

LncRNA HOTAIR Promotes Tumorigenicity in Glioblastoma

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Research Article

Keywords: HOTAIR, GBM, WHO CNS5, prognosis, therapy

Posted Date: February 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1340987/v1>

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Abstract

Background

Recently, the 2021 WHO Classification of Tumors of the Central Nervous System (fifth edition) was published. WHO CNS5 incorporates numerous molecular changes to the accurate classification of CNS neoplasms. GBM, as a special type of glioma, is confined to IDH-wildtype in WHO CNS5, which has a short medical history and poor prognosis. LncRNA *HOTAIR* has been found to be responsible for the poor prognosis of several human cancers in previous studies. The clinical significance, the prognostic and treatment value of *HOTAIR* for GBM in the new WHO CNS5 classification remain unclear.

Methods

We created a Random Forest-based prediction model to assess the *IDH1* mutational status of patients in REMBRANDT cohorts. Then we analyzed the role and the potential mechanism of *HOTAIR* in TCGA, CGGA, and REMBRANDT cohorts. GSVA and GSEA analysis were used to detect the potential biological functions and signaling pathways. The infiltration of immune cells was quantified by Cibersort. The effectiveness of targeted therapy in the clinical application was sought through drug response analysis. Furthermore, we verified that *HOTAIR* affected the proliferative activity of that glioma cells.

Results

We found that *HOTAIR* is highly expressed in all cohorts. The patients' samples with high *HOTAIR* expression have poorer overall survival. The results of the functional analysis indicated that the cell cycle and proliferation-related processes were enriched in the high *HOTAIR* expression group. The infiltration of immunocytes is different in the two groups. In multiple immune checkpoints, the risk score showed a strong correlation. The analysis of drug treatment response present that the high *HOTAIR* expression group has a better treatment response and better curative effect on the treatment of temozolomide, sorafenib, lenalidomide, bexarotene, and axitinib.

Conclusion

In conclusion, We found that *HOTAIR* could lead to poor prognosis of GBM mainly through regulation of cell cycle and apoptosis. And *HOTAIR* could be used as a marker to guide chemotherapy in GBM patients rather than immuncheckpoint inhibitor therapy.

Introduction

Gliomas are the most common primary malignant tumor in the brain that arise from glial cells. Glioblastoma (GBM), which is characterized by highly malignant and rapidly progressive, has a median overall survival (OS) of only 15months after being treated with radiation and adjuvant temozolomide chemotherapy (Stupp protocol)[1, 2]. The fifth edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (WHO CNS5) was recently released[3].

The distinction between adult and pediatric diffuse gliomas is being made for the first time. Adult-type diffuse gliomas are classified as three types: astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; glioblastoma, IDH-wildtype[3]. These provide adequate conditions for selecting more homogeneous subjects for future clinical studies via reclassifying the pathologic types with the same molecular characteristics and similar prognosis into one type. With further research on the development of GBM, the role of new molecular markers in pathological diagnosis targeted therapy and immunotherapy and prognostic evaluation needed to be further explored. In this study, GBMs included were under the WHO CNS5 classification standard.

HOX transcript antisense RNA (*HOTAIR*) is a well-studied carcinogenic lncRNA located on human chromosome 12q13. It was the first lncRNA to be discovered to influence gene expression in *trans* fashion, and it is now suspected of playing a role in the carcinogenesis of several human cancers[4]. It has been reported that *HOTAIR* regulated the invasion and metastasis of breast carcinomas[5] and colorectal cancer[6] in a PCR2-dependent manner. Moreover, *HOTAIR* could block the expression of microRNA to promote bladder cancer[7], esophageal cancer[8], and gall bladder cancer[9] proliferation and invasion as a competing endogenous RNA. *HOTAIR* expression levels were found to be significantly positively correlated with tumor grade in a study of 295 glioma samples from the Chinese Glioma Genome Atlas (CGGA), Repository of Molecular Brain Neoplasia Data (REMBRANDT), and GSE4290 datasets. Glioma patients with high *HOTAIR* levels had shorter overall survival[10]. Concordantly, another study based on The Cancer Genome Atlas (TCGA), four datasets from the Oncomine database, and two independent Portuguese and French glioma series, confirmed that *HOTAIR* expression is far more frequent in grades III and IV than in grade II gliomas and normal brain[11]. They all demonstrated that *HOTAIR* could be a clinically-relevant biomarker of prognosis in GBM (2016 WHO CNS)[10, 11]. Additionally, *HOTAIR* may be responsible for malignant progression and poor prognosis of glioma patients by regulating cell cycle[10].

However, the prognostic value of *HOTAIR* for GBM in the new WHO CNS5 classification and its molecular mechanisms underlying remain elusive. In this context, we aimed to clarify the clinical significance and function of *HOTAIR* in GBM samples by analyzing clinical and molecular pathology features.

Methods

Publicly available sample collection

All datasets used throughout this study are available to the public. Transcriptome data and clinical information of 205 patients of the CGGA cohort[12, 13] were obtained from the CGGA website (<http://www.cgga.org.cn>). The clinical phenotype of 564 patients and expression data of normalized combined the TCGA brain lower-grade glioma (LGG) and glioblastoma multiforme (GBM) were obtained from UCSC Xena website[14] (<http://xena.ucsc.edu>). The CGGA and TCGA cohorts were the training cohorts. 179 patients from the REMBRANDT cohort[15] and associated clinical information were obtained from the GliVis website (<http://gliovis.bioinfo.cnio.es>) as a validation cohort. The

transcriptome data from GSE104722 was taken to construct the *IDH1* mutant prediction model obtained from the GEO website (<http://www.ncbi.nlm.nih.gov/geo>). The details of these datasets are listed in Table 1.

***IDH1* mutation status prediction**

We created a Random Forest (RF)-based prediction model by R package “randomForest”[16] to assess the *IDH1* mutational status of patients in REMBRANDT cohorts. The RNA-seq data of GSE104722 have been made to develop this prediction model, and samples with available *IDH1* mutational status in TCGA was used as external validation for evaluating the model performance (Since the normalization method of transcriptome data of CGGA is different from that of GSE104722 and TCGA, CGGA is not taken as a validation set.). Differential expression analysis between *IDH1* mutant and *IDH1* wild-type samples was performed to select informative genes which served as input into the RF model ($\text{abs}(\text{Log}_2\text{FC}) > 1$ & adjust $P < 0.05$). Specifically, using the out-of-bag (OOB) error as a minimization criterion, at each iteration, the least important 20% of genes were removed until the OOB error rate reached its minimum. The final RF prediction model was established with the genome with the lowest OOB error rate. Subject operating characteristic (ROC) curves were used to evaluate the predictability of the model in training and validation tests.

