INTRODUCTION

Glioblastoma (GBM) is the most common and aggressive primary central nervous system (CNS) malignant tumor in adults, accounting for ~48.6% of all malignant CNS tumors [1]. Although the current standard treatment of GBM includes safe resection of tumor to the maximum extent or biopsy, followed by administration of temozolomide (TMZ) combined with radiotherapy and maintenance TMZ for 6–12 months [2], the median survival time of GBM patients is only 14–16 months, and the 5-year overall survival (OS) remains <10% [3]. Although management of GBM frequently involves the use of TMZ, due to severe hematological toxicity and low blood-brain barrier permeability, GBM treatment requires more breakthroughs.

During the last decade, new clinical trials for GBM have continuously emerged, including targeted drug therapy (such as EGFR-V1600E [4], BRAFV600E [5], MET [6], and immune checkpoint inhibitors (PD-1/PD-L1 [7–9], CTLA4 [10])), vaccination [11, 12], virotherapy [13–15], and CAR T-cell [16]. However, most of these trials have failed to prolong the OS of GBM patients in phase I–III clinical trials. Encouragingly, one phase III clinical trial of Tumor-Treating Fields (TTFields) for newly diagnosed GBM recruited 695 patients, and the results showed that patients treated with TMZ plus TTFields exhibited significantly longer overall survival (OS, 20.9 months vs. 16.0 months) and progression-free survival (6.7 months vs. 4.0 months) than those treated with TMZ alone [3]. Therefore, additional targeted molecules that are important for glioma progression need to be discovered.

Transmembrane protein 158 (TMEM158), also known as RIS1, P40BBP, BBP, and HBBP, a member of the TMEM family, was identified as an upregulated candidate tumor suppressor gene during Ras-induced senescence in haploid fibroblasts infected with RAS V12 lentivirus [17]. The expression level and biological mechanisms of TMEM158 have since been reported in many tumors [18–21]. Mohammed Ael et al. identified TMEM158 as a powerful predictive marker for cisplatin therapy efficacy in nonsmall cell lung cancer [18]. In addition, TMEM158 is upregulated in ovarian cancer and significantly promotes the proliferation, invasion, cell adhesion, and tumorigenesis of ovarian cancer cells [19]. TMEM158 was also found to be significantly overexpressed in pancreatic cancer and is associated with larger tumor size and poorer prognosis. Knockdown of TMEM158 inhibits the proliferation, migration, and invasion of pancreatic cancer cells [20]. Moreover, loss of function of TMEM158 significantly decreases the proliferation and migration of colorectal cancer cells and inhibited multidrug resistance [21]. However, the roles of TMEM158 in glioma have not been explored.

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Signal transducer and activator of transcription 3 (STAT3) may be the key point of multiple major oncogenic signaling pathways, such as epithelial growth factor receptor (EGFR), c-MET, and Janus family of kinases (JAK) [22, 23]. STAT3 is persistently activated in nearly 70% of human cancers [24], especially in gliomas [25]. Previous studies have shown that excessive activation of the STAT3 signaling pathway is involved in proliferation, metastasis, angiogenesis, stemness, therapeutic resistance, and the immunosuppressive microenvironment in GBM [26–28]. As a key molecule, activation of STAT3 promotes proliferation, migration, and epithelial-mesenchymal transition (EMT) of glioma cells [29]. However, there is currently no comprehensive analysis of the relationship between TMEM158 and the STAT3 signaling pathway.

In this study, we first investigated the clinicopathological and biological characteristics of TMEM158 in gliomas. The clinicopathological features that were evaluated included WHO grade, IDH mutation and 1p/19q status, gliomas tissue microarray, and OS. In addition, the effects of TMEM158 loss- and gain-of-function on the proliferation, migration, and invasion of glioma cells were assessed. Mechanistically, we further confirmed that TMEM158 enhanced glioma cells proliferation, migration, and invasion as well as the progression of EMT by activating STAT3 signaling. Importantly, TMEM158 downregulation inhibited glioma cell growth and decreased the expression of p-STAT3 and Ki-67 in vivo. Taken together, our findings revealed that TMEM158 is upregulated in GBM and promotes GBM tumorigenesis and aggressiveness by activating the STAT3 signaling pathway, highlighting that it may represent an effective therapeutic target in GBM.

