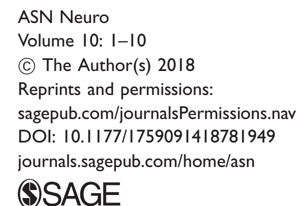
Original Paper



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Overexpression of CLEC18B Associates With the Proliferation, Migration, and Prognosis of Glioblastoma

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Abstract

C-type lectin domain family 18 member B (CLEC18B), encoding a superfamily of CLEC, has been found to be expressed in some of cancer cells, which possibly indicates it associated with cancer. However, the defined functional characterizations of CLEC18B in glioblastoma multiforme (GBM) progression still remain unclear. To this end, clinical relevance of CLEC18B expression with GBM patients' prognosis was analyzed both in The Cancer Genome Atlas dataset of 174 tissues and 40 GBM tumor tissues collected from our hospital by using the Kaplan–Meier survival and the Cox proportional hazard model. The role of CLEC18B in GBM was determined by loss-of-function assay using small interfering RNA approach *in vitro*. Functional and signaling analyses were also performed to understand how CLEC18B facilitated the aggressiveness of GBM at molecular and cellular levels using Cell Counting Kit-8 assay, wound-healing, transwell, and Western blot analyses. Results from our analyses showed that CLEC18B was markedly elevated in both GBM tissues and cells, and exhibited strong inverse correlation with overall survival in GBM patients. Moreover, CLEC18B was identified as an independent predictor of patient survival. Functionally, knockdown of CLEC18B inhibited the growth, migration, and invasion of GBM cells. Mechanistic studies revealed that silencing of CLEC18B resulted in downregulation of Wnt/β-catenin signaling activity. Collectively, our findings provide clinical, molecular, and cellular evidence of CLEC18B as a promising prognostic biomarker and therapeutic target for GBM.

Keywords

CLEC18B, glioblastoma, prognosis, proliferation, migration, Wnt/β-catenin

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Introduction

With accounting for approximately 70% of all gliomas, glioblastoma, also termed as glioblastoma multiforme (GBM), is the commonest and most deadly type of primary brain tumor of central nervous system (Omuro and DeAngelis, 2013; Alexander and Cloughesy, 2017). Although efforts to advance therapeutic regimens are ongoing, including surgery, chemotherapy, and radiotherapy for GBM, the median survival time of patients was less than 15 months from the time of diagnosis (Van Meir et al., 2010; Kawai et al., 2013; Louvel et al., 2016; Nalkiran and McDonald, 2017). Highly infiltrative intracranial proliferation and aggressiveness properties of GBM leading to recurrence was the chief culprit contributing to the dismal outcome, largely due to a paucity of understanding biological mechanisms attributes of malignancy. Accordingly, a deeper understanding of the basis of potential mechanisms and available biomarkers of the disease is of importance to GBM patient outcome improvement, prevention, and further clinical treatment.

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C-type lectin 18 (CLEC18), identified as a superfamily of CLEC, has been documented to bind to glycolipids, which was previously considered to play a potential role in modulating the host immunity (Huang et al., 2015; Pees et al., 2017). It has been reported that abnormal glycosylation, including increased level of O-glycans, glycoproteins, and glycolipids, is closely associated with tumor transformation and development (Hakomori, 1989; Hakomori and Cummings, 2012; Li et al., 2017). Importantly, essential roles of glycolipids in malignant properties of gliomas are shown, suggesting it plays a significantly regulatory role in neuroinflammation, metabolism, and gliomagenesis (Furukawa et al., 2017). However, hitherto molecular mechanism still remains poorly undetermined. Previous research has suggested that CLEC18A and CLEC18C are able to bind to polysaccharide, while insertion of the 9-amino acid (predicted CLEC18B characteristic) abolished this ability, which disturbs the function of CLEC18A and CLEC18C modulating the host immunity (Hsieh, 2016). CLEC18B has been found to be expressed in some of cancer cells (Huang et al., 2015), which implied it might be linked with tumorigenesis. Nonetheless, there is no report for CLEC18B regulating GBM.

Therefore, intensive efforts are still needed to explore the biological function of CLEC18B. Herein, in this work, by analyzing the data collected from The Cancer Genome Atlas (TCGA) database, we found that CLEC18B was upregulated in GBM tissues compared with normal brain tissues, and overexpression of CLEC18B predicted shorter overall survival (OS). These implications prompted us to hypothesize whether CLEC18B function as an oncogenic role in GBM. To validate it, we further determined the prognostic significance of CLEC18B expression in GBM patients recruited from our hospital. Then, we also analyzed the growth, mobility, and invasion of GBM cells by silencing of CLEC18B. Meanwhile, the potential molecular mechanism was explored in this work. Altogether, our findings provide evidence that CLEC18B takes on both a prognostic biomarker and a novel potential therapeutic target for GBM.

