HAT1 regulates the immune microenvironment and promotes the malignant pathology of lower-grade gliomas

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Article

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Abstract
Histone acetyltransferase 1 (HAT1) is a key regulatory molecule in histone acetylation and promotes cancer malignancy. This study aimed to reveal the role of HAT1 in lower-grade gliomas (LGGs) and the potential mechanism by which it mediates pathological processes. We collected transcriptome data and detailed clinicopathological features of 906 LGG patients through TCGA RNA-seq and CGGA RNA-seq. Subsequently, various bioinformatics and molecular biology methods were used to reveal the impact of HAT1 on the prognosis, clinical characteristics, regulatory mechanism, and the potential value of antitumor immunotherapy in patients with LGG. RT-qPCR and immunohistochemistry showed that the expression level of HAT1 protein or mRNA in LGG tissue was significantly higher than that in non-tumor brain tissue. Furthermore, there is a positive expression relationship between HAT1 and clinical malignant patient characteristics such as IDH-wild and recurrent types. Kaplan–Meier and Cox regression revealed that high HAT1 expression could be used as an independent risk factor for reduced overall survival of patients. GSEA analysis showed that HAT1 overexpression promotes the activation of the cell cycle, adherens junctions, and RIG-I-like receptor signaling pathways in LGG. Finally, the TIMER database showed that HAT1 could promote the increase in multiple immune cell infiltration levels and showed a positive expression relationship with immune checkpoints in LGG. This study provides a potentially high-value target for the antitumor immunotherapy of LGG patients. For the first time, we explain the influence and mechanism of HAT1 on the prognosis of LGG patients to gain insights into the pathogenesis of LGG.

1 Introduction
The 2016 World Health Organization (WHO) Classification of Tumors of the Central Nervous System is used to determine the malignant degree of gliomas. Based on histology and molecular characteristics, gliomas were classified into grades I–IV, where grades I–III are defined as low-grade gliomas (LGGs)[1]. As a progressive and invasive disease, LGGs exhibit sustained growth and tend to develop into higher-grade gliomas, resulting in disability or death [2]. Clinically, the treatment of LGGs is mainly based on surgery, supplemented by postoperative radiotherapy and chemotherapy[3]. However, according to previous case reports, even after complete or ultra-complete resection, more than 50% of LGG patients often relapse or develop secondary higher-level gliomas, leading to patient death [4, 5]. Therefore, there is an urgent need to reveal the pathogenesis of LGG and find highly sensitive biological targets that may be used to hinder pathological progress and improve patient prognosis.

Identifying molecular markers with high value can not only help judge patient prognosis but can also be used to classify tumors. For example, the discovery of isocitrate dehydrogenase (IDH) mutation and the 1p/19q co-deletion state has led to the ability to group LGG patients into molecular subtypes and predict the prognosis of patients with LGG [6]. In addition, BCL7A, as a potential new tumor suppressor gene, can be used to evaluate the response of LGG patients to temozolomide chemotherapy in gliomas [7]. Moreover, CKS2 has been found to be involved in the cell cycle, and its overexpression may indicate a poor prognosis for LGG patients [8]. However, due to the high heterogeneity and miscellaneous pathogenesis of LGG, there may be various potential pathogenic genes that have not been identified.
Therefore, the further discovery of new prognosis-related genes and the use of multi-target combined therapy may improve the prognosis of LGG patients.

The acetylation of lysine is an important regulation mode of protein post-translational modification, especially in the pathological progression of tumors [9]. Histone acetyltransferase 1 (HAT1) is a type B histone acetyltransferase that mainly regulates the acetylation of H2A and H4 histones at Lys5 and Lys12 [10]. More importantly, abnormal HAT1 expression can promote the malignant process of many tumors. For example, Jin et al. found that HAT1 can promote the proliferation of hepatocellular carcinoma cells and enhance their resistance to cisplatin [11]. In addition, Yang et al. reported that HAT1 participates in modifying the chemokine receptor CCR4 in breast cancer, thereby increasing the invasion of T-regulatory cells at the tumor site [12]. In the tumor microenvironment, tumor cells and infiltrating immune cells have the function of mutual remodeling, which eventually leads to a significant reduction in the ability of immune cells to monitor and kill tumor cells. Many studies have reported the regulatory role of HAT1 in malignant tumors, but the regulatory effect of HAT1 on the prognosis and immune microenvironment of LGG patients has not been reported.