MATERIALS AND METHODS

Bioinformatics analysis

The Cancer Genome Atlas (TCGA) pan-cancer RNA-seq data (level 3) and GTEx RNA-seq data were obtained from the UCSC website (https://xenabrowser.net/datapages/), and the datasets were used by the UCSC team to remove batch effects and perform transcripts per million conversion. TMEM158 gene expression and clinical data were obtained from TCGA database (https://cancergenome.nih.gov) and the Chinese Glioma Genome Atlas (CGGA) database (http://www.cgga.org.cn). Kaplan–Meier survival curves were used to analyze differences in TMEM158 expression and their effects on OS in glioma patients. Differentially expressed genes (DEGs) were screened with R language using the “DESeq2” package. Then, GSEA of the HALLMARK gene set was performed based on the Log2FoldChange value ranking of the DEGs by R language using the “clusterProfiler” package. We also performed Gene Ontology (GO) analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using the online DAVID database (https://david.ncifcrf.gov).

Clinical sample collection

Clinical glioma tissues and glioma pathologic diagnoses were obtained from the Department of Neurosurgery, Tianjin Medical University General Hospital, China, from August 2011 to April 2017 [26]. All samples were histologically diagnosed by pathologists based on the World Health Organization (WHO) classification for brain tumors. Written informed consent was obtained from all donors or their relatives. This study was performed in accordance with the principles of the Helsinki Declaration and was approved by the ethical committee of Tianjin Medical University General Hospital.

Tissue microarray (TMA)

The TMA was run using tissues from 55 patients, including 2 non-tumor cases (temporal lobe epilepsy and cortical dysplasia), 1 WHO I case, 12 WHO II cases, 12 WHO III cases, and 28 WHO IV cases. For each tumor mass, we divided the tissues into intratumor, tumor border, and peritumor tissues. In this study, we excluded WHO I cases and only analyzed intratumoral and peritumoral staining.

H&E staining and immunohistochemistry (IHC) analysis

Paraffin-embedded tissues used for H&E staining and IHC analysis were prepared as previously described [30, 31]. Glioma tissues and mouse brain tissues were incubated with primary antibodies (TMEM158, 1:100, ab98335, Abcam, USA; p-STAT3, 1:100, 91455, Cell Signaling Technology, USA; and Ki-67, 1:100, 90275, Cell Signaling Technology, USA), IHC markers were detected using a goat anti-rabbit IgG two-step detection kit (PV-9000, ZSGB-Bio, China). Next, the slides were counterstained with Mayer Hematoxylin Solution (G1080, Solarbio, China) for nuclear staining. The IHC staining images were acquired using a VANOX microscope (Olympus, Japan). The intensity score was graded as 0 (negative), 1 (weakly positive, light brown), 2 (moderately positive, brown), or 3 (strongly positive, dark brown). The quantity score was graded as 0 (negative), 1 (<25%), 2 (26–50%), 3 (51–75%), or 4 (>75%). The total IHC staining score was calculated by adding the intensity and quantity scores.

Cell lines and cell culture

Human glioma A172, LN18, LN229, and T98G cell lines were purchased from ATCC (USA), SNB19, U251MG, and U87MG cells were purchased from the Chinese Academy of Sciences Cell Bank (China). The human glioma TJ905 cell line was isolated from human GBM tissue and cultured in DMEM/F12 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA). All other glioma cell lines were cultured in DMEM (Gibco, USA) supplemented with 10% FBS and incubated in 5% CO2 at 37 °C.

Lentivirus and plasmid transfection

We constructed shRNA-TMEM158 sequences (sh-1, 5′-ctggccaac ggcattggaaca-3′; sh-2, 5′-ggattttctgctgctagactt-3′) using the GV493 vector, and a scramble sequence (5′-tctccgacggtgcagct-3′) was designed as a negative control. We also constructed a TMEM158-overexpressing plasmid using the GV492 vector (GeneChem, China). Lentiviral transfection was conducted according to the manufacturer’s manual. After infection, U87MG, U251MG, and TJ905 cells were selected using 2.0 µg/ml puromycin solution. Plasmids were purchased from Hanbio (China). A STAT3-overexpressing plasmid was constructed using the pcDNA3.1 vector. Plasmids were transiently transfected into cells using Lipofectamine 3000 (InVitrogen, USA).