Materials and Methods

Clinical Data Acquisition

GBM expression profiles and information of survival rates were downloaded from the TCGA database containing 169 GBM and 5 normal brain samples. Furthermore, GBM expression profiles were collected from the Oncomine datasets comprising 24 GBM and 3 normal brain samples. Based on these data, we analyzed expression level of CLEC18B in GBM and normal brain tissues, as well as the relation between CLEC18B expression and probability of survival rates.

Patient Samples

Paired GBM samples and their respective adjacent noncancerous tissues (ANT) were obtained from 40 GBM patients who underwent surgical resection at The First Affiliated Hospital of Zhengzhou University during the period from January 2007 to December 2011. All the samples were collected using protocols approved by the Research Ethics Committee of The First Affiliated Hospital of Zhengzhou University, and informed consents were obtained from all patients or their guardians. OS was defined as the time from the date of the surgery to death or the last follow-up. Progression-free survival (PFS) was calculated from the date of treatment until disease progression or death.

Cell Culture

The human GBM cell lines H4 and U87 were purchased from American Type Culture Collection (Manassas, VA, USA). The human GBM cell lines U251 were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Human normal brain glial cell lines (HEB) were obtained from Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences (Guangzhou, China). These cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Gibco, Thermo Fisher Scientific, Inc.). All cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C.

Small Interfering RNA Transfection

Transfections of U87 and U251 cells with small interfering RNA (si-RNA) were carried out using Lipofectamine® 2000 (Invitrogen, Thermo Fisher Scientific, Inc.) according to manufacturer's suggested procedures. CLEC18B-targeted si-RNA#1: 5′-CAGGA AGGAG AGUUU CUUG-3′, CLEC18B-targeted si-RNA#2: 5′-GACAA UCAUC CCCUA UAAG-3′. Scrambled si-RNA was used as negative control (si-NC). si-RNA and si-NC were synthesized by GenePharma (Shanghai, China). After 48 hr, the mRNA and protein level of CLEC18B were tested by quantitative polymerase chain reaction (qPCR) and Western blot analysis.

Cell Counting Kit-8 and Colony-Formation Assays

Cell Counting Kit-8 (CCK-8) assay was conducted to evaluate the cell proliferation in accordance with manufacturer's instructions. Cells were seeded in 96-well plates (1×10^3 cells/well) and incubated for 0, 24, 48, 72, and 96 hr. Then, 10 μl of CCK-8 (Beyotime Institute of

Biotechnology, Shanghai, China) were added to each well and incubated for 1 hr. The optical density (OD) at 450 nm was tested using a spectrophotometer (Thermo Fisher Scientific, Inc.). Next, for performing the colony-formation assay, after 48 hr of transfection, cells (500 cells/well) were seeded in six-well plates. After 7 to 14 days, the cells were fixed in methanol and then stained with 0.1% crystal violet. The colonies were photographed under an inverted microscope (Olympus Corporation, Tokyo, Japan). Colony-forming efficiency was defined as the rate of the number of colonies formed in cultivation to the number of cells inoculated. Each experiment was performed in triplicate.

Wound-Healing and Transwell Assays

For conducting wound-healing assay, cells were plated and grown until reaching 90% confluence in six-well plate. The confluent monolayer of cells was scratched using a pipette tip. After 24 hr, the cells migrating into the wounded areas were observed. Images were captured using an inverted microscope at 0 hr and 24 hr after scratching. Then, for performing the migration of GBM cells, briefly, cells were plated on the top chamber of transwell assay inserts (Costar, Cambridge, MA, USA) with a membrane containing 8-μm pores in 200 μl serum-free medium, and the lower chamber contained medium with 10% fetal bovine serum. After 24 hr, the cells that did not migrate on the upper surface of the membrane were gently removed with a cotton swab. The cells on the lower surface were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 20 min. Migrated cell from five random fields were counted under an inverted microscope using a magnification of 200×. For invasion assay, the steps were similar to those of migration assay, except that the Matrigel (BD Biosciences, San Jose, USA) was added into the membrane. The assays were conducted in triplicate.

Quantitative Real-Time PCR

Total RNAs from cell lines were extracted with TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript™ RT reagent kit (Takara Bio, Shiga, Japan) according to the manufacturer's protocol. qPCR was performed using an SYBR Premix Ex Taq™ kit (Takara) and the ABI 7500 real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.). The reaction conditions were as follows: 95°C for 60 s, followed by 95°C for 30 s, and 60°C for 35 s for 40 cycles. Sequences of primers were as follows: CLEC18B: F: 5′-TGAGT GCTGC CATGG GGTT-3′, R: 5′-TGTAA CGGTT TCGGG TTTTG C-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): F: 5′-GGAGC GAGAT CCCTC CAAAA T-3′, R: 5′-GGCTG TTGTC ATACT TCTCA TGG-3′. All samples were normalized to GAPDH. qPCR quantification was performed with the $2^{-\Delta\Delta Ct}$ method. Each experiment was carried out in triplicate and repeated three times.

