APOBEC3C Served as a Novel Immune-Related Prognosis Biomarker in Glioma: A Pan-Cancer Analysis

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Abstract

Background: Apolipoprotein B mRNA-editing enzyme catalytic subunit 3C (APOBEC3C) belongs to the cytidine deaminase family, and its function remains unknown in some cancers. The purpose of this study was to elucidate the role of APOBEC3C in immune infiltration and tumor progression in glioma.

Methods: In this study, we comprehensively analyzed the transcriptional expression levels of the APOBEC3C among 33 cancer types by downloading data from TCGA (The Cancer Genome Atlas) and GTEx (the Genotype-Tissue Expression) databases. Moreover, correlation analyses of the APOBEC3C with cancer prognosis, immune infiltration, immune checkpoints, tumor mutation burden (TMB), microsatellite instability (MSI), mismatch repair (MMR) and methylation were explored. Finally, immunohistochemistry (IHC) staining and experiments in vitro were performed to validation our findings.

Results: Our study found that APOBEC3C was aberrantly expressed in multiple types of human cancers, and associated with immune infiltration and tumor progression. The overexpression of APOBEC3C was observed in human pan-cancer, and plays an important role in overall survival (OS) of glioma and disease-specific survival (DSS) on glioma according to survival analysis results. Besides, APOBEC3C expression is strongly associated with MMR defects and methylation in glioma. High APOBEC3C expression was associated with malignancy in glioma detected by IHC staining. APOBEC3C knockdown reduced the proliferation, migration and invasion of glioma cells. CCK8, clone formation assay, wound healing and Transwell assays confirmed the results in vitro.

Conclusion: Our results indicate that APOBEC3C could be a novel biomarker for predicting prognosis and immune response in glioma.

Introduction

Gliomas, including lower-grade gliomas (LGGs) and glioblastomas (GBMs), are the most common type of primary brain tumors in the central nervous system (CNS). Malignant gliomas are characterized by rapid progression, high post-operative recurrence rates, resistance to therapeutic anticancer, and poor prognosis[1]. Nowadays, standardized conventional treatment for gliomas is maximal safe surgical resection in combination with chemotherapy and radiotherapy[2]. Despite considerable progress in the diagnosis and treatment of gliomas, the median survival of patients with glioma is still unsatisfactory. Recently, immunotherapy including checkpoint inhibitors has gained great momentum and offers a fresh perspective on the management of patients with glioma[3, 4].

The tumor microenvironment (TME) is a very complex dynamic ecosystem. It has previously been observed that TME became a critical factor to influence tumor initiation and progression. The rapid progression and vulnerability to relapse of malignant glioma are closely associated with the immune-suppressive microenvironment[5]. Increasing evidence suggests that different infiltrating immune cells are related to the prognosis of immunotherapy patients through different mechanisms in the tumor

microenvironment. For example, regulatory T cells (Tregs) can prevent autoimmune response, and tumor-associated macrophages (TAMs) prompt tumor progression in glioma[6]. Currently, immune checkpoint therapy has been regarded as a promising treatment strategy for many human malignancies[7]. For instance, programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitors have improved clinical outcomes in lung cancer and melanoma[2, 8].

Apolipoprotein B mRNA-editing enzyme catalytic subunit 3C (APOBEC3C), a protein-coding gene, belongs to a member of the cytidine deaminase family that are capable of editing single-strand DNA and/or RNA sequences[9, 10]. APOBEC3C was identified to be overexpressed in myeloproliferative neoplasm (MPN) and primary effusion lymphoma (PEL), while is downregulated in breast cancer[11–13]. APOBEC3C promotes human pre-leukemia stem cell (pre-LSC) proliferation through activation of deaminase in MPN[11]. In PEL, APOBEC3C might be a propulsive force for carcinogenesis by inducing a high mutation burden[12]. APOBEC3C expression was correlated with breast cancer mutagenesis and clinical prognosis[13]. Additional evidence suggests that APOBEC3s are upregulated in multiple cancers and strongly correlate with prognosis, particularly in low grade glioma (LGG)[14]. The carcinogenesis of multiple cancers is likely to be driven by APOBEC3C-mediated mutagenesis[15]. However, the relationship between the biological functions of APOBEC3C and the occurrence and development of tumors in various cancers remains unclear. Moreover, whether the APOBEC3C expression is associated with TME in brain gliomas needs to be further explored, a comprehensive analysis is needed.

In present study, we conducted data mining and analysis on various databases, and comprehensively analyzed the expression of APOBEC3C in 33 cancer types. Then, we utilized the Gene Expression Profile Interactive Analysis (GEPIA) database to further explore the relationship between APOBEC3C expression and the prognosis of cancer patients. In addition, we further studied the relationship between APOBEC3C expression in the different tumor microenvironment and 6 tumor-infiltrating immune cells, immune checkpoints, tumor mutation burden (TMB), microsatellite instability (MSI), mismatch repair (MMR), and methylation respectively. More significantly, we performed in vitro cellular experiments and found that APOBEC3C contributed to the proliferation, motility, and strong invasive capacity of glioblastoma. The results of this study indicate that APOBEC3C is a new valuable biomarker and is closely related to the immune response in various tumors, particularly in glioma.

Methods And Methods

Data Collection and Processing

The Genotype-Tissue Expression (GTEx) database was used to analyze the expression of APOBEC3C in 31 normal tissues. Similarly, the expression data of APOBEC3C in 21 tumor cell lines were downloaded in Cancer Cell Line Encyclopedia (CCLE) database. We evaluated the results using the Kruskal-Wallis test after a screening of APOBEC3C expression levels in various normal human tissues and neoplastic cell lines. To compare the differential expression of APOBEC3C in normal and neoplastic samples,

differentially expressed genes were identified by using the data originated from the Cancer Genome Atlas (TCGA) and the GTEx database. Differences in expression were compared using the Student's t-test. All the gene expression data were downloaded in the form of log2(TPM + 1).

Survival Analysis

We utilized the GEPIA database to assess Overall survival (OS) and disease-specific survival (DSS) data of 33 different human cancers with different levels of APOBEC3C expression. We drew forest plots and the Kaplan-Meier survival curves to compare the prognostic value of different cancer types with different levels of APOBEC3C expression on OS and DSS.