RNA isolation and real-time polymerase chain reaction (RT-PCR)

Total RNA from cells or tissues was extracted using TRIzol reagent (15596018, Thermo Fisher Scientific, USA), and RNA (5 µg) was reverse transcribed to cDNA using the GoScript Reverse Transcription System (A5001, Promega, USA). RT-PCR was conducted as previously described [32]. Expression of TMEM158 and GAPDH (internal control) mRNA was detected using GoTaq qPCR Master Mix (A6001, Promega, USA). The primer sequences (Genewiz, China) were as follows: TMEM158 Forward: 5′-ggcatggaaca-3′; TMEM158 Reverse: 5′-cgattttctgctgctagactt-3′; GAPDH Forward: 5′-ttggcttcctctgtgctactc-3′; GAPDH Reverse: 5′-ggttcttcctgctgctagact-3′. Data were analyzed using the relative standard curve method and normalized to GAPDH.

Cell counting kit-8 assay (CCK-8)

Glioma cell viability in response to TMEM158 loss- and gain-of-function and rescue experiments was measured using CCK-8 (CK04, DOJINDO, USA) and was selected in 5% CO2 at 37 °C for 1, 2, 3, and 4 day. Then, the cells were incubated with CCK-8 solution for 1 h, and the absorbance was measured at 450 nm using a microplate luminometer (BioTek, USA).

Colony-formation assay

Glioma cells were seeded into six-well plates and incubated in 5% CO2 at 37 °C for 14 day. Then, the cells were washed with PBS, fixed in 4% paraformaldehyde (P1110, Solarbio, China), and stained with 2.5% crystal violet stain solution (G1061, Solarbio, China). The colony-forming efficiency was defined as the ratio of the number of colonies formed to the number of cells seeded.
Transwell assay
Cells (1.0 × 10^4 cells) were seeded in serum-free DMEM or DMEM/F12 into the upper part of the Cell Culture Insert (24-well format; 353097, Conning, USA) for Transwell migration assay, and the lower chamber was filled with medium supplemented with 10% FBS as a chemoattractant. For the Transwell invasion assay, first, Matrigel was added to the bottom of the chamber (5.0 × 10^4 cells) were then seeded in serum-free DMEM or DMEM/F12 into the upper chamber. After incubation for 24 h, the noninvading cells in the upper chamber were removed using cotton swab. Transwell chambers containing the invading cells were fixed with 4% paraformaldehyde and stained with 2.5% crystal violet stain solution. The number of invading cells on the lower surface of the filters was then quantified.

Western blotting
Cells were lysed in RIPA buffer (RO010, Solarbio, China) containing PMSE (dilution: 1:100; P0100, Solarbio, China), protein phosphatase inhibitor (dilution, 1:100; P1260, Solarbio, China), and protease inhibitor mixture (dilution, 1:100; P6730, Solarbio, China). Then, the total protein concentration was measured using a BCA Protein Assay Kit (PC0020, Solarbio, China) according to the manufacturer’s instructions. Subsequently, 30 µg of each protein sample were analyzed. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to separate the extracted proteins. Then, the proteins were blotted onto PVDF transfer membranes (ISEQ0010, Millipore, USA), and blocked with 5% skimmed milk. Next, the PVDF membranes were incubated overnight at 4°C with the primary antibodies. Then, the membranes were incubated with goat anti-rabbit/mouse IgG secondary antibody (dilution, 1:3000; ZB-2301, ZB-2305, ZSGB-BIO, China) for 1 h at room temperature. Protein expression was analyzed using GBOX (Syngene Company, UK) and a chemiluminescent HRP substrate (WBKLS0500, Millipore, USA). The primary antibodies used in this study targeted the following proteins (dilution, 1:1000): TMEM158 (ab98335) was obtained from Abcam (UK); E-cadherin (E-Ca; 31955), N-cadherin (N-Ca; 131165), vimentin (S7415), Snail (38795) and p-STAT3 (9145S) were purchased from Cell Signaling Technology (USA); STAT3 (A11867) was obtained from ABclonal (China); and β-actin (TA-09) was purchased from ZSGB-BIO (China).