To explore the influence of HAT1 on the pathological process of LGG and its mechanism of action, we adopted multi-database bioinformatics and molecular biology techniques. To the best of our knowledge, we prove for the first time that the high expression of HAT1 can be used as an independent prognostic risk factor, leading to a poor prognosis. Moreover, our findings suggest HAT1 as a potential biomarker for clinical LGG patients that can be used to further develop targeted therapies to block LGG malignancy. More importantly, the effect of HAT1 on the immune microenvironment of LGG patients was confirmed, and the expression relationship between HAT1 and programmed death-ligand 1 (PD-L1) was explained for the first time, which is critical to revealing the immune microenvironment of LGG and providing a potential target for further development of combined antitumor immunotherapy.

2 Results

2.1 The expression of HAT1 was abnormally increased in LGG.

First, we searched for the expression profile of HAT1 in various tumors from the GEPIA database and found abnormal HAT1 expression in various cancers (Fig. 1A). Our interest is in nerve tumors; therefore, we selected LGG for further research. We obtained 518 LGG and 207 normal brain tissues to map differential expression and found that HAT1 was significantly increased in LGG (Fig. 1B). Subsequently, high HAT1 expression in LGG was also observed in the GSE12907 dataset with 21 LGG tissues and 4 normal cerebellar tissues (Fig. 1C). Protein was one of the important ways to exert biological functions after mRNA translation. Therefore, we attempted to reveal whether the protein expression level of HAT1 changed in LGG using the HPA database and observed that the protein expression of HAT1 was markedly increased in LGG tissue (Fig. 1D). To increase the reliability of our results, we used RT-qPCR and
immunohistochemistry for verification. As expected, both HAT1 mRNA and protein expression levels were significantly increased in LGG (Fig. 1E–F).

### 2.2 High HAT1 expression contributed to the malignant clinical molecular characteristics of LGG patients

Different clinical and molecular characteristics are of great significance for the prognosis of LGG patients. Therefore, we attempted to explore the relationship between HAT1 expression and clinical molecular characteristics of LGG patients through TCGA RNA-seq and CGGA RNA-seq databases. The results indicated that the expression level of HAT1 was increased in the WHO grade III, chemotherapy, and IDH wild-type mutant groups in the TCGA RNA-seq database ($P<0.05$; Fig. 2A–C). This result was verified in the CGGA RNA-seq database, and observations revealed that the HAT1 expression level was increased in the WHO grade III, chemotherapy, recurrent type, and 1p19q non-codeletion status groups ($P<0.05$; Fig. 2D–G). Collectively, our results suggest that the high expression of HAT1 might also have an impact on the prognosis of LGG patients.

### 2.3 High HAT1 expression plays the role of a pathogenic gene in LGG

To determine whether high HAT1 expression could lead to poor patient prognosis, we first performed a Kaplan–Meier survival analysis on the data from TCGA RNA-seq and CGGA RNA-seq databases, and the results showed that HAT1 expression was negatively correlated with the survival rate of LGG patients (Fig. 3A, B). Subsequently, to verify the diagnostic value of HAT1 in LGG, we constructed the ROC curve and found that HAT1 had a high diagnostic value for the prognosis of LGG (Fig. 3C, D). Furthermore, we used univariate and multivariate Cox regression analyses to explore whether HAT1 was an independent prognostic factor and revealed that high HAT1 expression was a risk factor (HR>1) for the OS of LGG patients (Fig. 4A, B).