Immune infiltration analysis

Tumor Immune Estimation Resource (TIMER) database, an integrated website, is used to systemically evaluate immune infiltration and expression levels of the gene in different cancer types. We utilized the TIMER to measure abundance values of the immune infiltration of 6 major immune cells in different tumor tissues, which include B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells. Furthermore, we calculated the Spearman correlation coefficient to evaluate intercorrelation among the APOBEC3C expression and immune infiltrate levels. In this study, we used the Estimation of Stromal and Immune cells in Malignant Tumors using the Expression data (ESTIMATE) algorithm to evaluate the relationship between APOBEC3C expression and the scores of these infiltrating immunocytes downloaded from the TIMER database. Then, we calculated the correlations of APOBEC3C expression with the immune score, stromal score, and ESTIMATE score respectively by Spearman correlation coefficient analysis. A heatmap of expression was graphed to examine the associations of APOBEC3C levels with immune checkpoint markers in 33 types of cancer via Pearson correlation.

Mismatch Repair (MMR) Gene and Methylation Transferase Analysis

Mismatch repair (MMR) deficiency can contribute to tumor initiation. Correlations between APOBEC3C expression and MMR genes were assessed using Pearson correlation coefficient analysis. Additionally, Dysregulation of methylation transferases (DNMT1, DNMT2, DNMT3A, and DNMT3B) activity will disturb DNA transcription, leading to the occurrence of cancers. We evaluated the association between APOBEC3C and the expression levels of these four methyltransferases via Pearson correlation.

Clinical Samples

Glioma tissues were obtained from the department of neurosurgery in Xiangya hospital of Central South University. For immunohistochemical (IHC) staining, 80 glioma frozen tissues (stored at -80°C) of different grades were evaluated. None patients received any chemo- or radiotherapy before surgery. All patients signed informed consents and this study received the approval of the Ethics Committee of Xiangya hospital of Central South University.

Cell Lines and Cell Culture

Glioblastoma cell lines A172 and U87 were stored in our laboratory. A172 and U87 cells were maintained in high-glucose DMEM (Gibco) with 10% fetal bovine serum at 37°C with 5% CO_2.

Reverse Transcription and Quantitative Real-Time PCR

Total RNA was extracted using the Trizol reagent (Invitrogen, USA), and complementary DNA (cDNA) was synthesized from 1 μg of RNA with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA). Quantitative real-time PCR (qRT-PCR) was performed to detect the RNA levels of APOBEC3C according to the manufacturer's protocol (SYBR Green Master Mix, Vazyme). The expression levels of those genes were normalized by using $2^{-\Delta\Delta Ct}$ value methods. The primers were purchased from Sangon (Shanghai, China) and the sequences were designed as follows: For APOBEC3C, the forward primer was 5'-AGCGCTTCAGAAAAGAGTGG-3' and the reverse primer was 5'-AAGTTTCGTTCCGATCGTTG-3'. For GAPDH, the forward primer was 5'-CATTGACCTCAACTACATGGTT-3' and the reverse primer was 5'-CCATTGATGACAAGCTTCCC-3'.

RNA Interference

Small interfering RNA (siRNA) targeting APOBEC3C (siAPOBEC3C: 5'-CAACGATCGGAACGAAACT-3') was purchased by RiboBio Corporation (Guangzhou, China). Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific) was used for the siRNA transfection. A172 and U87 cells were plated into each well of the 6-well plate at least 24 h before transfection to achieve 50–60% confluency. Cells were collected 24 h after transfection for RNA isolation.

Cell Proliferation Assay and Clone Formation Assay

After being transfected with siRNA (50 nM) for 24 h, treated and control cells were plated into 96-well plates at 2×10^3 cells per well. At indicated time points, each well was added with 10 μl CCK-8 reagent. The plates were further incubated at 37°C for 1.5 h. The optical density (OD) at 450 nm was measured using a microplate reader (KHB ST-360, Shanghai, China). Cells lines infected with siRNA were seeded in six-well plates at a density of 1000 cells/well. After incubation at 37°C for 14 days, colonies were washed with PBS, fixed with 4% paraformaldehyde, and stained with 1% crystal for 15 min.

Cell Migration and Invasion Assay

Cell migration ability was examined by cell scratch assay. The invasion experiments were carried out using 8-μm transwell filters precoated with Matrigel matrix in 24-well plates. In brief, 5×10^4 cells were seeded into chambers coated with Matrigel for the invasion assay, in DMEM with 10% FBS. After incubated at 37°C for 48 h, cells that did not penetrate the filter were wiped off, and cells on the lower surface of the filter were stained with 0.4% crystal violet. The numbers of invading cells were counted under a light microscope.

Immunohistochemical (IHC) staining

Immunohistochemistry was performed using serial sections from patient tumor sections. 71 clinical samples were paraffin-embedded and sectioned for immunohistochemistry. The sections were

deparaffinized using xylene and rehydrated by decreasing concentrations of ethanol in the order of 100%, 95%, 85% and water. Antigen retrieval was treated using a low-boiled 10mM citrate buffer, pH 6.0. Endogenous peroxidase activity was quenched by 3% hydrogen peroxide. Blocking was performed using 1% BSA. The sections were incubated with the following primary antibodies at 4°C overnight: rabbit anti-APOBEC3C (1:100; Proteintech 10591-1-AP). Primary antibody staining was visualized using peroxidase-conjugated anti-rabbit IgG followed by the DAB substrate kit for peroxidase visualization of secondary antibodies. The Slides were then rinsed, dehydrated, mounted and cover slipped. The immunostaining results were examined by two researchers independently. Immunohistochemical evaluation was based on the intensity and percentage of membranous and cytoplasmic reactivity: Staining intensity was scored as follows: no staining (0); faint yellow staining (1); intermediate positive (2); and brown staining (3). Staining of APOBEC3C was scored by the percentage of positive cells (0, < 10%; 1, 10–25%; 2, 26–50%; 3, 51–75%; 4, > 75%). The final immunoreactive score (FIS) was calculated as: staining intensity × percentage of positive cells. We defined FIS (0–4) as low expression and FIS (6–12) as high expression[16].