Intracranial xenograft model in nude mice
All animal experiments were approved by the Ethical Committee of the Tianjin Medical University General Hospital. An intracranial xenograft mouse model was established as previously described [31]. U251MG cells were stably transfected with sh-TMEM158 and OE-TMEM158 lentivirus, and control cells were injected into the brains of mice. Tumor burden was monitored by bioluminescence imaging every week starting on day 7 after implantation using an IVIS Spectrum Live Imaging System (Perkin Elmer, USA). Body weight and OS of mice in both groups were monitored every 12 days. The brains of the mice were carefully extracted when the mice died, fixed in 10% formalin, and embedded in paraffin for H&E staining and IHC staining.

Statistics
All experimental data were examined at least three times. Statistical analysis was performed using SPSS 20. All quantitative data are presented as the mean ± SD. Survival analyses were conducted using the log-rank (Mantel–Cox) test in GraphPad Prism 8.01. An unpaired t-test was used to compare the means of two groups, and a two-tailed p value of <0.05 was considered statistically significant.

RESULTS
TMEM158 mRNA is highly expressed in IDH1-WT GBM
First, we analyzed the expression profile of TMEM158 across cancers. TMEM158 expression values in 33 types of cancers were extracted from TCGA database and compared to TMEM158 expression values in tissues from non-lesion sites obtained from GTEx database (Fig. 1A). Among 33 cancers, mesothelioma (MSEO) and uveal melanoma did not match their corresponding normal tissue. In the remaining 31 cancers, there were no statistically significant difference in TMEM158 expression in 7 cancers (adrenocortical carcinoma, ACC; bladder urothelial carcinoma, BLCA; lower grade glioma, LGG; liver hepatocellular carcinoma, LIHC; sarcoma, SARC; testicular germ cell tumors, TGCT; thymoma, THYM). TMEM158 expression was significantly downregulated in 10 cancers (cervical squamous cell carcinoma and endocervical adenocarcinoma, CESC; kidney chromophobe, KICH; kidney renal clear cell carcinoma, KIRK; kidney renal papillary cell carcinoma, KIRC; acute myeloid leukemia, LAML; pheochromocytoma and paraganglioma, PCPG; prostate adenocarcinoma, PRAD; thyroid carcinoma, THCA; uterine corpus endometrial carcinoma,UCEC; uterine carcinosarcoma, UCS), and significantly upregulated in 14 cancers (breast invasive carcinoma, BRCA; cholangiocarcinoma, CHOL; colon adenocarcinoma, COAD; lymphoid neoplasm diffuse large B-cell lymphoma, DLBC; esophageal carcinoma, ESCA; glioblastoma multiforme, GBM; head and neck squamous cell carcinoma, HNSC; lung adenocarcinoma, LUAD; lung squamous cell carcinoma, LUSC; ovarian serous cystadenocarcinoma, OV; pancreatic adenocarcinoma, PAAD; rectum adenocarcinoma, READ; skin cutaneous melanoma, SKCM; stomach adenocarcinoma, STAD), especially in GBM (GBM: GTEX = 5.38: 3.54, p < 0.0001. LGG: GTEX = 3.32: 3.54, p > 0.05) (Fig. 1A).

Furthermore, we performed a stratified analysis based on the WHO classification and IDH1 mutation status of glioma samples in the TCGA and CGGA databases. We found that TMEM158 mRNA expression increased with tumor grade, and its expression in IDH1-WT-glyoma was 14.1 times higher than that in IDH1-mutant (IDH1-Mut) gliomas. Importantly, TMEM158 exhibited the highest expression level in IDH1-WT GBM (Fig. 1B–D). Adding 1p/19q status analysis, we found that the status of 1p/19q was also correlated with TMEM158 mRNA expression levels. Compared to lower grade gliomas with IDH1 mutation and non-1p/19q codetection (IDH1-Mut-noncodel, LGG), TMEM158 mRNA expression was higher in lower grade gliomas with IDH1 mutation and 1p/19q codetection (IDH1-Mut-codel, LGG) (Fig. 1E–G). These results suggested that TMEM158 may be preferentially expressed in oligodendrogliomas. Moreover, we found that TMEM158 mRNA expression levels in IDH1-WT LGGs were only inferior to that in IDH1-WT GBMs and were higher than that in any other type, including IDH-Mut GBMs (Fig. 1E–G). These results suggest that TMEM158 is closely related to IDH1 status and is preferentially expressed in IDH1-WT GBMs.