Western Blot Assay Analysis

Cells were lysed with radioimmunoprecipitation buffer (Beyotime) and quantified with bicinchoninic acid (BCA) Protein Assay Kit (Beyotime). Equal amount (20 μg/lane) of protein samples were separated by 10% to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes. The membranes were incubated overnight with the primary antibodies including CLEC18B (1:1000; OriGene Technologies Inc., Rockville, MD, USA), nucleus β-catenin (1:1000), Histone (1:1000), C-Myc (1:1000), and Cyclin D1 (1:1000; Cell Signaling Technology, Danvers, MA, USA). Actin (Beyotime, 1:1000) was used as an internal control. Primary antibody incubation was followed by incubation with horseradish peroxidase-conjugated secondary antibody (Beyotime, dilution 1: 1000) for 2 hr in a dark place. Protein bands were detected by an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA). Densities of proteins bands were measured with Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical Analysis

SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 standard deviation were used to carry out the statistical analyses. Survival analysis was determined by the Kaplan–Meier method and the log-rank test. The chi-square test was used to examine the relations between CLEC18B expression and categorical variables. Cox proportional hazards model was applied for univariate and multivariate analyses of the disease prognosis. Student's t test was employed for statistical analysis of remaining data. Data are presented as mean ± standard deviation. A value of $p < .05$ was considered statistically significant.

Results

Expression of CLEC18B in Human GBM Tissues or Cells

To investigate the functional effect of CLEC18B in GBM, we first assessed its expression in GBM tissues and normal brain tissues or ANT samples. Through analyzing the data from the TCGA datasets comprising 169 GBM tissues and 5 normal brain tissues, we found that CLEC18B was significantly overexpressed in GBM tissues compared with normal brain tissues (Figure 1(a), $p < .01$). To further verify it, by analyzing data from other datasets of Oncomine, high level of CLEC18B

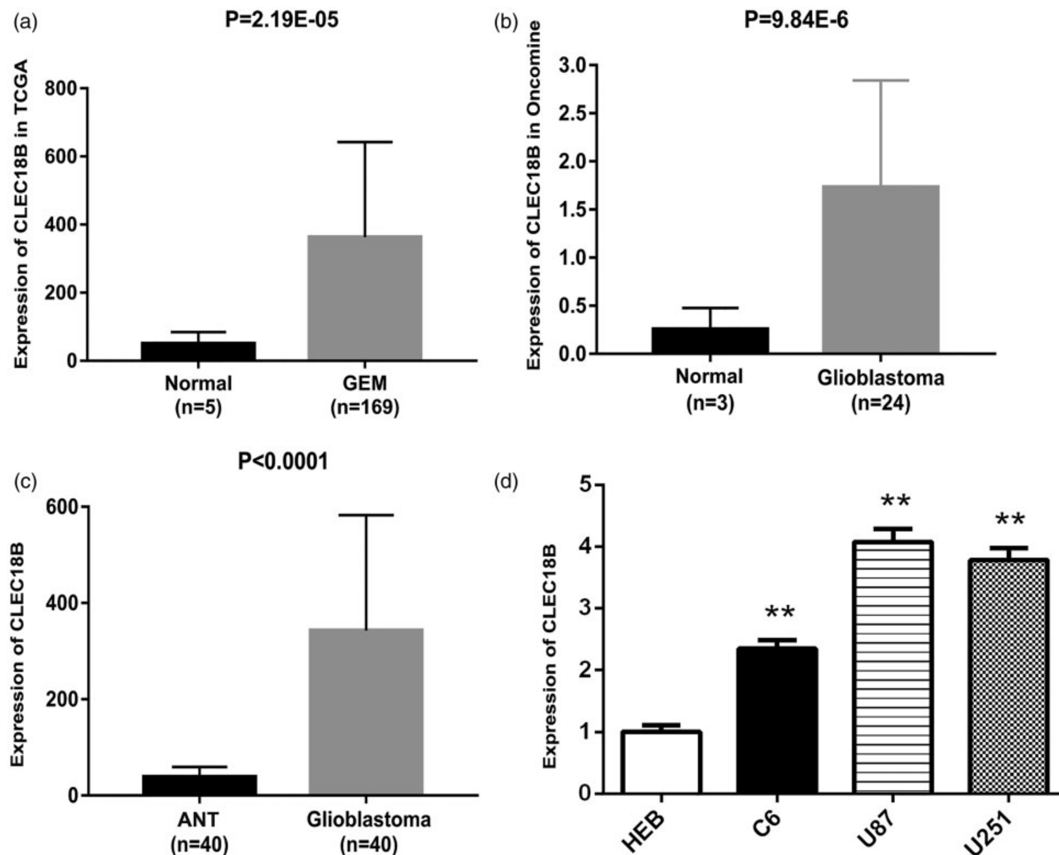

Figure 1. Elevated expression level of CLEC18B was observed in GBM tissues and cells. (a) Box plots of The Cancer Genome Atlas (TCGA) RNA expression analysis for CLEC18B. (b) Box plots of Oncomine datasets RNA expression analysis for CLEC18B. (c) RNA expression level of CLEC18B in clinical samples. (d) RNA expression level of CLEC18B in GBM cells and normal brain glial cell lines, HEB. All values are shown as mean ± SD, $^{**}p < .01$ versus normal, noncancerous tissues (ANT) or normal brain glial cell lines, HEB. Assays were repeated three times.
CLEC18B = C-type lectin domain family 18 member B; GBM = glioblastoma multiforme; HEB = human normal brain glial cell lines; ANT =adjacent noncancerous tissues; SD = standard deviation.