Results

Aberrant Expression of APOBEC3C Across Cancers

APOBEC3C expression in 31 kinds of normal human tissues was determined by analyzing the data obtained from the GTEx database. APOBEC3C expression was relatively up-regulated in several normal tissues, including the adrenal gland, bone marrow, and testis tissues (Fig. 1A). Furthermore, APOBEC3C levels in 21 different cancer types were detected. According to the obtained results from the CCLE database, all 21 kinds of cancer cells expressed APOBEC3C gene (Fig. 1B). To further determine whether APOBEC3C gene was differentially expressed in paired normal and tumor tissues, we integrated the GTEx and TCGA databases, analyzing 27 groups of tissue data. Results revealed APOBEC3C expression levels were significantly up-regulated in 22 tumors including ACC, BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, SKCM, STAD, TGCT and THCA samples as compared with normal samples. In contrast, it was expressed much lower in KICH, PRAD, READ, and UCEC compared with normal samples (Fig. 1C). Collectively, we conclude that APOBEC3C gene is aberrantly over-expressed across different tumors.

Associations Between APOBEC3C Expression and Prognostic Value in Human Pan-Cancer

To evaluate whether APOBEC3C expression is associated with the survival of cancer patients, we compared the OS and DSS of patients with different APOBEC3C expression levels (Fig. 2). Particularly, higher expression of APOBEC3C seemed to be a risk predictor for OS in patients with COAD, GBM, LGG, PAAD, and THYM, but reversely in BRCA and CESC (Fig. 3A-G). Moreover, higher expression of APOBEC3C was an indicator of unfavorable outcomes for DSS in patients with COAD, GBM, LAML, LGG, PAAD, and THYM, but oppositely in BLCA, BRCA, CESC, MESO, and SARC (Fig. 4A-K).

Correlations of APOBEC3C Expression with Levels of Immune Infiltration, Immune Checkpoint Marker, TMB, and MSI

To explore whether APOBEC3C level was related to immune infiltration levels in diverse cancer types, we analyzed domain scores related to 6 kinds of infiltrating immune cells (B cells, $CD8^+$ T cells, $CD4^+$ T cells, neutrophils, macrophages, and dendritic cells) across cancers from TIMER. The infiltration degree of 6 immune cells was visibly positively associated with the APOBEC3C expression levels in BRCA, KIRC, LGG, LIHC, LUAD, and PAAD (Fig. 5). Next, we calculated the stromal, immune, and ESTIMATE scores of tumor samples for three outcome predictors to analyze the immune and stromal components in pan-cancer. Our results show that the top three tumors undoubtedly positively correlated with APOBEC3C expression were LGG, KICH, and GBM (Fig. 6).

The appearance of immune checkpoint inhibitors (ICIs) offered those patients with various types of malignancy a potential treatment. Therefore, we determined whether APOBEC3C expression levels directly correlate with immune checkpoint gene expression in 33 cancer types. Particularly, in BRCA, HNSC, KIRC, LGG, LIHC, LUAD, PAAD, and THCA, a heatmap showed there were significant positive correlations between APOBEC3C expression and various immune checkpoint molecules, such as CTLA4, CD40, TIGIT, and LAIR1, etc (Fig. 7A). In addition, the association between TMB/MSI and APOBEC3C expression was also assessed. We found that APOBEC3C was positively correlated with TMB in LGG but conversely in THYM, THCA, STAD, PRAD, LUSC, LUAD, LIHC, GBM, and ESCA (Fig. 7B). APOBEC3C was positively correlated with MSI in COAD, and negatively correlated with MSI in UCEC, THCA, STAD, SKCM, SARC, PRAD, LUAD, LGG, and CESC (Fig. 7C). In summary, APOBEC3C gene plays a crucial role in regulating reactions of tumor immunity.

Correlation Analyses of APOBEC3C Expression with MMR Defects and Methylation Transferases in Different Cancers

Mismatch repair (MMR) defects will result in replicating faulty DNA that cannot be repaired[17], which will make somatic hypermutation and oncogenesis[18]. After establishing the relationship between APOBEC3C and mutation indexes TMB as well as MSI, assessment of the performance of APOBEC3C in oncogenesis was necessary, we detected its expression is associated with well-recognized mismatch repair genes. APOBEC3C expression was significantly correlated with these MMR genes (MLH1, MSH2, MSH6, PMS2, and EPCAM) in a wide range of cancer types (Fig. 8A). Epigenetic modification of methylation can change gene expression[19]. Changes in methylation state play a vital role in oncogenesis[20]. Next, we further investigated whether APOBEC3C expression is correlated with 4 methylation transferases such as DNMT1, DNMT2, DNMT3A, and DNMT3B. Obviously, these DNA methyltransferase genes were particularly co-expressed in LGG, LIHC, KIRP, KICH, NHSC, and COAD (Fig. 8B). In conclusion, these results illustrate that APOBEC3C may modulate DNA damage or methylation-mediated tumorigenesis.

High APOBEC3C Expression Associates with Malignancy in Glioma

Based on the TCGA and GTEx database, the APOBEC3C gene was highly expressed in LGG and GBM samples than in normal tissues (Fig. 1C). While APOBEC3C expression in gliomas remained poorly understood. Immunostaining indicated that APOBEC3C was elevated in gliomas compared with that of non-tumor tissues detected by IHC staining, the expression level of APOBEC3C showed a significantly correlation with WHO grade of glioma (Fig. 9A-B), suggesting that higher APOBEC3C level was paralleled with higher malignancy in glioma.

APOBEC3C Knockdown Inhibits Proliferation, Migration, and Invasion of Glioma Cells

To further confirm whether the APOBEC3C gene was involved in cancer progression, GBM cell lines A172 and U87 were selected to explore the biological behaviors of APOBEC3C in glioma progression. We used a small interfering RNA (siRNA)-mediated knockdown of APOBEC3C in A172 and U87 cells. Negative control (si-NC) and si-APOBEC3C were used to transfect A172 and U87 cells. The expression levels of APOBEC3C were downregulated dramatically in A172 and U87 cells after transfection with siAPOBEC3C (Fig. 10A). The CCK-8 assay was utilized to evaluate the effect of changes in the expression of the APOBEC3C gene on cell proliferation. Compared with the negative control (NC) group, the proliferation of A172 and U87 cells was suppressed in the si-APOBEC3C group (Fig. 10B). In a colony formation assay, knockdown of APOBEC3C in A172 and U87 cells significantly decreased the number and volume of colonies (Fig. 10C-D). Thus, the APOBE3C3 gene affects cell proliferation, further confirming its carcinogenic activity. Besides, we subsequently conducted scratch wound healing and Transwell assays to further confirm the migration and invasion of glioma cells after transfection with the siRNA. Wound healing and invasive activities were decreased after APOBEC3C knockdown (Fig. 10E-H). These results suggest that APOBEC3C is strongly associated with the proliferation, migration, and invasion in glioma.