To verify our results, we collected additional sample information to confirm the risk posed by high HAT1 expression on the prognosis of LGG patients through a meta-analysis. A total of 958 patients were obtained from four different data sets and included in the analysis. The combined HR and its 95% CI associated with high HAT1 expression and OS was 1.62 (1.07–2.45; Fig. 4C). These results suggest that high HAT1 expression is pathogenic and negatively associated with the prognosis of LGG patients. Moreover, to determine whether the impact of HAT1 on LGG prognosis was accompanied by its mutation, we obtained the transcriptome mutation data of LGG patients from the TCGA database and found only one patient with a HAT1 mutation in the pathological process of LGG (Supplementary Fig. 1). Therefore, the influence of HAT1 mutation on the prognosis of LGG was excluded. It can be inferred that the high HAT1 expression can be used as a biological target to determine the prognosis of LGG.

### 2.4 Mechanism of high HAT1 expression in LGG

Gene co-expression analysis can reveal associated genes that have synergistic effects with target genes in the disease process. Therefore, we used Pearson correlation analysis to reveal the co-expression of
genes with *HAT1* based on TCGA RNA-seq. The results revealed the top five genes with the most positive correlation (*POLD3*, *USP1*, *CDK2*, *UBE2A*, and *RPA1*) and those with the most negative correlation with *HAT1* (*JPH4*, *ATP6V0A1*, *CASKIN1*, *FAIM2*, and *SCAMP5*; Fig. 5A, B). These genes might have antagonistic or synergistic regulatory effects with *HAT1* in LGG.

Moreover, GSEA analysis uncovered the effect of target genes on the activity of cellular signaling pathways in the disease pathology. Therefore, we proceeded to investigate the effect of *HAT1* on cell signals using GSEA analysis based on TCGA RNA-seq and CGGA RNA-seq. The results showed that the high expression of *HAT1* could promote the activities of the cell cycle, adherens junctions, DNA replication, and RIG-I-like receptor signaling—the results of the two databases were consistent (Fig. 6, Table 1). Collectively, our findings partially revealed the possible regulatory mechanism of *HAT1* on the poor prognosis of LGG.

### 2.5 Correlation of HAT1 expression with immune cells and checkpoints

Based on the influence of *HAT1* on the function of immune cells and the increase in antitumor immunotherapy, we sought to demonstrate the impact of *HAT1* on the immune microenvironment of LGG using the TIMER database [13]. The results indicated that the expression level of *HAT1* was positively correlated with the infiltration levels of B cells (partial. cor = 0.477), CD8 + T cells (partial. cor = 0.422), CD4 + T cells (partial. cor = 0.387), macrophages (partial. cor = 0.501), neutrophils (partial. cor = 0.461), and dendritic cells (partial. cor = 0.515) in LGG tissues (Supplementary Fig. 2A). Subsequent Kaplan–Meier survival analysis showed that the high-infiltration capacity of the six immune cells and high *HAT1* expression could shorten the OS of the LGG patients (Supplementary Fig. 2B). The basis of antitumor immunotherapy is to find key immune checkpoints as targets. Therefore, we further explored the expression relationship between *HAT1* and existing well-known immune checkpoints to establish whether *HAT1* had potential value in antitumor immunotherapy. We observed that *HAT1* had a high positive correlation with many well-known immune checkpoints such as *CD274*, *PDCD1*, *HAVCR2*, and *CTLA4* (Supplementary Fig. 2C–F), suggesting that high *HAT1* expression had an important regulatory effect on the immune microenvironment and is a potentially valuable antitumor target.

Although the TIMER database can quickly resolve the impact of target genes on the immune microenvironment, there are still some shortcomings in the specific typing of immune cells. Therefore, we determined the LGG tumor microenvironment (TME) score of the sample transcript spectrum in the TCGA cohort, including stromal, immune, and ESTIMATE scores. The results demonstrated that the expression level of *HAT1* positively correlated with the three scores (Fig. 7A). To further evaluate the correlation between TME and immune infiltrating cells in patients with LGG, we explored the correlation between *HAT1* and 22 immune cell subtypes. The results indicated that M0 and M2 macrophages increased with an increase in *HAT1* expression (Fig. 7B–D). In addition, we verified the cellular markers of seven types of immune cells (B, CD8 + T, and CD4 + T cells, M1 and M2 macrophages, neutrophils, and dendritic cells). We found that these cell markers, except M1 macrophages, were positively correlated with *HAT1*
expression (Fig. 7E–G, Supplementary Fig. 3A-G), consistent with the TIMER database results. Surprisingly, the M1 macrophage marker was negatively correlated with HAT1 expression. These results consolidated those of the TIMER database.