was also found in GBM tissues in comparison with normal brain tissues (Figure 1(b), $p < .01$). Consistently, by determining the CLEC18B expression in 40 paired ANT and GBM tissues from our hospital using the qPCR assay, indeed, we uncovered that CLEC18B in GBM tissues was obviously higher than in ANT (Figure 1(c), $p < .01$). At an *in vitro* level, compared with normal brain glial cells lines (HEB), high level of CLEC18B was detected in GBM cells, such as H4, U87, and U251; especially, this trend was more obvious in U87 and U251 (Figure 1(d), $p < .01$). Thus, U87 and U251 were used in the following functional test. All these data suggest that CLEC18B might contribute to the growth and recurrence of GBM.

Overexpressed CLEC18B Correlates With Poor Clinical Outcomes in GBM Patients

To shed light on the relation between expression and patient outcome, we performed statistical correlation and qPCR analysis of CLEC18B on 40 GBM tissues with follow-up records. In survival analysis of GBM patients, we found that patients with higher CLEC18B expression levels had poorer OS than those with lower CLEC18B expression levels (Figure 2, $p < .01$). Furthermore, we analyzed the correlation between CLEC18B and clinicopathological characters. As shown in Table 1, the analysis showed that the higher level of CLEC18B associated with PFS ($p = .006$) and OS ($p = .025$). There were no significant changes between the CLEC18B expression and age, gender, and Karnofsky performance status (KPS; all $p > .05$). Moreover, in univariate survival analysis, all clinicopathological parameters were included. Only the CLEC18B expression appeared to have visible difference (Table 2, $p = .000$). On the multivariate analysis, only the CLEC18B expression maintained the significance (Table 2, $p = .000$) as an independent prognosticator in GBM patient. Altogether, these results suggest that CLEC18B is a prognostic factor of GBM.

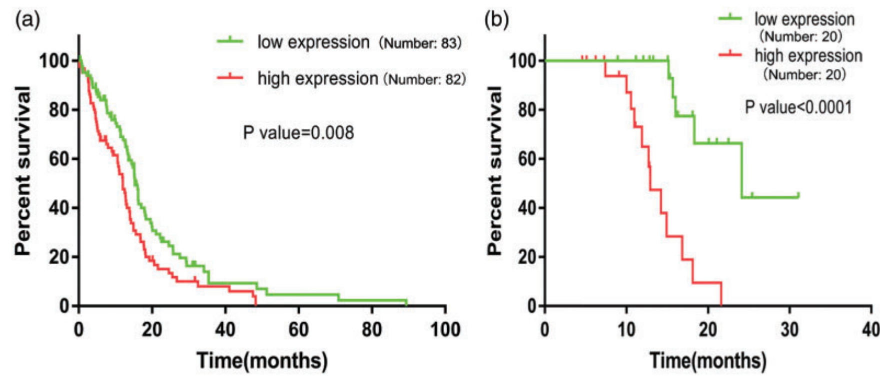

Figure 2. High level of CLEC18B predicted shorter overall survival (OS) in GBM patients. (a) Kaplan–Meier curves for OS according to CLEC18B expression in patients with GBM collected from TCGA. (b) Kaplan–Meier curves for OS according to CLEC18B expression in GBM patients collected at our institution.
CLEC18B = C-type lectin domain family 18 member B; GBM = glioblastoma multiforme; TCGA = The Cancer Genome Atlas.

Table 1. Clinicopathological Variables of 40 Patients With GBM.

	Expression of CLEC18B		
Characteristics	Low	High	*p* Value
Age			.752
<60	10	9	
≥60	10	11	
Gender			1.000
Female	6	6	
Male	14	14	
KPS			1.000
≤80	15	16	
>80	5	4	
PFS (months)			.006*
<12	10	18	
≥12	10	2	
Death			.025*
Yes	5	12	
No	15	8	

Note. GBM = glioblastoma multiforme; CLEC18B = C-type lectin domain family 18 member B; KPS =Karnofsky performance status; PFS = progression-free survival.

