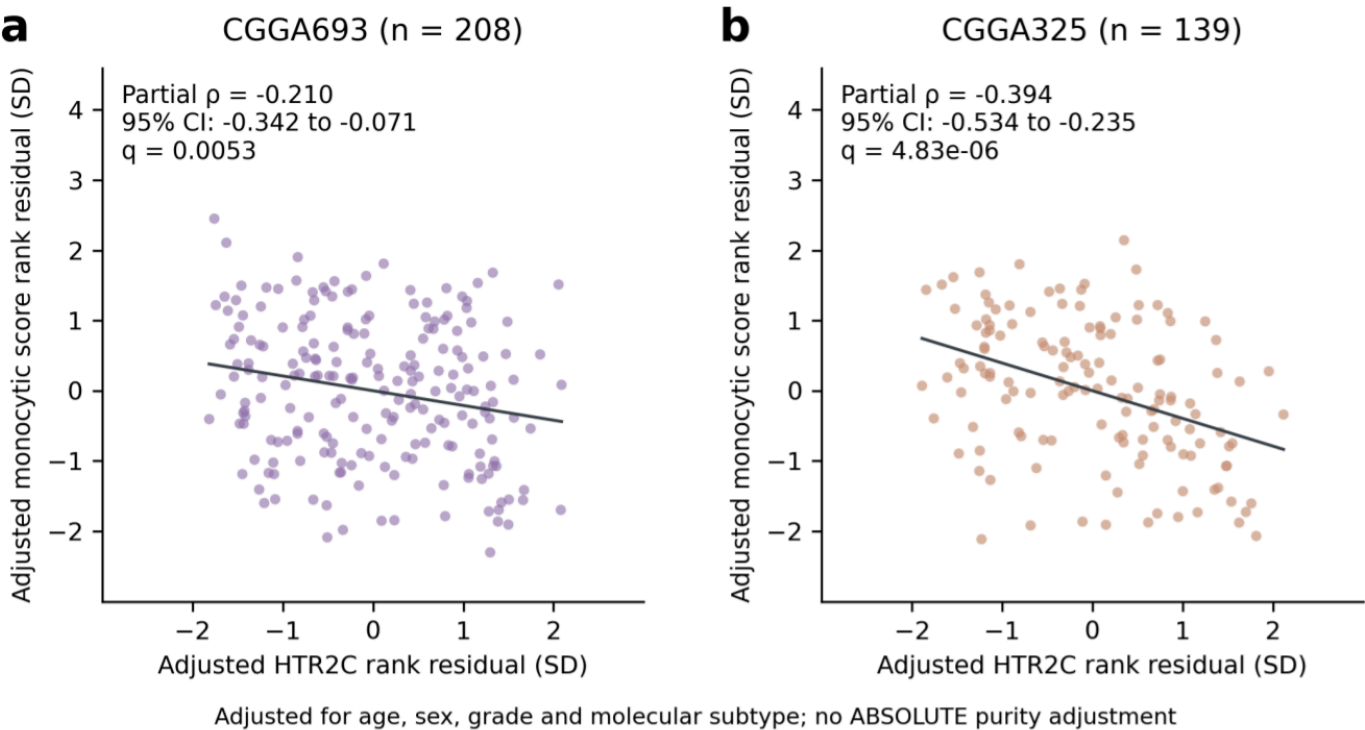


Figure 6

Association of HTR2C expression with the MCP-counter monocytic-lineage score in two CGGA cohorts

Adjusted rank residuals are shown for HTR2C expression and the monocytic-lineage score in (a) CGGA693 (n = 208) and (b) CGGA325 (n = 139). All seven monocytic-lineage markers were available in both cohorts. Scores were calculated as the arithmetic mean of $\log_2(\text{FPKM} + 1)$ expression across these

markers. Expression and score ranks were separately adjusted for age, sex, historical grade and molecular subtype, and residuals were scaled to unit standard deviation for display. Each point represents one patient; lines summarize the residual associations. Partial rank correlations and percentile 95% confidence intervals from 2,000 patient-level bootstrap samples are shown. The BH-adjusted P values use Benjamini–Hochberg correction across the two evaluated scores within each cohort, including the incomplete myeloid dendritic-cell score reported in Supplementary Table S4. CGGA models did not include ABSOLUTE purity. The two cohorts were analyzed separately

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementtable.docx](#)