Upregulated TMEM158 expression is correlated with poor prognosis in glioma patients
To determine the prognostic value of TMEM158 gene expression in glioma patients, Kaplan–Meier (K–M) survival curves were performed using data from the TCGA and CGGA clinical information, RNA-seq, and microarray datasets. The results showed that the OS of glioma patients with higher TMEM158 expression was shorter than that of glioma patients with lower TMEM158 expression in the TCGA RNA-seq database (p < 0.0001) (Fig. 1H). Moreover, in the CGGA RNA-seq database (n = 693 and n = 325), glioma patients with higher TMEM158 expression had a worse prognosis than those with lower TMEM158 expression (p < 0.0001) (Fig. 1I–J). Importantly, GBM patients with higher expression of TMEM158 also had a worse prognosis than those with lower expression of TMEM158 in the TCGA agilent dataset (n = 488) and TCGA U133A dataset (n = 525) (TCGA agilent dataset: p = 0.0021; TCGA U133A dataset: p = 0.011) (Fig. 1K–L). These results indicate that TMEM158 may be a prognostic factor for glioma patients.

TMEM158 is related to glioma grades and was preferentially expressed in the core of glioma tissues
To further explore the expression pattern of TMEM158 in gliomas, we used IHC and RT-PCR to determine the relationship between the expression of TMEM158 in glioma tissue and glioma grade. Therefore, we took advantage of clinical human glioma samples to detect the protein pattern of TMEM158 in a tissue microarray (TMA) (Fig. 2A). The TMA data (nontumor, n = 2; WHO grade II,
Fig. 1  TMEM158 mRNA is highly expressed in IDH1-WT GBMs and is correlated with poor prognosis in glioma patients. A Expression profile of TMEM158 in 31 kinds of cancers and their paired normal tissues from TCGA database. B–D Relationship between TMEM158 mRNA expression and WHO glioma grades and IDH1 mutation status of glioma samples in the TCGA and CGGA databases. E–G Expression of TMEM158 in IDH1-Mut-codel LGG, IDH1-Mut-noncodel LGG, IDH1-WT LGG, IDH1-Mut GBM, and IDH1-WT GBM. H–J TCGA and CGGA datasets were used for survival analysis of the two groups of glioma patients with higher TMEM158 expression and lower TMEM158 expression in glioma patients. K–L Kaplan–Meier survival curves were used to analyze the overall survival of GBM patients with higher expression of TMEM158 and lower expression of TMEM158 in the TCGA Agilent and U133A databases. (ns \( p > 0.05 \), * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \), **** \( p < 0.0001 \)).
Fig. 2  **TMEM158 is related to glioma grade and is preferentially expressed in the core of glioma tissues.**  
A TMEM158 protein expression was detected in a tissue microarray (TMA) (nontumor, n = 2; WHO grade II, n = 12; WHO grade III, n = 12; WHO grade IV, n = 28).  
B Total IHC staining score of TMA was related to WHO glioma grades, and nontumor samples exhibited the lowest protein expression of TMEM158.  
C The intratumoral and peritumoral expression patterns of TMEM158.  
D–G Representative image of TMEM158 expression on nontumor, WHO II, WHO III, and WHO IV tumor samples.  
H–K Partial enlarged image of (D–G).  
L RT-PCR results show the expression of TMEM158 mRNA in different grades of gliomas.  
M Western blotting was used to determine expression of TMEM158 in 8 different glioma cell lines.  
N Expression of TMEM158 in glioma cell lines in the CCLE database. (ns *p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).
To confirm whether TMEM158 affects the migration and invasion of glioma cells, a Transwell assay was performed. First, we used a Transwell migration assay to determine the effect of TMEM158 on the migration ability of glioma cells. The results showed that glioma cell migration ability was enhanced when TMEM158 expression was upregulated (p < 0.001) (Fig. 3I, K), while downregulating TMEM158 expression significantly decreased the motility of glioma cell (p < 0.001) (Fig. 3J, L). Next, we added Matrigel into the bottom of the chamber to conduct the Transwell invasion assay. The results also revealed that overexpression of TMEM158 expression increased the invasion ability of glioma cells (p < 0.001) (Fig. 3M, O). Downregulating TMEM158 expression using shRNA significantly inhibited the invasion ability of glioma cells compared to the control group (p < 0.001) (Fig. 3N, P). These results demonstrate that overexpression of TMEM158 significantly promotes the motility of glioma cells.