Inhibiting CLEC18B Expression Results in Decreased Proliferation in GBM Cells

To determine the functional role of CLEC18B on GBM cell, we first used the si-RNA approach to downregulate CLEC18B. The expression levels of CLEC18B in U251 and U87 cells were tested by qPCR and Western blot analysis, respectively. As predicted, the expression level of CLEC18B at both mRNA and protein levels were dramatically decreased in U251 and U87 cells transfected with si-CLEC18B compared with si-NC group (Figure 3 (a) to (c), $p < .01$). Next, to evaluate the impact of CLEC18B on GBM cell growth, we carried out the CCK-8 and colony-formation assays. By measuring the OD values of U251 and U87 cells using the CCK-8 assay, we observed a remarkable decrease in OD values in si-CLEC18B group compared with that in si-NC group (Figure 3(d), $p < .01$). Of note, a detectable reduction in ratio of colony formations in si-CLEC18B group compared with si-NC group was observed (Figure 3(e), $p < .05$). Overall, these observations indicate that CLEC18B displays an oncogenic role in GBM cell.

Downregulation of CLEC18B Expression in GBM Cells Results in Diminished Migration and Invasion

To further determine the biological effect of CLEC18B expression on migration and invasion in GBM cells, we performed the wound-healing and transwell assays. The results of wound-healing assay showed that the migration index of U251 and U87 cells in si-CLEC18B group was obviously lower than that in si-NC group. By transwell assay, the number of U251 and U87 cells migrating/invading through the membrane/Matrigel following si-CLEC18B transfection was apparently lower than that of the si-NC group (Figure 4, $p < .01$). These data suggest that CLEC18B plays a suppressive role in migration and invasiveness of GBM cell.

Activity of Wnt/β-Catenin Signaling Is Depressed by Knockdown of CLEC18B

Canonical Wnt/β-catenin signaling, known as a highly conserved developmental pathway, displays a role in modulating cellular survival and growth, differentiation, and development. In an effort to gain insight into the mechanism by which CLEC18B regulated tumorigenesis of GBM, markers of Wnt/β-catenin signaling were

Table 2. Univariate and Multivariate Cox Regression Models for Estimating Overall Survival (OS).

	Univariate analysis			Multivariate analysis		
Variables	*p* Value	HR	95% CI	*p* Value	HR	95% CI
CLEC18B expression (high/low)	.000*	8.665	[2.731, 27.488]	.000*	8.939	[2.660, 30.044]
Age (<60/≥60)	.991	0.995	[0.379, 2.612]			
Gender (female/male)	.966	0.979	[0.358, 2.676]			
KPS (≤80/>80)	.860	1.107	[0.358, 3.148]			
PFS	.021*	0.261	[0.084, 0.813]	.031*	0.263	[0.078, 0.885]

Note. CLEC18B = C-type lectin domain family 18 member B; KPS = Karnofsky performance status; PFS = progression-free survival; HR = hazard ratio; CI = confidence interval.