Pathway enrichment analysis

Based on the expression profile of each sample between high and low *HOTAIR* expression groups, Gene Set Variation Analysis (GSVA) and Gene set enrichment analysis (GSEA) were conducted to estimate the score of gene-set in each group. GSVA was applied to enrich hallmark gene sets using the “GSVA”[17] R package, while the kegg gene sets were as the reference in GSEA using the “clusterProfiler”[18] R package. The enrichment score of each gene-set more than 1.2 and adjusted P -value less than 0.05 was regarded as significantly enriched.

The exploration of the immune microenvironment

To further explore the immune microenvironment in GBM, the relative abundance of 22 immune cells in each patient was computed by “Cell type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT)”[19]. Moreover, single-sample GSEA (ssGSEA)[20] in the “gsva” R package was performed to calculate the relative abundance of 28 immune cells. Sets of characteristic genes for each immune cell type were obtained from the previous studies[21]. We calculated the correlation between *HOTAIR* expression level and the abundance of 28 immune cells.

The response to immune checkpoint blockade prediction

Based on the tumor that before immuncheckpoint inhibitor including anti-PD1 and anti-CTLA4 treatment expression profiles, the TIDE module can estimate patient response to immune checkpoint blockade[22] (<http://tide.dfci.harvard.edu>).

Drug Sensitivity Analysis

Based on the GDSC2 dataset in Genomics of Drug Sensitivity in Cancer (GDSC) [23], the largest open pharmacogenomics database, a ridge regression model was constructed to predict the drug treatment response of each sample based on the transcriptome data using “pRRophetic” package. In the present study, we estimated the half-inhibitory concentrations (IC50) of temozolomide, sorafenib, lenalidomide, bexarotene, and axitinib of each individual.

Cell culture and cell transfection

The human glioma cell line U251 was purchased from the College of Life Science of Wuhan University (Wuhan, China). All cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, HyClone, USA) with a standard humidified incubator under 5% CO₂ at 37°C.

The full-length complementary cDNAs of human *HOTAIR* were synthesized and cloned into the pcDNA3.1 vector (Sangon Biotech). Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific) was used to transfect the plasmids into U251 cells according to the manufacturer's protocol. The cells transfected for 48h were used for subsequent experiments.

Real-time PCR

RNA was extracted from U251 cells by using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Isolated RNA was reverse transcribed to cDNA using the cDNA synthesis kit (Thermo Fisher, TOYOBO, Japan). The resulting cDNAs were subjected to quantitative real-time PCR with a Bio-Rad CFX Connect real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The primer sequences are shown in Table 2. The relative quantitative value for each gene was calculated using the $2^{-\Delta\Delta Ct}$ method.

CCK-8 and colony formation assays

Transfected U251 cells were seeded in 96-well plates and cell viability was assessed using the Cell Counting kit-8 (CCK-8; Vazyme, Nanjing, China) according to the manufacturer's protocol. The absorbance at 450nm was measured using a spectrophotometer.

In the colony formation assay, cells were seeded in six-well plates and cultured for 14 days after transfection. Then, we used PBS to wash the resulting colonies twice, and used 4% formaldehyde to fix them for 15 min, finally used 0.1% crystal violet to stain for 15 min.

Statistical analysis

All statistical tests were conducted in R statistical software (Version 4.0.2) and GraphPad Prism 8.4.0 (GraphPad Software, San Diego, CA, USA) software. We used Student's t-test, Wilcoxon rank-sum test, and Permutation test in this study. The relationship between *HOTAIR* and other continuous variables was measured by the Spearman method. Survival analysis was carried out using Kaplan–Meier curve, and the log-rank test was used to determine the statistical significance of differences. Multivariate cox proportional hazard regression was used to explore the related independent predictors of the prognosis. All reported *P*-values were two-sided, and the statistical significance was set at 0.05.

Results

Clinical characteristics of rearrangement of included populations

According to the latest classification criteria for gliomas from WHO[3], WHO grade 4 astrocytomas, IDH mutant in WHO CNS5 is mainly consistent with glioblastoma, IDH-mutant in the 2016 World Health Organization (WHO) Classification of Tumors of the Central Nervous System (2016 CNS WHO)[24]. Glioblastoma, IDH-wildtype in WHO CNS5 includes the glioblastoma, IDH-wildtype in 2016 CNS WHO. Therefore, IDH-wildtype glioblastoma patients in the previous classification were reserved for GBM in this study. IDH-mutant, WHO grade II-III gliomas were retained as IDH-mutant gliomas in the previous classification. While IDH-wildtype, WHO grade II-III gliomas combination of *TERT* promoter mutation or *EGFR* gene amplification or the combined gain of entire chromosome 7 and loss of entire chromosome 10 (+7/-10) were classified as GBM[25]. In this study, all gliomas included were reclassified according to WHO CNS5. A total of 948 glioma patients with prognostic information were included from three clinical cohorts (TCGA, CGGA, REMBRANDT). The detailed information of all clinical cohorts in this study is summarized in Table 1, and the flow chart of this study design is shown in Figure 1.

A robust model predicts *IDH1* mutations based on transcriptome data

Utilizing the RF method, an effective prediction model of *IDH1* mutation was constructed on the GSE104722 cohort. As shown in Figure 2a, this prediction model can accurately divide the 20 samples of GSE104722 into 2 groups (*IDH1* wild-type and *IDH1* mutant). The prediction accuracy of the model was 100% in the GSE104722 cohort and 95.7% in the TCGA cohort. The AUC of the model was 1.00 in the

training cohort and 0.96 in the validation cohort, respectively, indicating that the model could effectively predict *IDH1* mutation in other transcriptome cohorts (Figure 2b, 2c). Next, we applied the model to GSE104722 to identify the estimated *IDH1* wild-type samples. For subsequent studies, GBM samples from TCGA and CGGA cohorts were invoked as a training set, and GBM samples estimated from the REMBRANDT cohort were used as an independent test set.

The expression of *HOTAIR* in GBM is higher and the higher the expression level, the worse the prognosis

Firstly, lncRNAs with abnormal expression in GBM than other types of glioma were screened out from TCGA and CGGA cohorts respectively (Figure 3a). *HOTAIR* is highly expressed in both cohorts and verified in REMBRANDT cohorts (Figure 3b, c). According to the median expression of *HOTAIR*, patients in the training and test sets were split into the high *HOTAIR* expression group and the low *HOTAIR* expression group. Kaplan-Meier survival analysis showed that GBM patients with low expression of *HOTAIR* had significantly higher OS than patients with high expression of *HOTAIR* ($P < 0.05$; Figure 3d). Multivariate analysis was performed on GBM patients in TCGA, CGGA, and REMBRANDT cohorts to assess whether *HOTAIR* was an independent prognostic factor. Variables with statistical significance ($P < 0.05$) were used as independent prognostic factors in multivariate analysis. *HOTAIR* was found to be an independent prognostic factor in TCGA and CGGA cohorts (Figure 3e). Figure 3f illustrates the relationship between *HOTAIR* and routine clinical and molecular features in TCGA, CGGA, and REMBRANDT cohorts, respectively, there is a significant correlation between the *HOTAIR* subgroup and TCGA subgroups in CGGA.