Discussion

Pan-cancer analysis for APOBEC3C can comprehensively reveal its functional significance with abnormal expressions between tumor tissues and normal ones, providing new insights into the pathogenesis and prevention of cancer[21]. In this study, we utilized multiple public databases to perform comprehensive bioinformatics analysis of the expression of the APOBEC3C gene in normal tissues and different types of tumors. In addition, we examined the association between APOBEC3C expression and survival outcomes (OS and DSS) and found that APOBEC3C expression levels had different prognostic impacts across the different cancer types. APOBEC3C was associated with immune features (including immune infiltration, immune checkpoints, TMB, and MSI), MMR Gene Mutation, and epigenetic modification methylation. Finally, we conducted several in vitro experiments with GBM cells to further explore the biological functions of APOBEC3C in tumor progression.

APOBEC3C belongs to the cytidine deaminase (APOBEC) family that can convert cytosine to uracil or thymine (C-to-U or T)[22]. Recent studies have shown that APOBEC3C may be involved in the occurrence and development of human cancer[11]. However, its role in most types of cancer is still quite unclear. By analyzing 27 cancer data sets, we found that the expression of APOBEC3C was significantly different in several new tumor types, such as CHOL, GBM, and LGG. OS and DSS are important prognostic indicators in survival analysis. Through the analysis of these 2 prognostic indices, we found that the expression of APOBEC3C can be regarded as a tumor marker for predicting unfavorable prognosis, especially in glioma. Therefore, our studies strongly suggest that APOBEC3C may provide a new sight for tumor prognosis and treatment.

The tumor microenvironment, including neoplastic cells, non-cancerous cells, and some non-cellular components, is a key factor in conducting complex interactions with tumor cells, such as assisting malignant cells to escape from immune clearance, and promoting tumor inflammation, invasion and metastasis. With in-depth studies, therapies targeted against the TME have become an ingenious choice[23]. Increasing evidence suggests that tumor-infiltrating lymphocytes (T and B cell lineage) and other immune cells (dendritic cells, macrophages, and neutrophils) play an indispensable role in the tumor immune response. At present, there are few studies on the relationship between APOBEC3C expression levels and immune infiltration. As shown in this research, the expression of APOBEC3C was significantly correlated with the level of immune cell infiltration in BRCA, KIRC, LGG, LIHC, LUAD, and PAAD. The immune score, which is composed of the immune cell type, density, and location, is considered to be a simple, quantitative and standardized routine tool for evaluating the immune environment, showing great potential in predicting the prognosis of immune or matrix components and evaluating the value of biomarkers[24]. Similarly, we calculated matrix scores, immune scores, and estimated scores of APOBEC3C gene expression in different cancers. Our results suggested that APOBEC3C expression positively correlated with the matrix and immune components in LGG and GBM. Immune checkpoint inhibitors (ICIs) have emerged as a new pillar of cancer treatment, pointing to new directions for tumor immunotherapy[25]. Patients with high TMB or MSI are more likely to receive the better efficacy of immunotherapy. TMB and MSI can be used as predictors of immunotherapy for cancer patients[26, 27]. Previous studies have shown that APOBEC3 was abnormally expressed in many malignant tumors and led to genome instability and idiosyncratic DNA mutations by the deamination of cytosine to thymine (C-to-T)[28–31]. Jiang et al. reported that APOBEC3C was upregulated in myeloproliferative neoplasm (MPN) patients, and the burden of C-to-T mutations increased, subsequently inducing the proliferation of human pre-leukemia stem cells (pre-LSCs) and facilitating the transformation into acute myeloid leukemia stem cells (LSCs)[11]. With this in mind, our work examined the correlation between APOBEC3C expression and TMB and MSI in all TCGA patients. The results suggested that APOBEC3C expression was associated with TMB and MSI respectively.

Mismatch repair can correct errors missed by the proofreading function of DNA polymerase, thereby ensuring the stability of DNA replication, while MMR gene mutations lead to defects in the mismatch repair function (dMMR), subsequently resulting in genome or microsatellite instability[32]. A body of

evidence showed that mismatch repair defects (dMMR) could be used to predict the efficacy of immune checkpoint inhibitors (ICI)[33]. Abnormal methylation plays important role in the development and metastasis of tumors[34]. In our research, we found that APOBEC3C was linked with the expression of 5 MMR genes and 4 DNA methyltransferases in human cancer. These results indicate that the aberrant APOBEC3C expression may play an essential role in oncogenesis by regulating MMR activity and methylation. Based on the above bioinformatics analysis results, we also carried out IHC and in vitro cell experiments to study the biological functions of APOBEC3C in GBM cell lines. The results suggested that higher APOBEC3C level was paralleled with higher malignancy in glioma and the upregulation of APOBEC3C promoted the proliferation, migration ability as well as invasion of glioblastoma. There are studies indicating that APOBEC3B triggers DNA replication stress and chromosomal instability through incomplete replication of genomic DNA[35]. Therefore, we speculate that APOBEC3C may play an important role in malignancy in glioma by APOBEC3C-mediated mutagenesis.

Although we used multiple databases to perform pan-cancer analysis on APOBEC3C, there is still much room for improvement in our research. First of all, we have not conducted a comprehensive analysis of mutational signatures, especially in glioma. Due to this, our understanding of the biological process of mutation is limited, which means that more detailed studies are needed. Secondly, this study only conducted limited experiments, we will conduct more experiments in vivo or in vitro, especially in glioma. Third, our work proved that APOBEC3C expression was associated with tumor immunity and disease prognosis, but how APOBEC3C regulates tumor microenvironment and immune-related pathways to influence clinical survival and prognosis requires further mechanism research. Future research on the APOBEC3C gene may solve these scientific problems.

Conclusions

The APOBEC3C expression is significantly correlated with tumor prognosis and immune infiltration across cancers, especially in glioma. APOBEC3C might act as a new potential biomarker for prognostic prediction and immune cell infiltration of glioma.