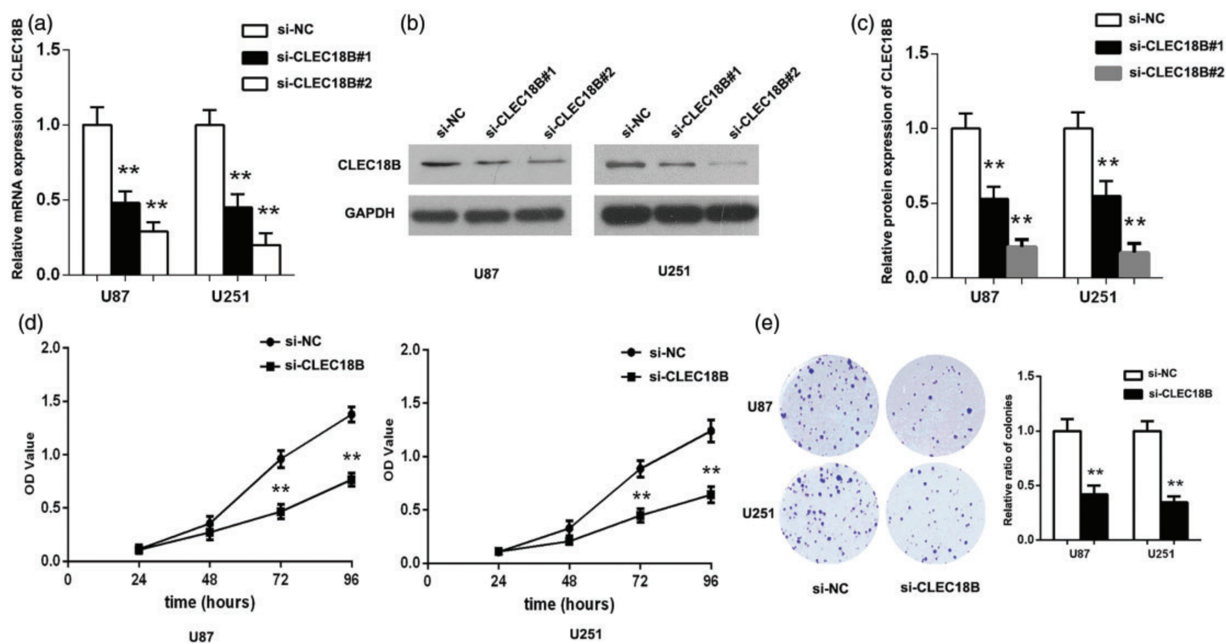

Figure 3. Growth and colony-formation abilities of GBM cells were inhibited by CLEC18B knockdown. (a to c) Transfection efficacy of CLEC18B si-RNA in U87 and U251 cells was analyzed by qPCR and Western blot assay, respectively. (d) Assessing the effects of CLEC18B downregulation on the proliferation ability of U87 and U251 cells using CCK-8 assay. (e) Evaluating the effects of CLEC18B knockdown on the colony-formation ability of U87 and U251 cells. All values are shown as mean ± SD, $^{**}p < .01$ versus si-NC group. Assays were repeated three times.
CLEC18B = C-type lectin domain family 18 member B; GBM = glioblastoma multiforme; si-RNA = small interfering RNA; qPCR = quantitative polymerase chain reaction; CCK-8 = Cell Counting Kit-8; si-NC = small interfering negative control; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; SD = standard deviation.

identified, including key modulator nuclear β-catenin and downstream molecules c-Myc and Cyclin D1 that are involved in cell proliferation. The results of Western blot analysis showed that nuclear β-catenin, c-Myc, and Cyclin D1 were evidently downregulated in U251 and U87 cells treated with si-CLEC18B compared with that in si-NC group (Figure 5, $p < .01$). To sum up, these results indicate that Wnt/β-catenin participates in the oncogenic effect of CLEC18B in GBM.

Discussion

In the present study, we explored the expression status of CLEC18B in GBM tissues and its relation with clinicopathological variables and progression of GBM. A negative association between CLEC18B expression and poorer OS was found. High CLEC18B expression, closely correlated with PFS and OS rate, was an independent prognostic factor for OS in GBM patients. Moreover,