It is worth noting that M2 macrophages are the main component of tumor-associated macrophages and promote the proliferation, migration, and invasion of tumor cells in various ways [14, 15]. Accordingly, to verify the relationship between HAT1 and M2 macrophages, we used immunohistochemical staining. As a result, we found that CD163 (an M2 macrophage marker) and HAT1 had a positive expression relationship in the same samples (Figs. 8A and 1F). It should also be emphasized that the positive correlation between HAT1 and PD-L1 was also verified by immunohistochemistry (Fig. 8B). Since PD-L1 is the most important evaluation index and well-known immune checkpoint for the efficacy of antitumor immunotherapy[16, 17], it can be inferred from the above results that HAT1 may have a synergistic regulatory effect with PD-L1, and may also be a new target of combined immunotherapy.

3 Discussion

Acetylation and deacetylation of histone mediate the dynamic balance of chromatin regulation, while the target anchored by HAT1 is lysine at a specific position in histone. Acetylated histones lose their positive charge, and this change in chromatin structure can increase the accessibility of chromatin to RNA polymerase and transcription factors [18]. Therefore, the abnormal function of histones has an important impact on the proliferation, migration, differentiation, and other links to tumor cells [19, 20]. More importantly, HAT1, as the main molecule of histone regulation, has been confirmed to have an impact on prognosis in cancer. For example, when the expression levels of HAT1, which is involved in the malignant behavioral characteristics of various tumors, are significantly increased, there is a significant reduction in the prognosis of patients with liver and pancreatic cancers and nasopharyngeal carcinoma [11, 21, 22]. Nevertheless, there are no reports exploring the regulatory effect of HAT1 on LGG pathology.

The abnormal expression of genes during tumor malignancy often has an important regulatory effect on the prognosis of cancer patients [23]. Therefore, to clarify whether HAT1 could regulate the pathological process of LGG, we first explored the expression level of HAT1 in LGG and demonstrated that HAT1 expression is abnormally increased, suggesting that HAT1 may have an impact on the prognosis of LGG patients. Subsequently, we adopted clinical correlation analysis and showed that the expression level of HAT1 was higher in WHO grade III, recurrence, and chemotherapy groups, whereas it was negatively correlated with the IDH mutation and 1p19q co-deletion groups. Based on these results, we speculated that HAT1 is a pathogenic molecule for LGG patients. Furthermore, the OS of patients classified as WHO grade III, recurrence, IDH-wildtype, and 1p19q non-codeletion groups was significantly reduced [24]. More importantly, high HAT1 expression could shorten the OS of LGG patients. This conclusion is reached for the first time in LGG, but similar findings have been reported for other cancers. For example, HAT1 is a known risk factor in esophageal squamous cell carcinoma [25]. Therefore, we believe that the high expression of HAT1 plays the role of a pathogenic gene in LGG, providing insights into the complex
pathological mechanism of LGG. Furthermore, *HAT1* could be considered a valuable biological target for the clinical diagnosis and treatment of LGG.