Abbreviations

Adrenocortical carcinoma: ACC; bladder urothelial carcinoma: BLCA; breast invasive carcinoma: BRCA; cervical squamous cell carcinoma: CESC; cholangiocarcinoma: CHOL; colon adenocarcinoma: COAD; lymphoid neoplasm diffuse large B cell lymphoma: DLBC; esophageal carcinoma: ESCA; glioblastoma multiforme: GBM; brain lower grade glioma: LGG; head and neck squamous cell carcinoma: HNSC; kidney chromophobe: KICH; kidney renal clear cell carcinoma: KIRC; kidney renal papillary cell carcinoma: KIRP; acute myeloid leukemia: LAML; liver hepatocellular carcinoma: LIHC; lung adenocarcinoma: LUAD; lung squamous cell carcinoma: LUSC; mesothelioma: MESO; ovarian serous cystadenocarcinoma: OV; pancreatic adenocarcinoma: PAAD; pheochromocytoma and paraganglioma: PCPG; prostate adenocarcinoma: PRAD; rectum adenocarcinoma: READ; sarcoma: SARC; skin cutaneous melanoma: SKCM; stomach adenocarcinoma: STAD; testicular germ cell tumors: TGCT; thyroid carcinoma: THCA;

thymoma: THYM; uterine corpus endometrial carcinoma: UCEC; uterine carcinosarcoma: UCS; and uveal melanoma: UVM

Declarations

Ethics approval and consent to participate

This study received the approval of the Ethics Committee of Xiangya hospital of Central South University. All patients signed informed consents.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used in the current study are included in the article. Other data and materials are available from the corresponding author upon reasonable request.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors' contributions

Conceived and designed this study: LW, CPR and XJJ. Wrote the manuscript: YRK, and ZQX. Data collection, analysis, and visualization: YRK, ZQX, WY, QWZ, YZ, YWG, ZPJ, CL, YDC, ZPW, HXH, DCX, HLH and CHZ. All authors read and approved the fnal manuscript.

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Figures

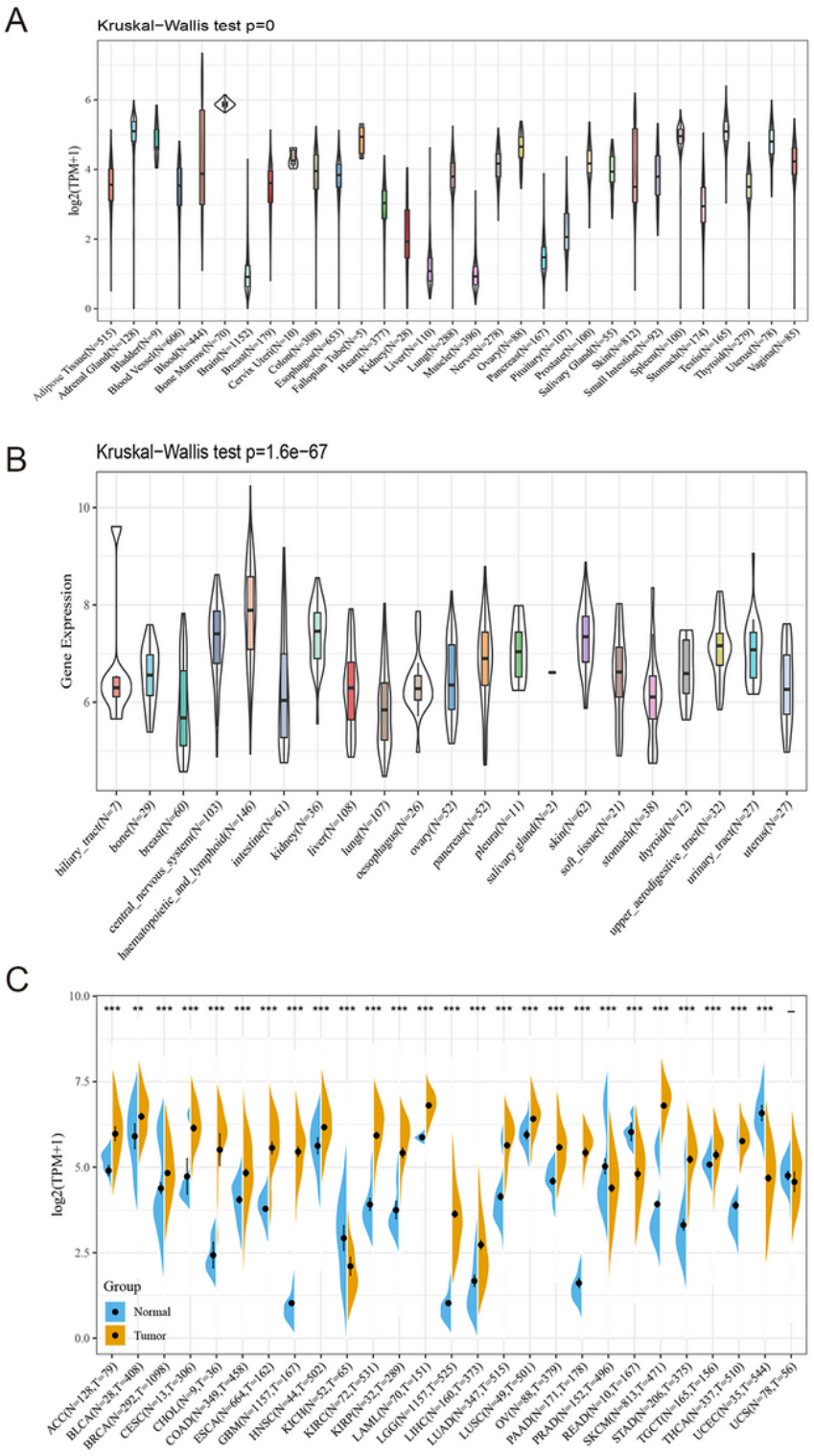

Figure 1

The expression levels of APOBEC3C in the human pan-cancer dataset. **(A)** APOBEC3C expression in 31 normal human tissues from the GTEx database. **(B)** APOBEC3C expression in 21 tumor cells from the CCLE database. **(C)** APOBEC3C was significantly upregulated in 22 cancer types from the TCGA and GTEx databases (**P*< 0.05, ***P*< 0.01, and ****P*< 0.001).