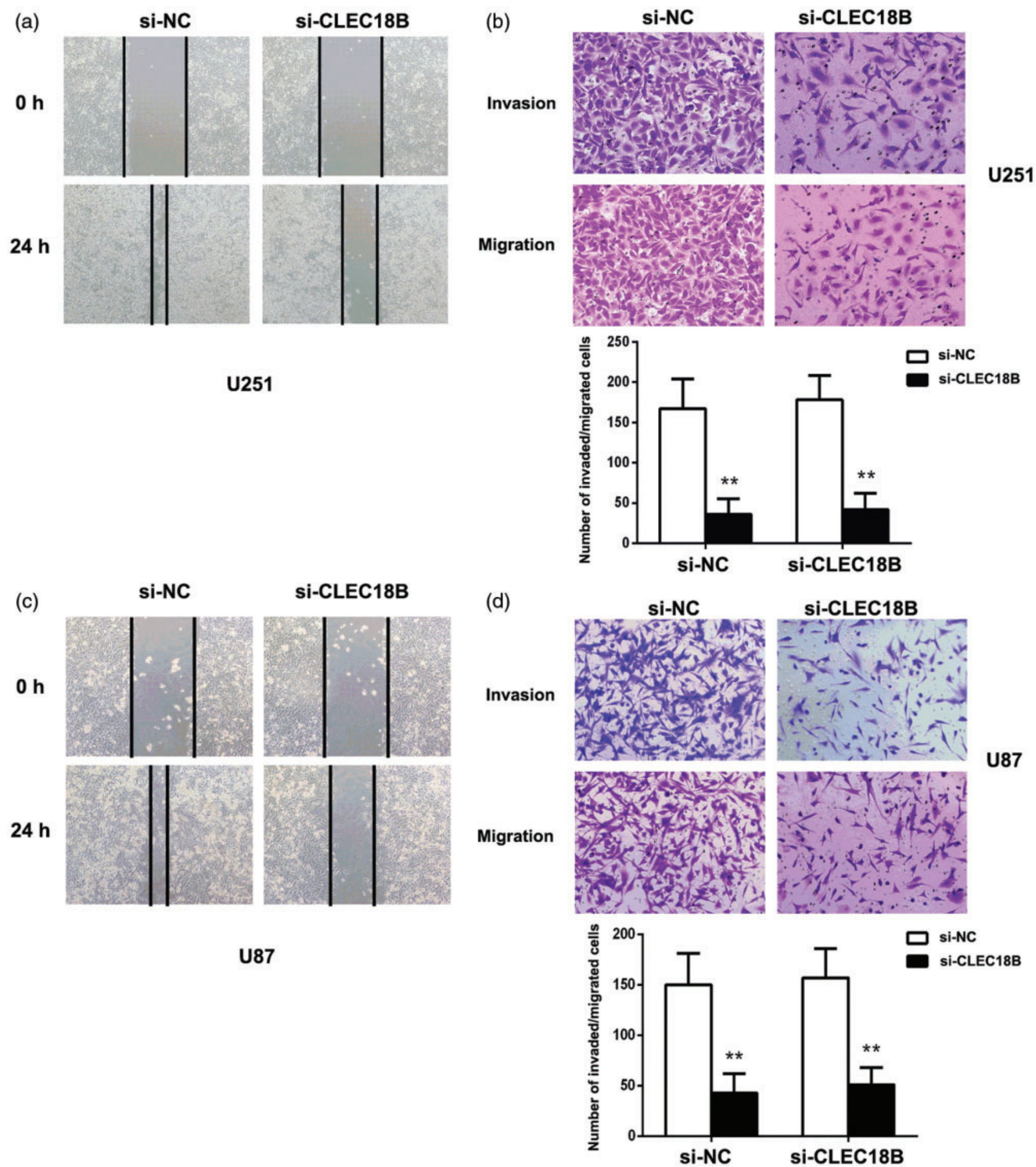

Figure 4. Migration and invasion capabilities of U87 and U251 cells were prohibited by CLEC18B knockdown. (a) The mobility of U87 cells was measured by wound-healing assay. (b) The migration and invasion of U87 cells were tested by transwell assay. (c) The mobility of U251 cells was measured by wound-healing assay. (d) The migration and invasion of U251 cells were tested by transwell assay. All values are shown as mean ± SD, $^{**}p < .01$ versus si-NC group. Assays were repeated three times.
CLEC18B = C-type lectin domain family 18 member B; si-NC = small interfering negative control; SD = standard deviation.

suppression of CLEC18B dampened GBM cell proliferation, migration, and invasion abilities, which was modulated by inactivity of Wnt/β-catenin signaling. Together, our findings demonstrated that CLEC18B had the potential to accelerate oncogenesis of GBM.

It has been reported that elevated toll-like receptor 4 levels were identified in GBM samples compared with adjacent normal tissue (Tewari et al., 2012). A previous report has shown that CLEC18, as a novel superfamily of CLECs, binds to toll-like receptor ligands (Hsieh, 2016), which offer us a clue that CLEC18 might work in GBM tumorigenesis. Here, our statistical correlation analyses strongly showed that CLEC18B was higher in GBM tissues in comparison with normal brain samples, and overexpression of CLEC18B correlated with shorter OS in GBM patients. In support, by evaluating the expression in samples collected from our institution and clinical relevance with long-term follow-up records, of note, the

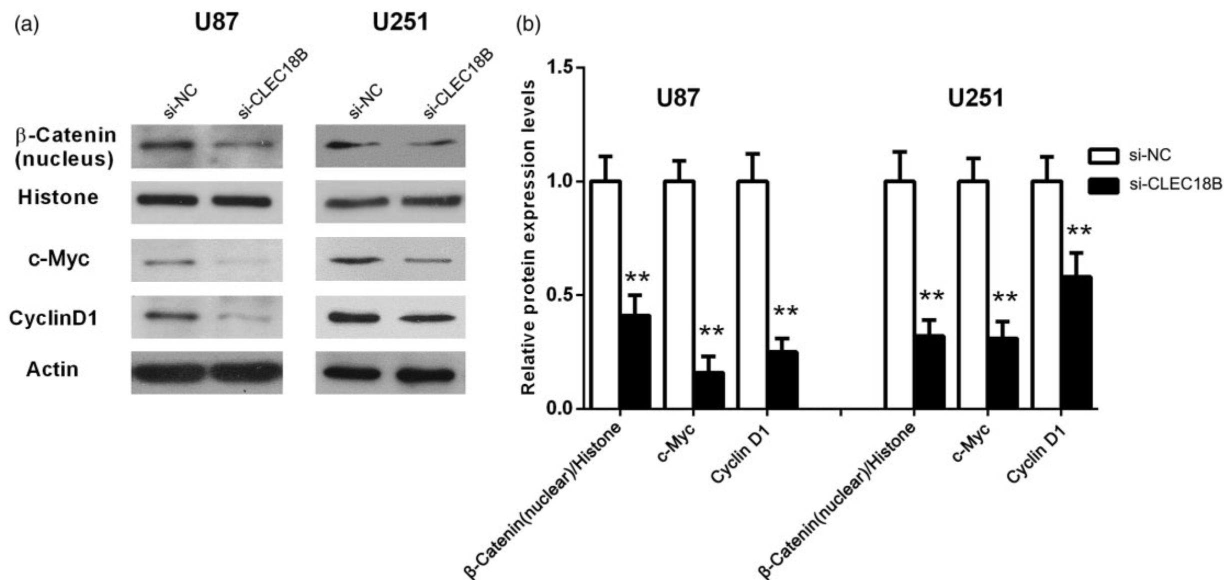

Figure 5. The Wnt/β-catenin signaling was suppressed by knockdown of CLEC18B. (a) The key markers of Wnt/β-catenin signaling were measured by Western blot assay. (b) Box–whisker plot presenting the difference in protein levels in si-CLEC18B and si-NC groups. All values are shown as mean ± SD, $^{**}p < .01$ versus si-NC group. Assays were repeated three times.
CLEC18B = C-type lectin domain family 18 member B; si-NC = small interfering negative control; SD = standard deviation.

high level of CLEC18B was observed in GBM samples in contrast with ANT samples, showing a poor prognosis in GBM patient and a dependent prognosis factor. These evidence suggests that CLEC18B plays a poor prognostic role in GBM, implying it possibly functions as a oncogenic part in GBM.