Identification of *HOTAIR*-related biological processes

Gene Set Variation Analysis (GSVA) and Gene Set Enrichment Analysis (GSEA) were carried out to investigate which biological processes were associated with poor prognosis in GBM patients with high *HOTAIR* expression. Firstly, the expression profiles of patients with high and low expression of *HOTAIR* were analyzed by GSVA. We believe that the biological process of enrichment in the high *HOTAIR* expression group is related to the poor prognosis of patients. The results reported that cell cycle-related processes, such as G2M checkpoint and E2F targets, showed the highest correlation with poor prognosis. Proliferation-related processes, such as Myc targets V1 and Myc targets V2, autophagy-related mTORC1 signaling, and angiogenesis were also related to poor prognosis. Meanwhile, the apoptosis and metabolism-related processes, such as cholesterol homeostasis, fatty acid metabolism, and bile acid metabolism, were enriched in the group with low expression of *HOTAIR* (Figure 4a). The results of GSEA also suggested that the disorder of cycle-related processes may be a possible factor resulting in poor prognosis in patients with high *HOTAIR* expression (Figure 4b). It has been reported that the knockdown of *HOTAIR* induced the cell cycle arrest in G0/G1 phase in glioma cells[26]. Thus, we analyzed the relationship of the expression of *HOTAIR* and cell cycle markers (*CCNB1*(cyclin B1), *CCND1*(cyclin D1),

CDC25A, and *CDC25C*) and apoptosis markers (*CASP3*, *BAX*, *BCL2*, *MCL1*). We found that the higher *HOTAIR* expression was positively correlated with these markers except *BAX* (Figure 4c, 4d). These results indicate that *HOTAIR* might promote the transformation of the cell cycle process and inhibit apoptosis, leading to a poor prognosis of GBM patients.

The relationship between *HOTAIR* and tumor immune microenvironment.

From the GSVA results of TCGA and CGGA cohorts, there is an interesting phenomenon that immune-related pathways, such as inflammatory response and interferon-gamma response, are enriched in different groups (Figure 4a). In CGGA, the inflammatory response pathway was enriched in high *HOTAIR* expression patients, while the result of TCGA was the opposite. Thus, we estimated the composition of 22 immune cells in each patient of CGGA and TCGA cohorts by CIBERSORT. Comparing the composition of the immune cells of high and low *HOTAIR* expression groups, the patients in the high *HOTAIR* expression group had a lower proportion of CD8+ T cells and monocytes in TCGA, and activated natural killer cells in CGGA, but a higher proportion of follicular helper T cells in TCGA, and activated memory CD4+ T cells, and activated dendritic cells in CGGA (adjusted $P < 0.05$, Figure 5a). Only M0 macrophages were statistically different between the two risk groups in both the TCGA and the CGGA cohort. Combined with the results of ssGSEA, there was no correlation between the expression level of *HOTAIR* and the activated immune cells (adjusted $P > 0.05$, Figure 5b). Since the enrichment of immune cells and immune pathways in TCGA and CGGA was different, we speculated this might lead to differences in immunotherapy. So, the relationship of *HOTAIR* and immune checkpoint markers was analyzed in two cohorts. Some immune checkpoint genes, such as *PDCD1*(PD1), *CTLA4*, and *LAG3* have a relatively higher expression in the high *HOTAIR* expression group in both two cohorts, especially *LAG3* ($P < 0.05$ in both TCGA and CGGA cohorts). However, *CD274*(PDL1) and *PDCD1LG2*(PD-L2) were negatively correlated with *HOTAIR* expression in TCGA, but it was the opposite in CGGA, especially *PDCD1LG2*(PD-L2) ($P < 0.05$ in both TCGA and CGGA cohorts) (Figure 5c). Due to the intensity of intratumoral CD8+ T cell infiltrates and tumor programmed cell death ligand 1 (PDL1) expression have been proposed as distinct biomarkers of response to anti-PDL1 therapies[27, 28]. In addition, in TCGA and CGGA cohorts, the density of CD8+ T cell and the expression of PDL1 were different in the *HOTAIR* subgroups, although it was not statistically significant. Hence, we evaluated the therapeutic response in two cohorts to immune checkpoint inhibitors via TIDE. The results showed that there was no significant correlation between the expression of *HOTAIR* and patients' response to immune checkpoint inhibitors. Interestingly, we found more patients responded to immune checkpoint blockade in CGGA than in TCGA (Figure 5d). The IC50 of other commonly used antitumor drugs was predicted in different groups by using the pRRophetic algorithm. The high *HOTAIR* expression group had higher IC50 of the chemotherapeutic agents (Figure 5e).

HOTAIR promotes the proliferation of glioma cells

Based on our findings and previous studies, *HOTAIR* promotes cell proliferation by regulating the cell cycle and apoptosis. The role of *HOTAIR* in GBM was confirmed by the cell experimental method. *HOTAIR* was overexpressed in glioma cell line U251, and the transduction efficiency was confirmed by qRT-PCR (Figure 6a). Cell proliferation assays, as well as colony formation assays, were performed to investigate the influence of *HOTAIR* overexpression on tumor malignancy in GBM cells. The results indicated that *HOTAIR* overexpression promoted cell growth (Figure 6b), showed a marked increase in colony-forming ability (Figure 6c).

Discussion

Glioblastomas were divided in the 2016 CNS WHO into GBM, IDH-wildtype, and GBM, IDH-mutant[24]. Compared to IDH-wildtype GBM, IDH-mutant GBM showed some molecular phenotypic changes, such as fewer *EGFR* amplification (rare vs 35%), *PTEN* mutations (rare vs 24%), and *TERT* promoter mutations (26% vs 72%), but more *ATRX* mutations (71% vs rare) and *P53* mutations (81% vs 27%)[24]. These differences suggested that IDH-wildtype and IDH-mutant GBMs carried different molecular contexts. Given that they had different driving genes, molecular characteristics, and clinical prognosis, all IDH-mutant diffuse astrocytic tumors are considered a single type in WHO CNS5, and the IDH-mutant GBM are classified as astrocytoma, IDH-mutant, CNS WHO grade 4, instead of GBM[3]. Gliomas exhibited pronounced heterogeneous are prone to confusion in clinical diagnosis and treatment[29]. The changes of classifying gliomas into more pathological types based on the combination of biological and molecular markers are more in line with the natural course of the disease. The new classification will enable clinicians to judge the prognosis of patients, choose the more suitable treatment and promote the exploration of novel treatments and evaluate the therapeutic effect of a new therapy. We aimed to explore the role and function of *HOTAIR* in GBM in WHO CNS5.