**TMEM158-associated genes in glioma are enriched in epithelial-mesenchymal transition and STAT3 signaling**

To further investigate the potential mechanism of TMEM158 in gliomas, we conducted a series of bioinformatics analysis. First, we ranked the glioma samples according to their expression levels of TMEM158 in the TCGA database and then took the 25% of the samples at both ends as the high and low expression groups. Next, we used the “DESeq2” R package for selecting DEGs and used the “enhanceVolcano” R package for visualization. We obtained a total of 776 upregulated genes and 1040 downregulated genes (Fig. 4A). Furthermore, we used the “clusterProfiler” R package to perform GSEA and HALLMARK gene set enrichment analysis based on the Log2FoldChange values. The results showed that TMEM158-associated genes were enriched in EMT, TNFa signaling via NF-κB, IL6-JAK-STAT3 signaling, and another pathway (Fig. 4B). In addition, we performed GO analysis and KEGG pathway enrichment analysis on the TMEM158-Low-Expression genes and TMEM158-High-Expression genes, respectively. The results indicated that TMEM158 was primarily associated with the modulation of chemical synaptic transmission, extracellular matrix organization, skeletal system morphogenesis, neuroactive ligand-receptor interaction, calcium signaling pathway, and cytokine-cytokine receptor interaction (Figure S1A-D). Then, we first analyzed the TMEM158-associated genes by GSEA enrichment analysis, and the results indicated that TMEM158 was associated with the process of EMT (NES = 2.89, p < 0.0001) and the IL6-JAK-STAT3 signaling pathway (NES = 2.20, p < 0.0001) (Fig. 4C-D). Collectively, these results show that TMEM158-associated genes in gliomas are enriched in EMT and the STAT3 signaling pathway.

**TMEM158 is positively correlated with the EMT process and STAT3 signaling**

Multiple studies indicate that EMT is frequently observed in invasive human cancers and plays a key role in cancer metastasis by increasing the motility of tumor cells [33, 34]. The mesenchymal subtype of GBM is related to a more aggressive and treatment-resistant phenotype and displays a poorer prognosis than other subtypes [35]. First, the GSEA results indicated that TMEM158 was related to the process of EMT (NES = 2.89, p < 0.0001) and the IL6-JAK-STAT3 signaling pathway (NES = 2.20, p < 0.0001) (Fig. 4C-D). Collectively, these results show that TMEM158-associated genes in gliomas are enriched in EMT and the STAT3 signaling pathway.

### Table 1. The clinicopathological relevance analysis of TMEM158 expression in patients with gliomas.

| Variable      | Total | TMEM158 expression | p value |
|---------------|-------|--------------------|---------|
|               |       | High   | Low   |         |
| Age           |       |         |       |         |
| ≤45 years     | 19    | 12     | 7     | 0.157   |
| >45 years     | 33    | 22     | 11    | 0.510   |
| Gender        |       |         |       |         |
| Male          | 26    | 19     | 7     | 0.003** |
| Female        | 26    | 21     | 5     |         |
| WHO grade     |       |         |       |         |
| LGG           | 24    | 14     | 10    |         |
| GBM           | 28    | 26     | 2     |         |