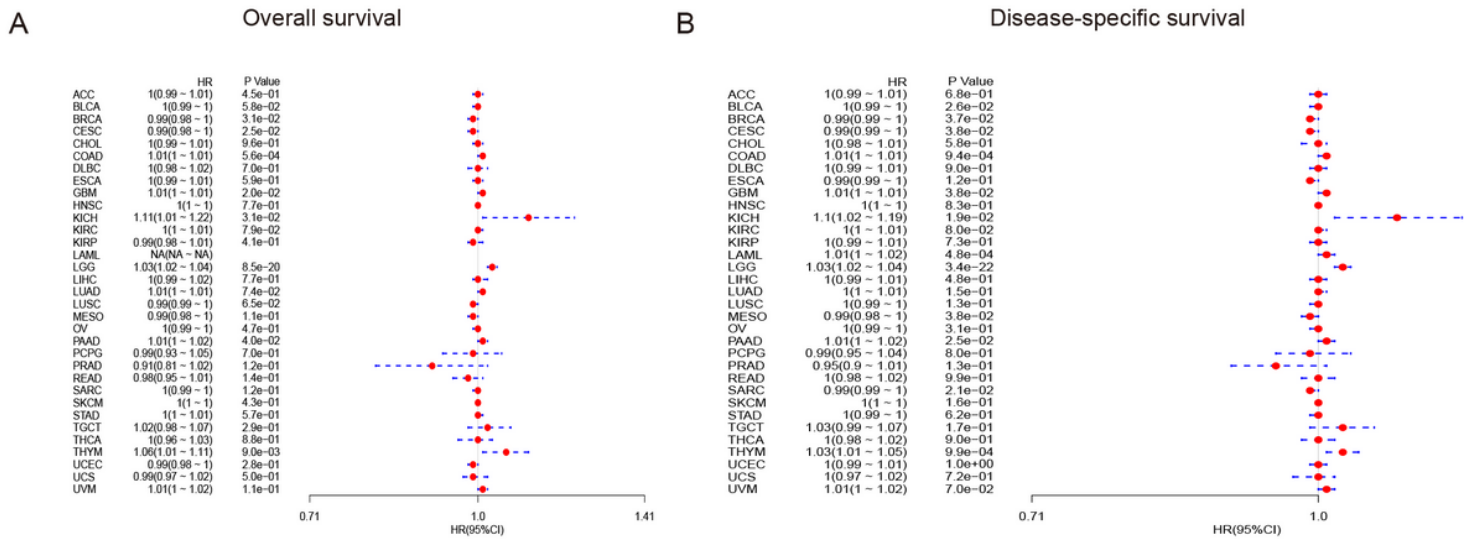

Figure 2

Relationship of APOBEC3C expression with patients' OS and DSS. **(A)** Forest plots showing the HRs related to APOBEC3C expression with patients' OS in 33 cancer types. **(B)** Forest plots showing the HRs related to APOBEC3C expression with patients' DSS in 33 cancer types.

Figure 3

Survival analyses based on differences in APOBEC3C expression in patients with different cancers. **(A-G)** Kaplan-Meier OS curves for patients stratified by different expression levels of APOBEC3C in 7 cancer types.

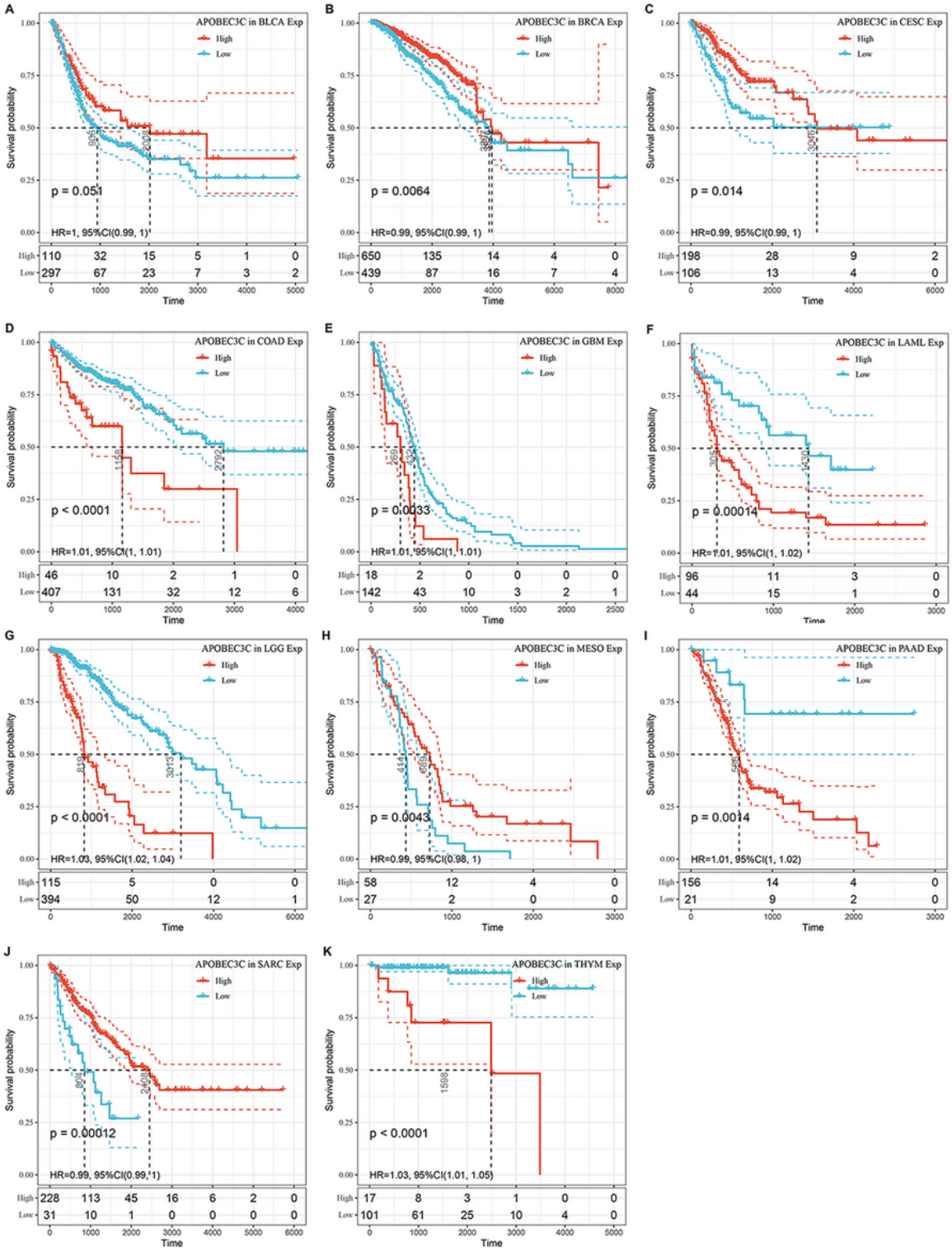

Figure 4

Survival analyses based on differences in APOBEC3C expression in patients with different cancers. **(A-K)** Kaplan-Meier DSS curves for patients stratified by different expression levels of APOBEC3C in 11 cancer types.