Previous studies suggest that the co-expression relationship between genes may have synergistic or antagonistic effects; therefore, it could predict the pathogenicity and regulatory mechanism of genes [26]. The results of the present study suggest that most genes with a positive expression relationship with *HAT1* are oncogenes, whereas those negatively related to *HAT1* are tumor suppressor genes. For example, *POLD3*, which has been found to be easily amplified in various tumors, is the helper subunit of DNA polymerase δ, and its increased expression reduces the OS of patients with gliomas [27, 28]. In addition, the high expression of *USP1* in glioblastoma not only maintains the characteristic of tumor stem cells to promote increased tumor cell viability but also improves tumor cell resistance to radiotherapy [29]. On the other hand, *CASKIN1* and *FAIM2* play the role of tumor suppressor genes in central nervous system tumors, promoting the apoptosis rate of tumor cells and significantly prolonging the OS of patients [30, 31]. Taken together, this evidence sheds light on the regulatory effect of *HAT1*, but as a pathogenic gene, its regulatory effect occurs variously in cancer pathology [32].

GSEA analysis is a common analytical method that reveals the biological functions of target genes based on whole-transcriptome data [33]. In the present study, we found that *HAT1* overexpression can promote the activity of various tumor cell signaling pathways. Histone acetylation, which can change gene expression and cell phenotype without changing the DNA sequence, plays inimitable roles in the cell cycle, DNA damage repair, and adherens junctions, thus affecting cell proliferation and apoptosis; this has been corroborated by our GSEA analysis results [34]. RIG-I is a cytoplasmic nucleic acid sensor for recognizing intracellular viruses [35]. As an important part of the innate immune perception system, when RIG-I-like receptor signaling is activated, it can trigger NF-κB, type I interferon (IFN), or other inflammatory body signaling pathways, which in turn lead to the production of various pro-inflammatory and antiviral cytokines and chemokines, evoking adaptive immune responses [36]. Interestingly, the positive correlation with RIG-I-like receptor signaling suggests that *HAT1* may be involved in immune microenvironment changes in LGG.

There is evidence that immune inflammatory cells can promote angiogenesis and provide bioactive molecules, thus promoting the proliferation and invasion of cancer cells [37]. To further clarify the effect of *HAT1* on the TME, we conducted immune correlation analysis through the TIMER database and TCGA RNA-seq. According to the quantities of the six immune cell types and related cell checkpoints, we confirmed that *HAT1* could significantly stimulate infiltration by the various immune cells in the LGG immune microenvironment. It is worth noting that there is a positive correlation between *HAT1* and M2 macrophages, which are recruited at initial tumor development and help cancer cells evade surveillance and attack by the immune system [38]. At the same time, M2 macrophages promote tumor cell growth through β-oxidation of fatty acids and the tricarboxylic acid (TCA) cycle, as well as induction of polyamines and L-proline [39]. In addition to the effect on immune cells, *HAT1* can also upregulate the expression level of many immune checkpoints, especially PD-L1. The blocking transmission path of PD-L1 is an important reference index to evaluate the effect of antitumor immunotherapy [40]. The positive
correlation between $HAT1$ and PD-L1 also indicates the prospect of targeted $HAT1$ inhibitors in immunotherapy.

Due to the blood-brain barrier, many antineoplastic drugs face difficulties reaching the tumor site, a problem that has always been challenging for scholars [41]. Nonetheless, the progress of immunotherapy has enabled the development of precision-targeted therapies for gliomas [42]. Furthermore, several studies have shown that the OS of mice with gliomas could be increased by suppressing the expression of immune checkpoints [43, 44]. However, it is well known that the research and development of new drugs in immunotherapy are fraught with challenges. In addition to improving the effectiveness of new immunotherapy drugs, there is also a need to reduce the extensive effects of drugs on the immune system [45]. One of the ways to solve this problem is to improve efficacy through collaborative networks between genes [46]. Currently, immunotherapy research on the synergistic effects of $HAT1$ has been carried out in other cancer fields. For example, in pancreatic cancer, the efficacy of PD-L1 inhibitors alone in treating pancreatic cancer is not satisfactory, but synergistic $HAT1$ knockout can reduce PD-L1 activity to improve the effectiveness of immune checkpoint blockade [13]. Collectively, it can be speculated that the in-depth study of $HAT1$ can provide a new gene target for the immunotherapy of LGG.