**p < 0.01.
Fig. 3  TMEM158 overexpression promotes the proliferation, migration, and invasion of glioma cells. A–B  RT-PCR results showing the transfection efficiency of lentiviral overexpression (OE-TMEM158) and knockdown (shRNA-TMEM158-1, sh-1; shRNA-TMEM158-2, sh-2) and the expression of TMEM158 in U87MG, U251MG, and TJ905 glioma cell lines. C–D  CCK-8 results showing the proliferation of U87MG, U251MG, and TJ905 glioma cells in response to upregulating and downregulating TMEM158 expression. E, G  Colony-formation assay results showing the colony formation efficiency of glioma cells after overexpression of TMEM158. F, H  Colony-formation assay results showing the colony formation efficiency of glioma cells after silencing TMEM158 expression. I–L  The migration ability in U87MG, U251MG, and TJ905 glioma cells in response to overexpression and knockdown of TMEM158 was detected by Transwell migration assay. K and L  Show the statistical histogram of the quantification of migrated cells after upregulation and downregulation of TMEM158, respectively. M–P  The representative images of Transwell invasion assay for glioma cell lines after gain- and loss-of-function TMEM158. The number of invaded cells is shown in (O and P). Data in (K, L, O, and P) are shown as mean ± SD. Scale bar = 100 μm. (ns  *p > 0.05,  **p < 0.05,  ***p < 0.01,  ****p < 0.001).
Fig. 4  TMEM158 was positively correlated with EMT process and STAT3 signaling. A Volcano plot showing the differentially expressed genes (DEGs) related to TMEM158. We identified a total of 776 upregulated genes and 1040 downregulated genes. B GSEA and HALLMARK gene set enrichment analysis were used to enrich the pathways related to TMEM158-associated genes. C GSEA showed that TMEM158-associated genes were enriched in the EMT process. (NES = 2.89, p < 0.0001). D TMEM158-associated genes were enriched in the STAT3 signaling pathway by GSEA. (NES = 2.20, p < 0.0001) E–F Western blotting results show changes in TMEM158 and EMT marker expression (E-Ca, N-Ca, vimentin, and Snail) in U87MG, U251MG, and TJ905 glioma cells after overexpression and knockdown of TMEM158. G–H TMEM158, STAT3, and p-STAT3 protein levels in U87MG, U251MG, and TJ905 glioma cells after overexpression and knockdown TMEM158 detected by western blotting.
To understand the molecular mechanisms underlying TMEM158-induced proliferation, migration, and invasion of glioma cells, we examined TMEM158-associated genes, which were enriched in the IL6-JAK-STAT3 signaling pathway as assessed using HALLMARK gene set enrichment analysis (Fig. 4B). We first investigated the relationship between TMEM158 expression and the IL6-JAK-STAT3 signaling pathway via GSEA (NES = 2.20, p < 0.0001) (Fig. 4D). Furthermore, we performed western blotting to detect the expression of key proteins of the STAT3 signaling pathway (STAT3; phosphorylated-STAT3, p-STAT3). The results revealed that p-STAT3 was significantly increased in TMEM158-overexpressing glioma cells, whereas total STAT3 levels did not vary significantly from that of β-actin (Fig. 4G). However, reduced p-STAT3 levels were further observed in U87MG, U251MG, and TJ905 glioma cells in response to TMEM158 downregulation (Fig. 4H). These findings in this aspect indicate that TMEM158 is positively correlated with STAT3 signaling in glioma cells.

TMEM158 mediates the proliferation, migration, and invasion of glioma cells by activating STAT3 signaling