Figure 5

APOBEC3C expression is positively correlated with immune cell infltration in cancers. (**A)** BRCA. (**B)** KIRC. **(C)** LGG. (**D)** LIHC. (**E)** LUAD. (**F)** PAAD.

Figure 6

Correlations of the immune score, stromal score, and ESTIMATE scores with APOBEC3C expression in the top three cancers. **(A)** LGG. **(B)** KICH. **(C)** GBM.

Figure 7

Correlations betweenAPOBEC3C expression and immunity, including immune marker sets, TMB, and MSI in cancers. **(A)** Correlation between APOBEC3C expression and immune marker sets. **(B)** Radar map of correlation between APOBEC3C expression and TMB. **(C)** Radar map of correlation between APOBEC3C expression and MSI.

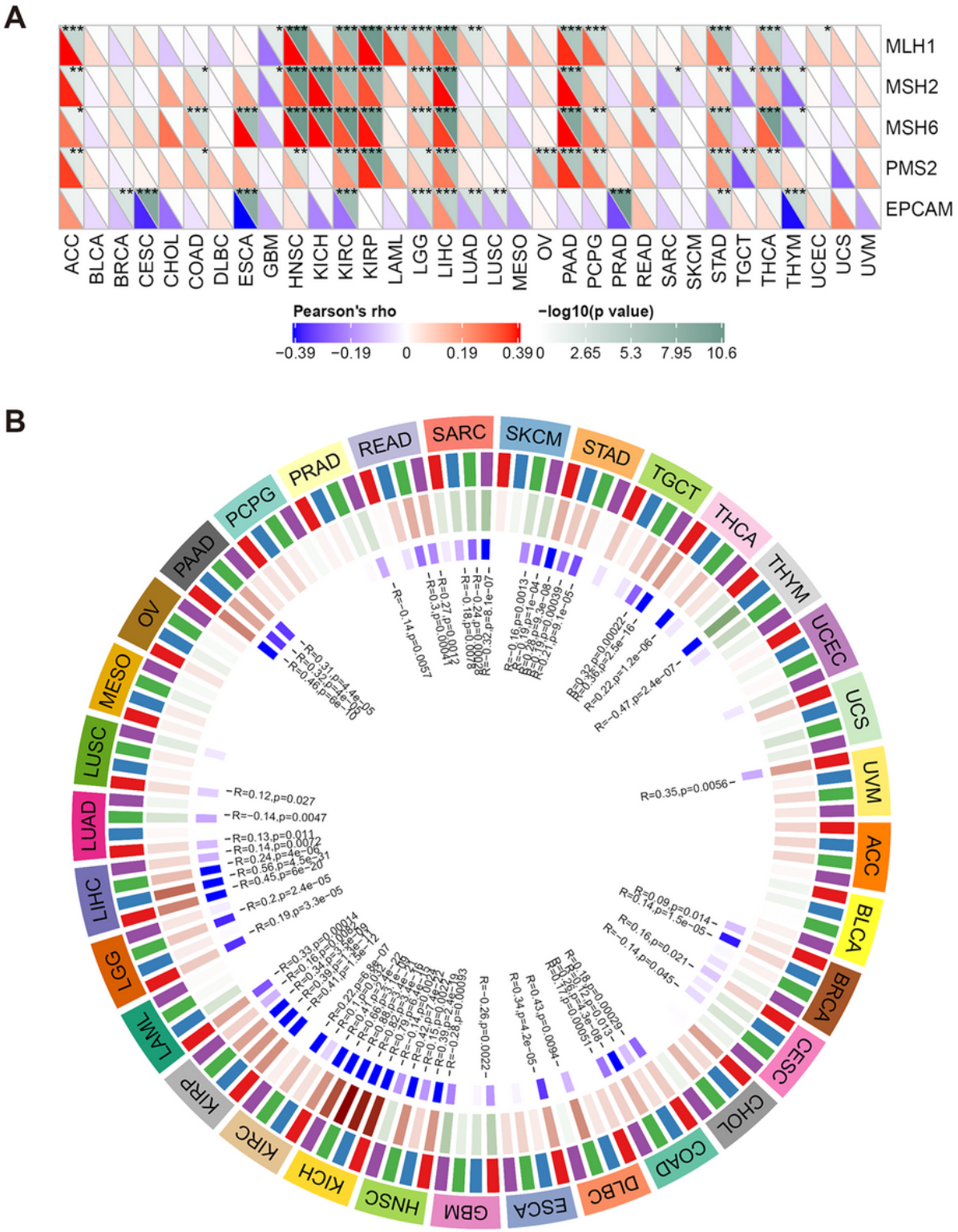

Figure 8

APOBEC3C is correlated with MMR gene mutation levels and DNA methyltransferases expression in human pan-cancer. **(A)** Pearson correlation analysis of APOBEC3C expression with mutation levels of MMR genes in human pan-cancer (*P < 0.05, **P < 0.01, ***P < 0.001). **(B)** Pearson correlation analysis of APOBEC3C expression with DNA methyltransferases in human pan-cancer.