In previous studies, *HOTAIR* positively regulated an 18 genes cell cycle-related mRNA network in human glioma samples obtained from the CGGA and verified in GBM cells[30]. The results of our study showed that *HOTAIR* played a pro-oncogenic role also mainly by accelerating cell cycle conversion and inhibiting apoptosis in GBM. It manifested that there was no difference in the functional mechanism of *HOTAIR* with the changes of classification in glioma, and further highlighting the critical role of *HOTAIR* in regulating the cell cycle during gliomagenesis.

Furthermore, we also found that the immune-related pathways were enriched in different *HOTAIR* subgroups in different cohorts, and *HOTAIR* was significantly correlated with CD4+ T cells, monocyte cells, and dendritic cells, and some immune checkpoint genes (*LAG3*, PD-L2), suggesting that *HOTAIR* might be involved in the immune regulation of GBM patients. It was reported that *HOTAIR* was required for the secretion of various cytokines and inflammatory factors including IL-6, iNOS, TNF α , and MIP-1B induced by LPS-treatment macrophage, and played a central role in NF- κ B activation upon stimulation with LPS[31]. Additionally, *HOTAIR* regulated glucose metabolism in macrophages potentially to meet the energy needs during the immune response[32]. Combined with our findings, *HOTAIR* may be involved in immune signaling and inflammatory responses in GBM, which may be one of the reasons for

the poor prognosis of patients. However, the predicted therapeutic response results in TIDE showed no difference between the two groups, manifesting that *HOTAIR* might not be responsible for immune checkpoint inhibitors in GBM. But possibly due to racial differences, the response rate of CGGA patients to immunotherapy was significantly higher than that of TCGA patients.

Recently, *HOTAIR* was found to mediate the GBM chemoresistance through mediating the expression of hexokinase 2 by targeting miR-125[33]. Exosome-mediated transfer of lncRNA *HOTAIR* regulated TMZ resistance by sponging miR-519a-3p in a study including 51 GBM patients receiving temozolomide treatment[34]. We also found significant differences in the sensitivity of temozolomide and sorafenib in different *HOTAIR* subgroups. In addition, *HOTAIR* was reported to be detected in GBM serum exosomes and observed a reduction of serum *HOTAIR* levels after surgery and a further reduction at the 2 weeks post-surgery follow-up in one recurrent GBM patient[35]. The si-*HOTAIR* has been shown to be successfully delivered via superparamagnetic iron oxide nanoparticles to promote the expression of *PDCD4* at the transcriptional level, thereby reducing the proliferation, invasion, and tumorigenicity of human glioma stem cells[36]. These findings manifested that *HOTAIR* could be a novel target for personalized treatment and monitoring the therapeutic effect of GBM.

The current study does, however, have certain limitations. To begin, we employed the machine learning method to predict *IDH1* mutation status in some individuals due to inadequate molecular information. However, in these patients, there may be a little disparity between the estimated and actual *IDH1* mutation status. Second, these patients were not included in GBM with deficient information on molecular signatures required for WHO grade II-III IDH-wildtype astrocytomas to be diagnosed as GBM. Third, the detection of the IC50 of drugs in the CCLE database was based on cell lines, and compared with cell lines, the transcriptome data of clinical tumor samples are contaminated by normal tissue and stroma components, which might reduce the accuracy of drug response prediction. Finally, although we verified that *HOTAIR* affected the proliferative activity of that glioma cells in vitro, further experimental and clinical validations are needed to support our hypothesis about the function of *HOTAIR*.

Conclusion

In conclusion, we analyzed the role of *HOTAIR* in GBM in WHO CNS5, and we found that *HOTAIR* might regulate the cell cycle process and inhibit apoptosis in GBM patients (no matter the fourth edition or the fifth edition). In addition, *HOTAIR* may not be involved in the immune regulation of GBM. Overall, this study suggests that *HOTAIR* could be a novel insight on prognosis prediction and a novel approach for precision therapy but not immunotherapy.

Abbreviations

GBM: Glioblastoma

WHO CNS5: The fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System

HOTAIR: HOX transcript antisense RNA

GSVA: Gene Set Variation Analysis

GSEA: Gene set enrichment analysis

CIBERSORT: Cell type Identification by Estimating Relative Subsets of RNA Transcripts

IC50: half-inhibitory concentrations

Declarations

Acknowledgment

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from UCSC-XENA (<http://xena.ucsc.edu>), CGGA (<http://www.cgga.org.cn>), GlioVis (<http://gliovis.bioinformatics.cn>), and GEO (<http://www.ncbi.nlm.nih.gov/geo>).

Funding

This work was supported by the Translational Medicine Fund of the Zhongnan Hospital of Wuhan University (No. ZLYNXM202011, ZNLH201901).

Authors' contributions

LZQ, LT, and SXY: designed the study, reviewed relevant literature, completed cell experiments, and drafted the manuscript. WD and YDH conducted all statistical analyses. LZQ, LT, and WGH: revise the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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Tables

Table 1

The clinical and molecular information of individuals in the TCGA, CGGA, and REMBRANDT cohorts.

	TCGA	CGGA	REMBRANDT
Number of patients	564	205	179
Age			
< 60y	411	183	113
≥ 60y	127	22	46
Gender			
Female	227	83	50
Male	311	122	80
Grade			
II	211	76	56
III	178	28	33
IV	175	101	85
TCGA subtype			
Classical	40	20	48
Mesenchymal	48	80	19
Neural	25	47	10
Proneural	26	58	8
IDH1 Status			
Mutant	175	101	
Wildtype	389	104	
1p19q Codeletion status			
Codeletion	160	16	8
Non-codeletion	400	49	32
MGMT promoter status			
Methylated	423	67	
Unmethylated	109	132	
TERT promoter status			
Mutant	138		
Wildtype	142		

+7/-10

Yes	130
No	430

+7/-10: Combined whole chromosome 7 gain and whole chromosome 10 loss

Table 2

The gene primer sequences used in study.