As a retrospective study, the current study has some limitations. First, our data were obtained from different medical institutions and might therefore be biased due to the different treatment procedures at these institutions. To offset this bias, we searched multiple databases to gather as much information as possible. The influence of $HAT1$ on LGG shows commonality among different races, which increases the credibility of this study. Second, the retrospective nature study means it lacks foresight. Therefore, we verified some analysis results through molecular biology techniques to make up for the shortcomings of this study as much as possible.

4 Conclusion

The present study demonstrated the pathogenicity of $HAT1$ in LGG pathology, broadening the recognition of $HAT1$ as a key pathogenic molecule. More importantly, we revealed the influence of $HAT1$ on the LGG immune microenvironment and elucidated its molecular regulation mechanism, partially revealing the pathogenesis of LGG and introducing a novel biological target for future antitumor immunotherapy of LGG.

5 Material And Methods

5.1 Data extraction

All RNA-seq and patient clinicopathological data included in this study were obtained from the Cancer Genome Atlas Program (TCGA) (https://portal.gdc.cancer.gov/) and the Chinese Glioma Genome Atlas (CGGA) (http://www.cgga.org.cn/) databases. After excluding patients with incomplete records, we
obtained the RNA-seq and clinical data of 906 patients with WHO grades II or III gliomas (TCGA RNA-seq, \(n = 503\), CGGA RNA-seq, \(n = 403\); Supplementary Tables 1 and 2, respectively).

A total of 518 LGG samples from the TCGA database and 207 normal brain tissue samples from the Genotype-Tissue Expression (GTEx) database were analyzed using the Gene Expression Profiling Interactive Analysis (GEPIA) network platform (http://gepia.cancer-pku.cn/), and the differential expression of \(HAT1\) in LGG and normal brain tissues was explored using a standardized processing pipeline.

To verify the different expression levels of target genes in normal and tumor tissues, the GSE12907 dataset based on the GPL96 platform was retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). In addition, we obtained GSE43378 and GSE50025 datasets based on the GPL570 and GPL13938 platforms, respectively. The gene transcriptome and patient survival data contained in these datasets were used to explore the effect of \(HAT1\) expression on the prognosis of LGG patients via meta-analysis.

The Human Protein Atlas database (HPA) has been used to construct the expression map of all human proteins based on various combinatorial techniques (https://www.proteinatlas.org/) [47]. We screened normal and LGG brain tissue samples of the same sex and similar age from the HPA database to examine \(HAT1\) protein expression.

Brain tissues, including five cases each of LGG and epilepsy, were obtained from the operating room and stored in a refrigerator at -80°C until use. All patients signed informed consent forms before the operation. The study was performed in accordance with relevant guidelines and regulations.

### 5.2 RNA extraction and the reverse transcriptionpolymerase chain reaction (RTqPCR)

Total RNA was extracted using a Total RNA Kit I (R6834-02; Omega Bio-Tek Inc, Norcross, GA, USA) according to the manufacturer's instructions. RNA quality was assessed using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was synthesized from isolated total RNA using a NovoScript Plus All-in-one 1st Strand cDNA Synthesis SuperMix (Novoprotein Scientific Inc, Jiangsu, China), and RT-qPCR was performed using NovoStart® SYBR qPCR SuperMix Plus (Novoprotein Scientific Inc). The thermal cycling conditions were 40 cycles at 95°C for 10 min, 95°C for 10 s, and 60°C for 30 s. 18S RNA was used as the reference gene. Fold change was determined using the \(\Delta\)CT method relative to the calibrator and verified by an unpaired \(t\)-test at \(P<0.05\). The sequences of the RNA-specific primers used were as follows: internal reference gene 18s: 5′-GTAACCCGTTGAACCCATT-3′ (F) and 5′-CCATCCAATCGGTAGTAGCG-3′ (R). The primer sequences for \(HAT1\) were 5′-AGGCAGATGATGTTGAGG-3′ (F) and 5′-AGTAAGGTTCCGAATGCG-3′ (R).