To further elucidate the underlying mechanism of the involvement of STAT3 signaling in TMEM158-mediated GBM progression, we conducted a series of experiments to demonstrate whether overexpression of STAT3 by plasmid transfection rescues TMEM158 knockdown-mediated proliferation, migration, and invasion. The CCK-8 assay showed that downregulating TMEM158 knockdown-mediated proliferation, migration, and invasion. Western blotting also showed that overexpression of STAT3 rescued the reduced proliferation caused by TMEM158 knockdown (Fig. 5A–C). The colony-formation assay also confirmed this result (Fig. 5D). Furthermore, we conducted a Transwell assay to demonstrate that upregulating STAT3 expression rescues the change in TMEM158 downregulation-mediated migration and invasion (Fig. 5E–F). Western blotting also showed that overexpression of STAT3 reduced the expression of E-Ca and increase the expression of N-Ca, vimentin, and Snail in glioma cells in response to TMEM158 knockdown (Fig. 5G). These results indicated that TMEM158 mediates the proliferation, migration, invasion, and EMT process of glioma cells by activating STAT3 signaling.
Fig. 5 TMEM158 mediates the proliferation, migration, and invasion of glioma cells by activating STAT3 signaling. A–C CCK-8 results showing the proliferation of sh-NC + pcDNA, sh-NC + STAT3 pcDNA, sh-TMEM158 + pcDNA, and sh-TMEM158 + STAT3 pcDNA in the four groups of glioma cells. D Colony-formation assay results showing the colony formation efficiency of glioma cells after silencing TMEM158 and upregulating STAT3. E–F Transwell assay results showing that overexpression of STAT3 rescued the reduced migration and invasion caused by TMEM158 knockdown. G Western blotting results showing the expression of TMEM158, STAT3, p-STAT3, E-Ca, N-Ca, vimentin, and Snail in the four groups of glioma cells. (ns p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).
Fig. 6  Silencing TMEM158 impairs the invasion of GBM cells in vivo and suppresses tumor growth. A Schematic diagram of experimental grouping, implantation, and bioluminescence imaging of the orthotopic xenograft model. B–C Bioluminescence imaging of tumor growth in animals. Signal intensities were quantified on days 7, 14, 21, and 28 after implantation. n = 7 per group. (****p < 0.0001) D Kaplan–Meier survival curves indicating the percentage survival of mice. E Representative images of H & E staining of the mouse cerebrum showing the tumor border. Immunohistochemical (IHC) staining for TMEM158, p-STAT3, and Ki67 in the samples.
The exact pathways that TMEM158 may regulate in glioma remain unclear. Therefore, we further performed GSEA and showed that TMEM158-associated genes were positively related to EMT and TNFα signaling via NF-κB and IL6-JAK-STAT3 signaling (Fig. 4A–D). EMT is a complex cellular process that allows epithelial cells to acquire a mesenchymal phenotype and is frequently observed in invasion human cancers [34, 53, 54]. The mesenchymal transition of glioma cells is correlated with a treatment-resistant and more aggressive phenotype, leading to worse outcomes and rapid progression in patients with GBMs [55–57]. Our data indicated that overexpression of TMEM158 decreases the expression of E-Cad, increases the expression of N-Ca, Snail, and vimentin and promotes the EMT process (Fig. 4E–F). Furthermore, our analysis confirmed that TMEM158 knockdown inhibits the expression of p-STAT3 (Figs. 4G–H, 6E). However, loss- or gain-of-function of TMEM158 did not affect the expression of p65 (data not shown). Many studies have reported that STAT3 transcriptionally regulates numerous downstream target genes that are crucial for tumor cell growth, migration, invasion, and immune evasion [58, 59]. Importantly, our results also demonstrated that overexpression of STAT3 rescues the TMEM158 knockdown-mediated proliferation, migration, invasion, and EMT process, indicating that TMEM158 promotes the proliferation, migration, and invasion of glioma cells by activating STAT3 signaling (Fig. 5).

Despite these findings, the current study is only a preliminary examination of TMEM158 in GBM, which has certain limitations. First, the underlying mechanism of TMEM158-mediated malignant carcinogenesis involved in STAT3 signaling remains unclear. Second, the upstream factors (such as transcription factors, cytokines, microRNAs, IncRNAs, or circRNAs) that may participate in the activation of TMEM158 in GBM are still unknown. Finally, there is currently a lack of drugs targeting TMEM158. Therefore, it is necessary to conduct more research in the future to clarify the direct upstream and downstream mechanism of TMEM158 in GBM and to develop fat-soluble, small molecule-targeted drugs targeting TMEM158 for further in vivo and in vitro studies to determine whether they have clinical translational value.

In conclusion, our work describes the molecular and clinical characterization of TMEM158 in glioma. TMEM158 is positively associated with glioma grade, IDH1 mutation status, and poor prognosis. For the first time, we associated TMEM158 with glioma cell growth, migration, and invasion via EMT and STAT3 signaling, suggesting that it may represent a useful therapeutic target for GBMs.

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AUTHOR CONTRIBUTIONS
JL, XW, LC, CZ, and XY designed the study. JW, LT, XF, GS, and XR performed the bioinformatic analysis. JL, JZ, YZ, XR, and JS conducted the in vitro experiments. TL, LT, LY, and HZ prepared the gliomas tissue microarray. JL, LC, GS, XR, HZ, SY, and HM assisted with the experiments. JL, XW, LC, SY, CZ, and XY performed data analysis and interpretation. JL, XW, LC, and XR conducted the rescue experiment. JL, XW, XR, GS, and HZ performed the animal experiment. JL, TF, TL, LY, CZ, and XY wrote the draft of paper. All authors have been involved in the writing of the paper and have read and approved the final version.

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COMPETING INTERESTS
The authors declare no competing interests.

ETHICS APPROVAL
For human glioma samples, written informed consent was obtained from all donors and their relatives. The study was carried out in accordance with the principles of the Helsinki Declaration and approved by the ethical committee at Tianjin Medical University General Hospital (March 03, 2018).

ADDITIONAL INFORMATION
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