Genes	Forward (5'-3')	Reverse (5'-3')
HOTAIR	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
GAPDH	GCTCTCTGCTCCTCCTGTTC	CGACCAAATCCGTTGACTCC

Figures

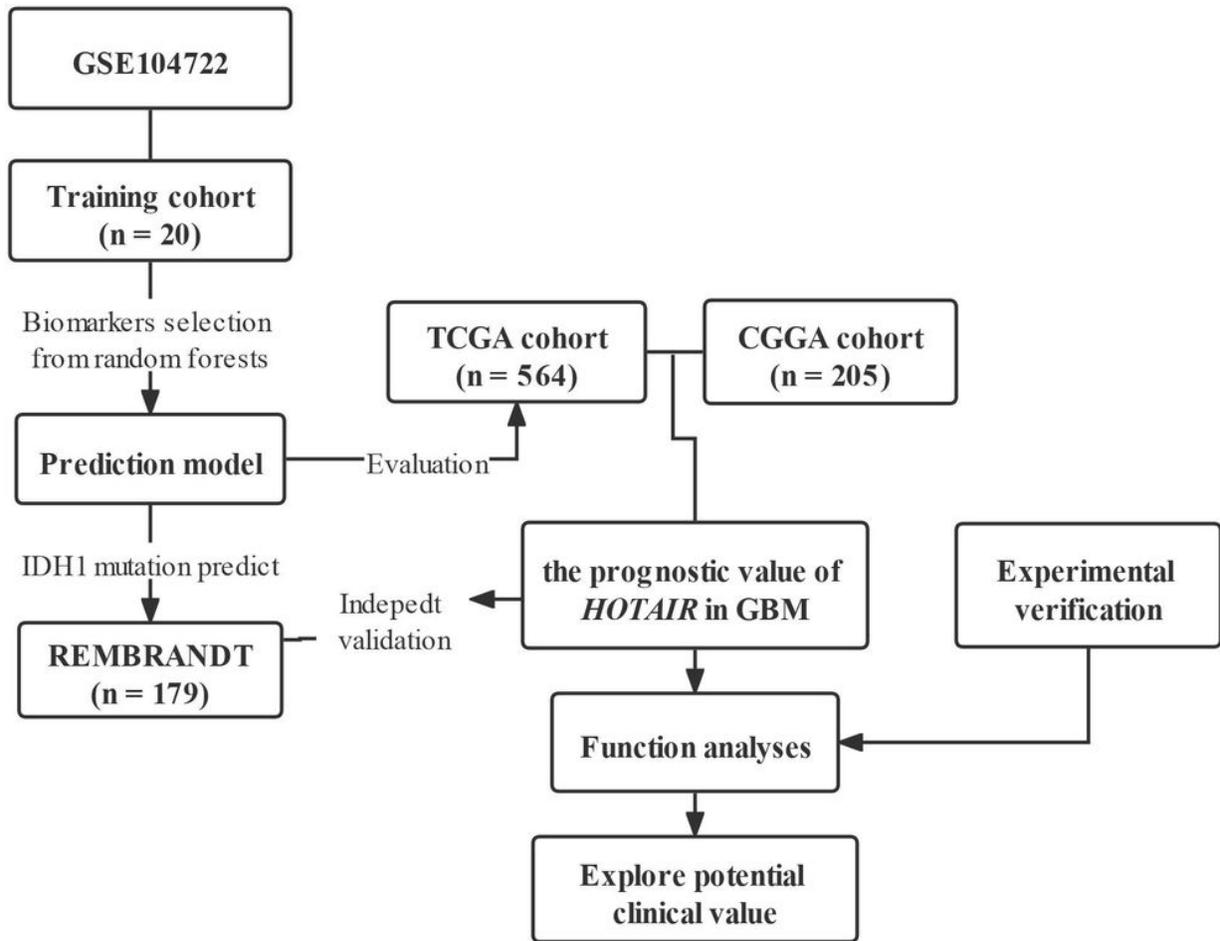


Figure 1

Study flow diagram.