It is widely recognized that proliferation, migration, and invasion are identified as the common properties of cancers, and thus, targeting this natures could be the pivotal issue of addressing tumor. Several reports have demonstrated that CLEC-2 promotes tumor metastasis in hematogenous tumor (Shirai et al., 2017), lung carcinoma (Takagi et al., 2013), and renal cell carcinoma (Xiong et al., 2016). Another research has reported that CLEC-like domain of CLEC14A (CLEC14A-CTLD) has the potency to mediate the angiogenesis; notably, antibody targeting of CLEC14A-CTLD suppresses the multiple tumor angiogenesis, including tumor angiogenesis (Kim et al., 2018). However, no studies have defined an association of CLEC18B with tumor progression. Strikingly, we showed herein for the first time that CLEC18B downregulation dramatically inhibited GBM cell proliferation, migration, and invasion. These data provide compelling implication for a crucial role for CLEC18B in GBM tumor progression.

Having established that Wnt/β-catenin signaling is of critical importance in the carcinogenesis (Barker, 2008), to gain further insights into molecular mechanism of CLEC18B modulation of GBM tumorigenesis, we detected markers of Wnt/β-catenin signaling. Surely, the presence of WNT signals led to prohibiting multiprotein destruction complex and thus hampering phosphorylation of β-catenin; subsequently, stabilized β-catenin translocates to the nucleus, where it binds T-cell factor/lymphocyte enhancer-binding factor transcription factors to activate specific target genes including Cyclin D1, c-Myc, and so forth, which have been identified to be vital to tumor development, such as regulation, cell proliferation, and survival (Rowlands et al., 2004; Gavert and Ben-Ze'ev, 2007). Thereby, nuclear accumulation of β-catenin is deemed to be one of the pivotal ties of Wnt/β-catenin signaling transduction that facilitates transcription of growth and metastasis-associated genes (Bowman and Nusse, 2011). β-catenin, known as a core component of this signaling, has been determined to be a pivotal contributing factor in a wide range of cancers, including GBM; besides, silencing of β-catenin decreased the expression of c-Myc and Cyclin D1 in GBM cells (Liu et al., 2011). It has been documented that Cyclin D1 and c-Myc were involved in regulation of proliferation in GBM (Cobanoglu et al., 2016; Zhang et al., 2017). Consistent with this, indeed, we found that nuclear β-catenin, c-Myc, and Cyclin D1 were inhibited by depletion of CLEC18B. Overall, these investigations provide a potential insight that Wnt/β-catenin was involved in gliomagenesis mediated by CLEC18B. To be sure, our current work is only a preliminary study in cellular level. Thus, future research on animal level *in vivo* and deeper mechanism still needs to be carried out. It has been documented that glutamine is a major metabolic driver of cancers, and c-Myc oncogene regulates glutamine metabolism in cancers, including

GBM (Yang et al., 2009). Thus, it is worthy for us to explore whether CLEC18B modulates glutamine metabolism in GBM in the further studies. Moreover, having determined that Secondary structures of Scherer confirmed to be involved in GBM metastasis by Zagzag et al. (2008), the effect of CLEC18B on it will be a worthy exploration in the further animal model. Furthermore, there are reports suggesting that GBM is also inversely linked to the content of macrophages/microglia, and some of the neoplastic cells in GBM are derived from neoplastic macrophages/microglia (Huysentruyt et al., 2011). In the current work, our data showed that overexpression of CLEC18B was associated with poor prognosis; however, whether and what extent CLEC18B correlates with the content of macrophages/microglia remains unclear, which is a worthwhile matter to be investigated in the subsequent study.

In summary, our study was the first report that elevated expression of CLEC18B remarkably associated with clinical poor prognosis and progression of GBM *in vitro*. The results of this study suggested that elevated CLEC18B was closely related with poorer patient survival in GBM patient. Notably, CLEC18B downregulation results in decreased in proliferation, migration, and invasion in GBM cells, which was likely to be regulated by suppression of Wnt/β-catenin signaling. Our results together provide biologically significant insight into the tumorigenic role of CLEC18B in GBM, indicating promising therapeutic agents designed to effectively treat GBM patients.

Declaration of Conflicting Interests

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