### 5.3 Immunohistochemistry
Immunochemically-stable surgical specimens of normal brain tissue and LGG patients were used to prepare paraffin sections with a thickness of 3 µm. After degumming and hydration in xylene, fractionated alcohol and paraffin sections, pH 8.0 EDTA antigen repair solution was used to recover antigens for 15 min. Thereafter, 10% serum solution was used to block non-specific antigens for 30 min, after which an appropriate amount of primary antibody working solutions (antibody-\textit{HAT1}, 1:50; Proteintech, Rosemont, IL, USA; antibody-CD163, 1:250; Proteintech; antibody-PD-L1, 1:250; Proteintech) was added to the sections and incubated overnight at 4°C. The next day horseradish peroxidase-labeled immunoglobulin polymer (HRP) was incubated as the secondary antibody at room temperature for 1 h. The staining results were visualized and acquired under light microscope. The immunohistochemistry results and data were analyzed using Image-ProPlus software (version 6. 0; Media Cybernetics, Rockville, MD, USA).

5.4 Relationship between \textit{HAT1} and immune cell infiltration

The tumor immune microenvironment plays an indispensable role in the prognosis of cancer patients \cite{48}. The TIMER database (https://cistrome.shinyapps.io/timer/) is an online platform to study the relationship between immune cell infiltration and tumor tissues based on RNA-seq expression profile data. We used the “Gene” module in the TIMER database to explore the correlation between six immune cells (CD4 + T, CD8 + T, and B cells, macrophages, neutrophils, and dendritic cells) and \textit{HAT1} expression. At the same time, the “Survival” module was used to explore the effect of immune cell infiltration on the prognosis of LGG patients, and the “Correlation” module was used to determine the relationship between some important immune checkpoints (PDCD1, HAVCR2, CTLA4, and CD274) and \textit{HAT1} expression. Subsequently, we used the ESTIMATE algorithm to calculate the immune and stromal scores. Finally, using the expression data in the TCGA database, the relationship between the aforementioned immune cell infiltration and \textit{HAT1} expression in LGG was analyzed using CIBERSORT, and the expression of common immune examination sites was analyzed.

5.5 Gene set enrichment analysis

Gene set enrichment analysis (GSEA) can be used to predict gene function \cite{49}. After standardizing the data set, it was divided into high and low expression groups according to the median expression level of \textit{HAT1}. Afterward, GSEA 4.0 jar software was used to identify the signal pathways in which \textit{HAT1} was significantly involved. The number of permutations was set to 1,000, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) cell signal pathway was selected as the gene set database. An FDR < 0.25 and \(P < 0.05\) were regarded as statistically significant.

5.6 Meta-analysis

After searching the PubMed database, we found few previous studies on the relationship between \textit{HAT1} and LGG prognosis. We excluded studies with incomplete clinical data, and R software (v.4.0.3 version) was used for meta-analysis. The risk ratio (HR) and its 95% confidence interval (CI) were used to evaluate the relationship between \textit{HAT1} expression and the clinical prognosis of LGG patients. Cochran's Q and
Higgins I-squared ($I^2$) tests were used to examine data heterogeneity. When $I^2 < 50\%$, the fixed-effect model was used, and if this was not the case, then the random effects model was used.

### 5.7 Statistical analysis

All data in this study were statistically analyzed using R software (v.4.0.3 version). A one-way ANOVA was used to analyze the expression difference of *HAT1* between LGG and normal tissues, and the chi-squared test was used to determine the correlation between *HAT1* and the clinical characteristics of LGG patients. Kaplan–Meier and Cox regression were used to analyze overall survival (OS). A receiver operating characteristic (ROC) curve was drawn to evaluate the diagnostic value of *HAT1*. GenVisR software was used to explore gene mutations, and Pearson's correlation coefficient was used to explore the co-expression relationship between *HAT1* expression level and other genes. Statistical significance was considered at $P < 0.05$.

### Declarations

#### Acknowledgments

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#### Ethical approval

This study was approved by the Ethics Review Committee of Henan People's Hospital (2020107).

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#### Conflict of interest

The authors declare that they have no conflict of interest.