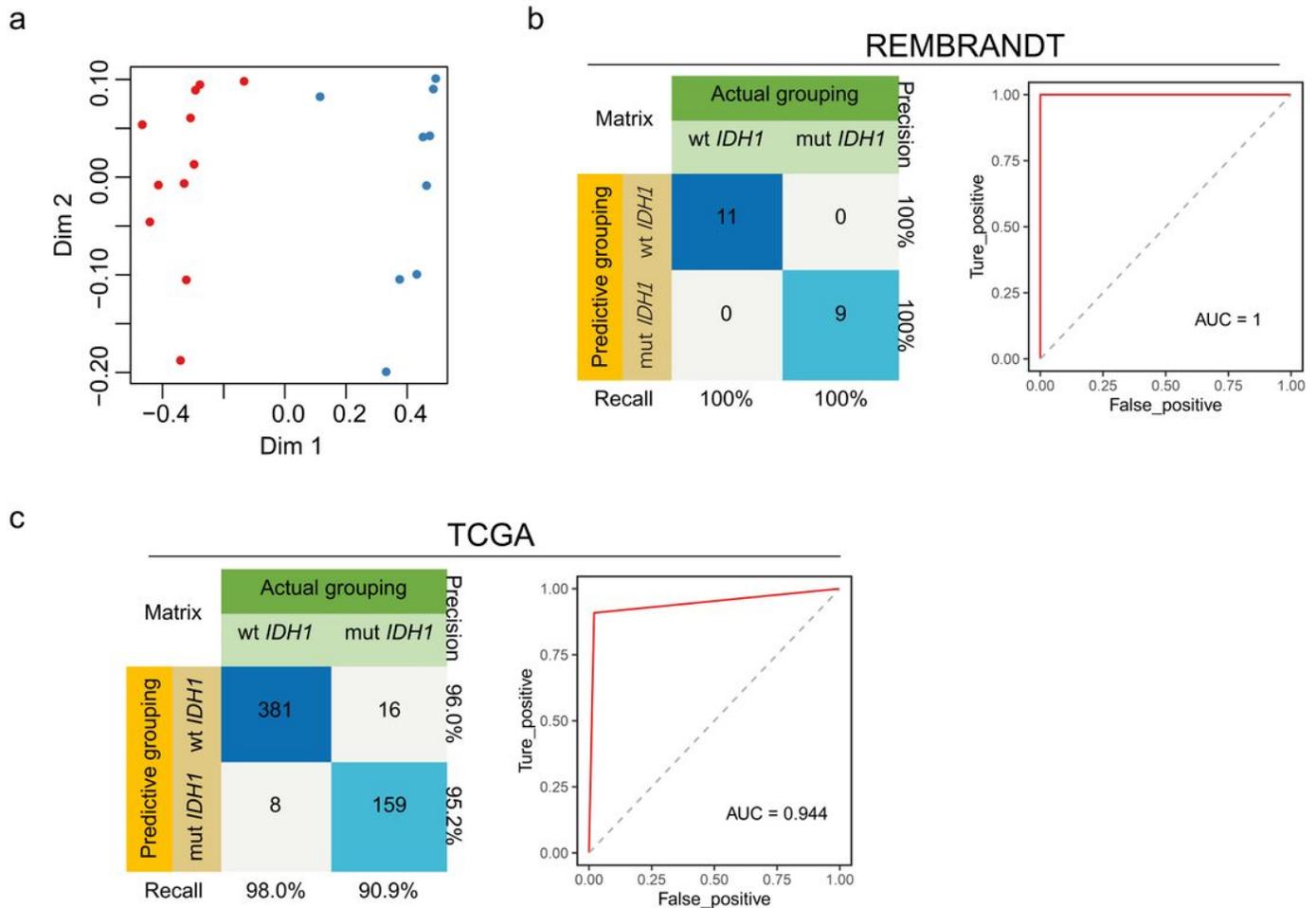


Figure 2

Evaluation of the performance of the prediction model. (a.) The distribution of predicted patients after the RF model in the GSE104722 cohort. Red dots are *IDH1*-mutant GBMs, blue dots are *IDH1*-wildtype gliomas. Confusion matrix and the ROC curve of the prediction results in the GSE104722 cohort **(b.)** and TCGA cohort **(c.)**. The closer the AUC value is to 1, the higher the true positive rate is, and the lower the false positive rate is.

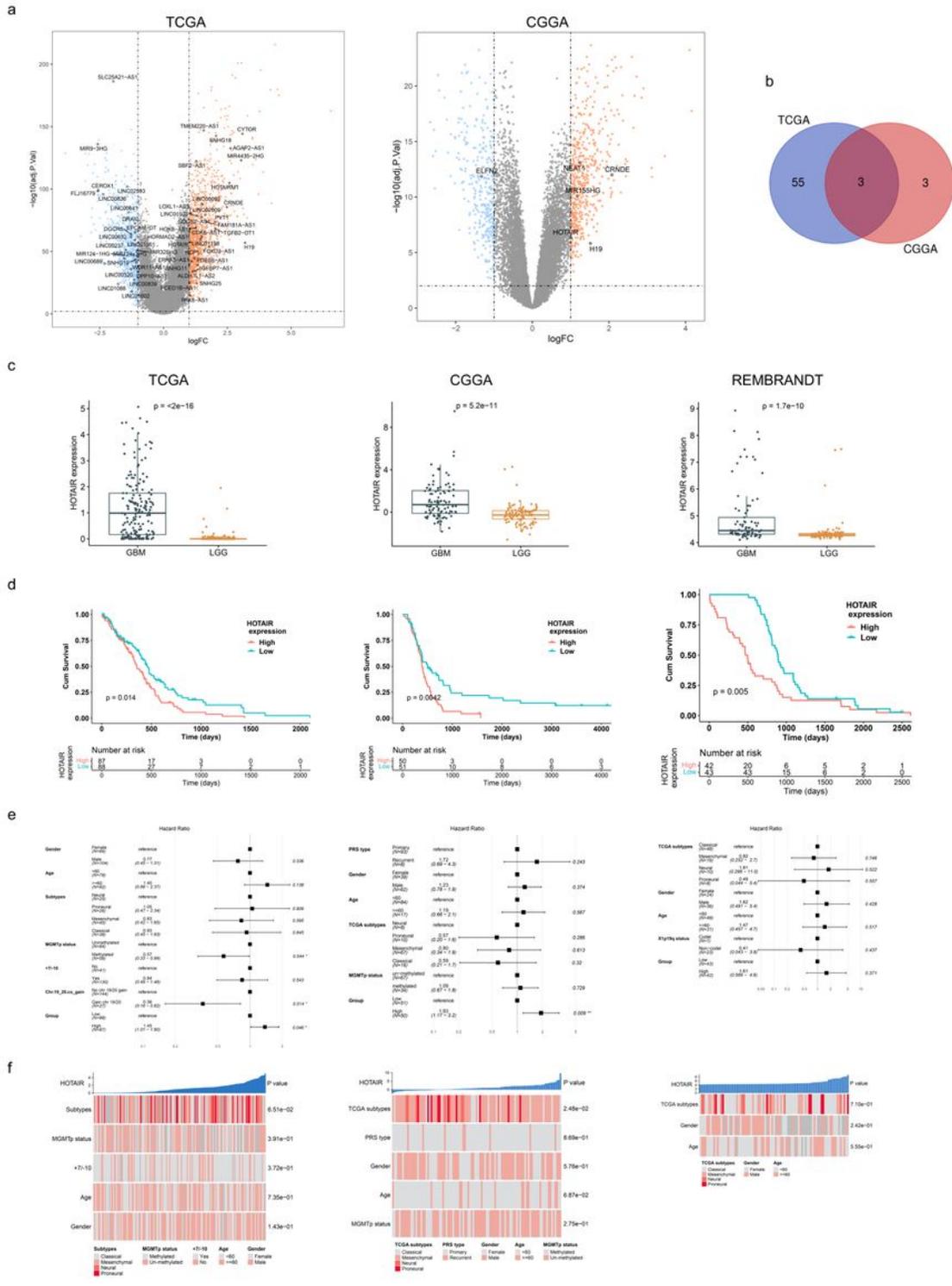


Figure 3

LncRNA *HOTAIR* is highly expressed in GBM and correlated with poor prognosis. (a.) Volcano map of differentially expressed lncRNAs between IDH1 wildtype (GBM) and IDH1 mutant in TCGA cohort and CGGA cohort. Orange dots are the up-regulated genes and azure dots are the down-regulated genes. **(b.)** Venn diagram for the abnormal expression of lncRNAs in both TCGA and CGGA. There are three lncRNAs: *HOTAIR*, *H19*, and *CRNDE*. **(c.)** Box plot for the expression of *HOTAIR* between GBM and other types of

glioma in TCGA, CGGA, and REMBRANDT cohorts. Each tumor sample is represented by a point. **(d.)** Kaplan–Meier survival curve analysis indicated that GBM patients with lower *HOTAIR* expression showed prolonged survival compared with patients with high levels of *HOTAIR* in TCGA ($P < 0.05$), CGGA ($P < 0.01$), and REMBRANDT ($P < 0.01$). **(e.)** Results of the multivariate Cox regression analysis in the TCGA, CGGA, and REMBRANDT. *HOTAIR* was regarded as an independent prognostic factor in TCGA and CGGA cohorts. **(f.)** An overview of the association between *HOTAIR* and clinical and molecular characteristics in TCGA, CGGA, and REMBRANDT. **+7/-10:** Combined whole chromosome 7 gain and whole chromosome 10 loss. The statistical significance of the difference was determined using the Permutation test.