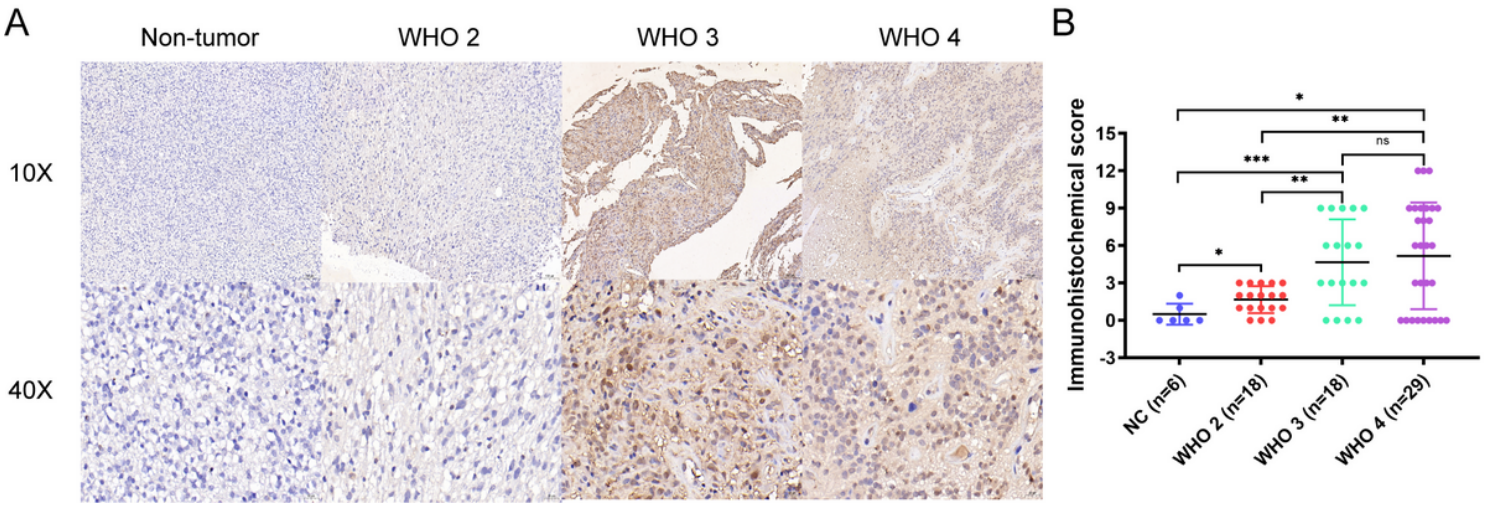

Figure 9

IHC staining was used to detect APOBEC3C expression in glioma tissues and non-tumor tissues. (A) The expression level of APOBEC3C detected by IHC. (B) Statistical analysis of IHC results of APOBEC3C (*P < 0.05, **P < 0.01, ***P < 0.001).

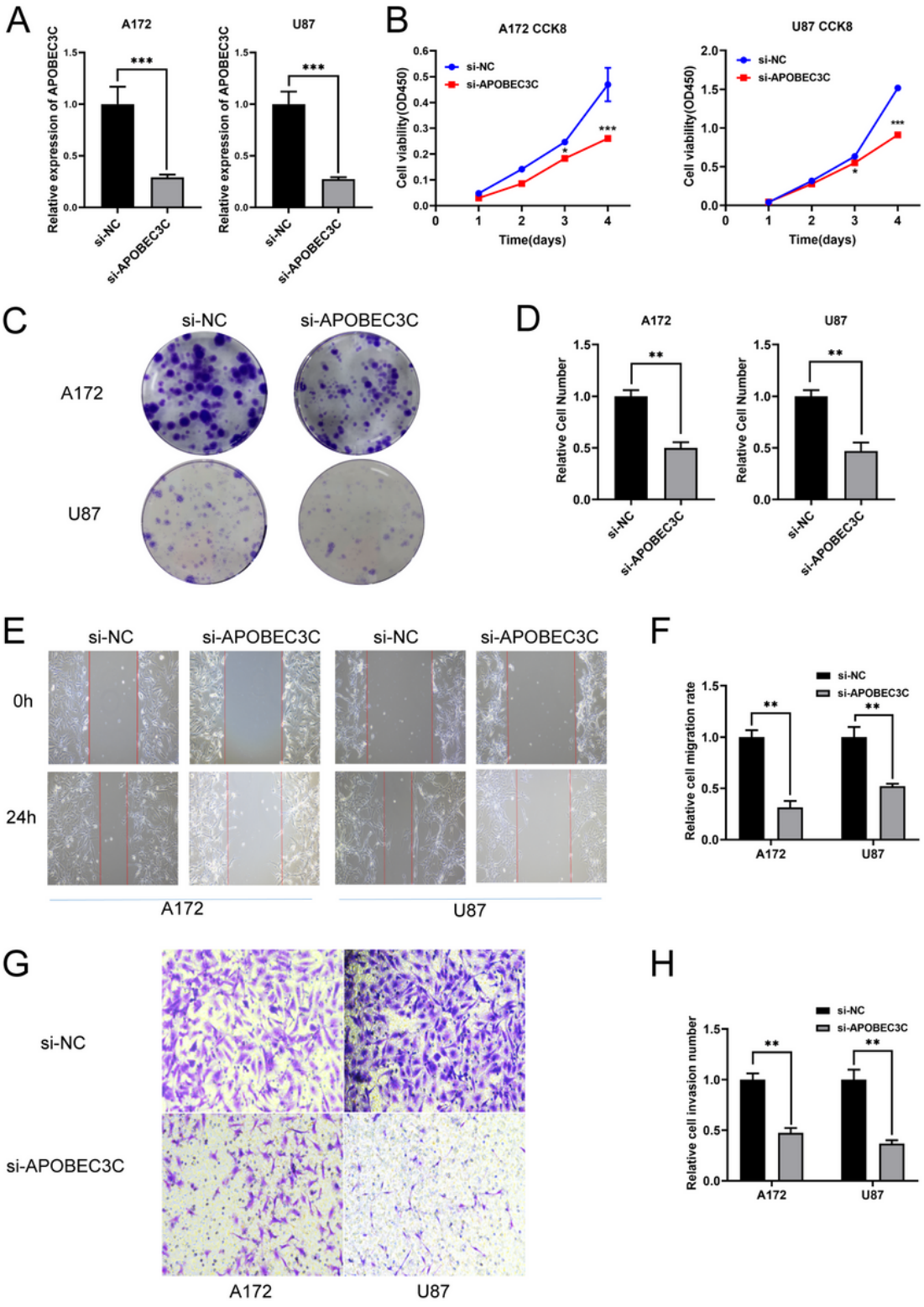

Figure 10

Knockdown of APOBEC3C inhibits proliferation, migration, and invasion. A172 and U87 cells transfected with NC-siRNA or APOBEC3C-siRNA were treated. **(A)** The transfection efficiency of the APOBEC3C-siRNA in A172 and U87 cells, as measured using RT-qPCR. **(B)** CCK-8 assays were used for proliferation ability detection. **(C-D)** Colony formation experiments were used for cell viability detection. **(E-F)** Wound healing

assays were used for migratory abilities detection. **(G-H)** Transwell assays were used for invasion detection. Data are represented as the mean values ± SD. (* $p < 0.05$, ** $p < 0.01$ and *** $P < 0.001$)