#### Author contribution

XBC and ZDL designed the experiment and analyzed the data. QNW and PXL was responsible for experiments, data analysis, manuscript writing and data preparation. WJL, HBW and QYZ contributed to the experiment and provided technical and theoretical support. YZG conducted the final review.

### Data Availability Statement

Data on the results of this study are available from the corresponding author upon reasonable request.

### References


**Table**

**Table 1.** The gene set enriches the high *HAT1* expression phenotype
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NES: normalized enrichment score; NOM: nominal. FDR: false discovery rate. Gene sets with NOM P-value <0.05 and FDR q-value <0.25 were considered as significantly enriched.

**Figures**
Figure 1

The expression of HAT1 between LGG and normal tissues. (A) The expression of HAT1 in various cancers. The red color indicates that HAT1 is higher than the corresponding normal control group. (B) HAT1 is significantly upregulated in LGG. (C) In the GSE12907 dataset, the expression of HAT1 is increased in 21 LGG and 4 normal brain tissues. (D) Protein expression level of HAT1 is increased in LGG tissue compared with that in normal brain tissue in the HPA database. (E) RT-qPCR shows that HAT1 is
highly expressed in LGG compared with that in non-tumor brain tissue. (F) Immunohistochemistry shows that HAT1 is highly expressed in LGG compared with that in non-tumor brain tissue. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

**Figure 2**

Association between HAT1 expression and clinicopathological features in LGG based on TCGA RNA-seq (A–C) and CGGA RNA-seq (D–G). (A, D) WHO grades. (B, E) Chemotherapy status. (C) IDH mutation status. (F) Primary, recurrent, and secondary types. (G) 1p19q co-deletion status.
Figure 3

(A–B) Kaplan–Meier survival analysis shows that HAT1 is associated with the poor prognosis of LGG patients based on TCGA RNA-seq and CGGA RNA-seq. (C–D) ROC shows that HAT1 has a high diagnostic value for LGG patients based on TCGA RNA-seq and CGGA RNA-seq.
Abnormally high expression of HAT1 is an independent risk factor for the prognosis of LGG based on TCGA RNA-seq and CGGA RNA-seq. (A) Univariate analysis shows that HAT1 is a hazard factor for LGG ($P < 0.001$; hazard ratio > 1.0). (B) Multivariate analysis shows that HAT1 is an independent prognostic factor for LGG ($P < 0.001$; hazard ratio > 1.0). (C) Meta-analysis of the four datasets shows that the high expression of HAT1 is a prognostic risk factor for LGG (HR = 1.62, 95%CI: 1.07–2.45).
Figure 5

Results of gene co-expression analysis. (A) The expression relationship diagram of genes related to HAT1 expression in LGG. (B) Co-expression networks show the top five genes that are positively or negatively correlated with HAT1, including gene names, correlation coefficient values, and $P$-values.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Correlation coefficient</th>
<th>P-value</th>
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<tbody>
<tr>
<td>JPH4</td>
<td>-0.591</td>
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<td>ATP6V0A1</td>
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<td>CASKIN1</td>
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<td>FAIM2</td>
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<td>RPA1</td>
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</tbody>
</table>
Figure 6

Results of GSEA analysis. (A) Cell-cycle. (B) Adherens junctions. (C) DNA-replication. (D) RIG-I-like receptor signaling
Figure 7

(A) The results show that the expression level of HAT1 positively correlates with the TME score, including stromal, immune, and ESTIMATE scores. \(*P < 0.05, \text{***}P < 0.001\). (B) The correlation between HAT1 and the 22 immune cell subtypes. (C) The expression of HAT1 positively correlates with M0 macrophages. (D) The expression of HAT1 positively correlates with M2 macrophages. (E–G) M2 macrophage cell markers, including CD163, VSIG4, and MS4A4A, positively correlate with HAT1 expression.
Figure 8

Results of immunohistochemistry between HAT1 and immune checkpoints. (A) CD163. (B) PD-L1. **** $P < 0.0001$.

Supplementary Files

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