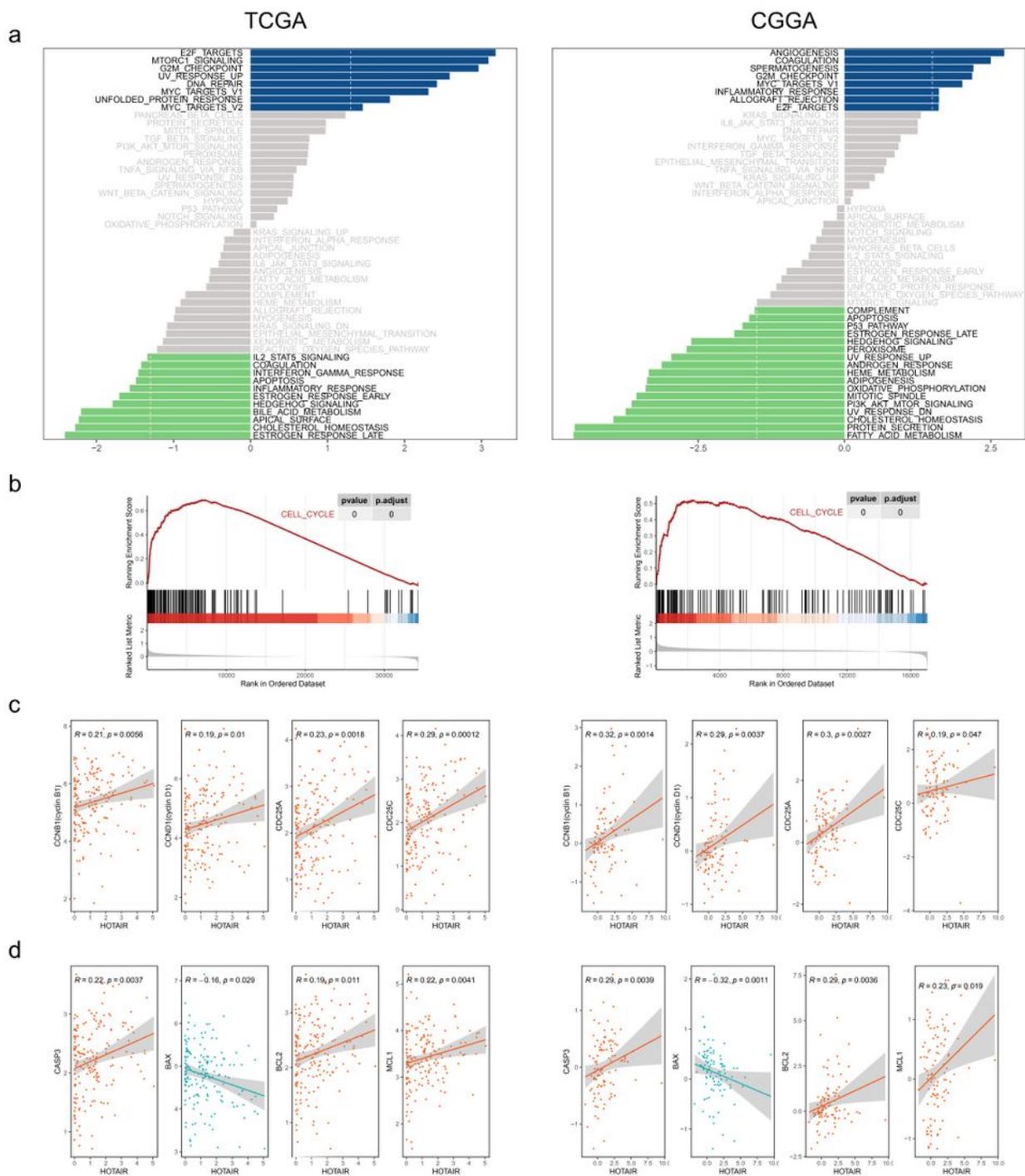


Figure 4

Function analysis of *HOTAIR* in GBM. (a.) Scores of GSEA for pathway activities between high *HOTAIR* expression group and low *HOTAIR* expression group in TCGA and CGGA. (b.) GSEA enrichment plots of the “CELL CYCLE” kegg pathway in TCGA and CGGA. (c.) Scatter plots of Spearman’s correlations and significance between *HOTAIR* and cell cycle markers expression. (d.) Scatter plots of Spearman’s correlations and significance between *HOTAIR* and apoptosis markers expression. Orange dots indicate

the significant positive correlations ($R > 0$ & $p < 0.05$). Azure dots indicate the significant negative correlations ($R < 0$ & $p < 0.05$).

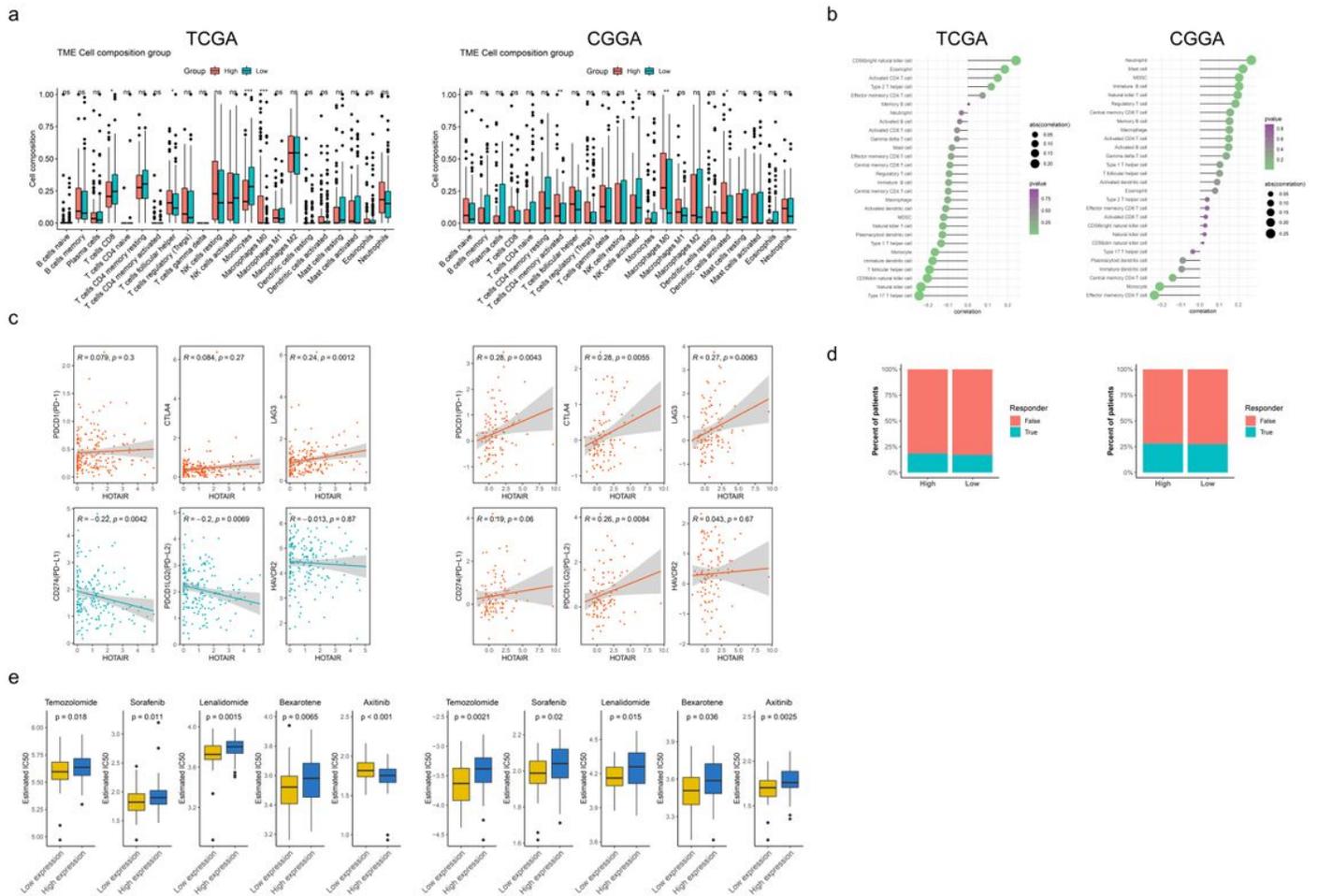


Figure 5

The tumor microenvironment analysis in the TCGA and CGGA cohorts. (a.) The proportion of the 22 immune cells inferred by CIBERSORT in different *HOTAIR* subgroups. The adjusted P -values are shown in the analyses. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. **(b.)** The correlation of the 28 immune cells identified by ssGSEA with the expression of *HOTAIR*. **(c.)** Scatter plots of Spearman's correlations and significance between *HOTAIR* and immune checkpoint markers expression. Orange dots indicate the positive correlations ($R > 0$). Azure dots indicate the negative correlations ($R < 0$). **(d.)** A histogram represents the distribution of patients who respond and do not respond to immune checkpoint blockade therapy as predicted by TIDE in different *HOTAIR* subgroups. **(e.)** The boxplot shows the predicted clinical temozolomide, sorafenib, lenalidomide, bexarotene, and axitinib sensitivity for GBM in different *HOTAIR* subgroups. The predictions were made using the pRRopheticPredict function for only glioma cell lines.

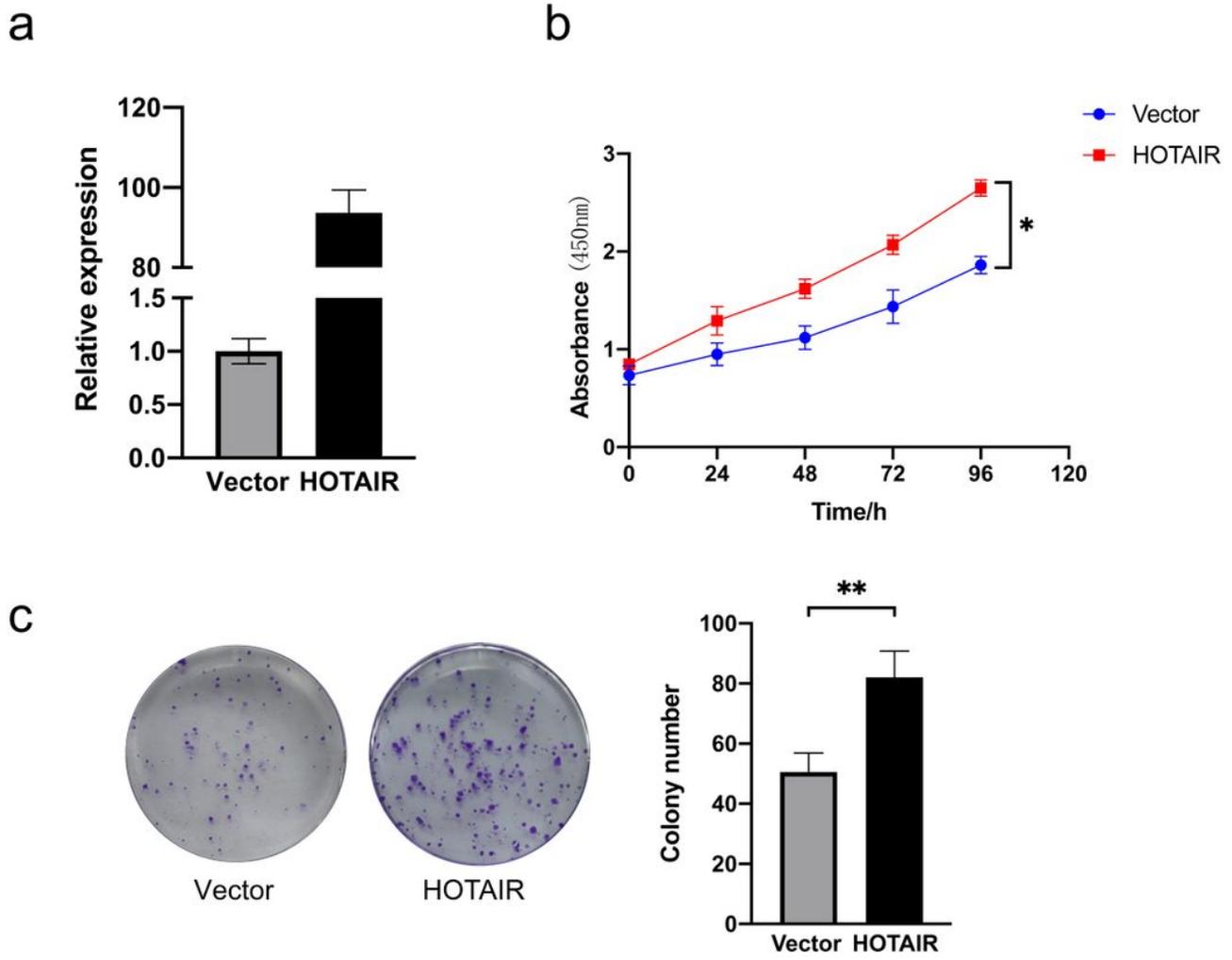


Figure 6

***HOTAIR* overexpression promotes glioma proliferation.** (a.) qRT-PCR analysis of *HOTAIR* expression in U251 cells transfected with vector or pcDNA-*HOTAIR*. (b.) Time-dependent survival curve of U251 cells transduced with vector or pcDNA-*HOTAIR* (* $P < 0.05$, with paired t-test). (c.) The colony formation ability of U251 cells pre-treated with vector or pcDNA-*HOTAIR* (** $P < 0.01$, with student t-test).