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ORIGINAL ARTICLE

Identified a Novel Biomarker, Sarcoglycan Beta Correlated with Poor Prognosis in Glioblastoma Multiforme

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Background: Glioblastoma multiforme (GBM) is the most aggressive and lethal in glioma. The most common chemotherapy is temozolomide. However, drug resistance increased patient recurrence and mortality rates. Sarcoglycan beta (SGCB) is a transmembrane protein involved in the dystrophin-glycoprotein complex of muscle fibers and affects tumor progression in several cancers. **Aim:** We found that SGCB is a potential biomarker in the development of GBM therapeutics. The study aimed to investigate the role and function of SGCB in GBM. **Methods:** We collected the mRNA expression of SGCB from The Cancer Genome Atlas databases for bioinformatics analyses, including the expression difference, Kaplan–Meier survival, and Cox survival analysis. Next, the single-cell sequencing databases were analyzed to investigate the role of SGCB in glioma. Then, the Gene Set Enrichment Analysis was performed to identify the signaling pathways of SGCB in glioma. Finally, to identify the effect of SGCB on the tumor microenvironment of GBM, we used CIBERSORT analysis. **Results:** It was shown that SGCB was highly expressed in tumor tissue compared with the normal group and was correlated with poor prognosis. Moreover, SGCB is mainly expressed in the tumor component. We also found that SGCB was correlated with cell cycle, DNA duplication, and the regulated release of protein in glioma. CIBERSORT analyses revealed that high levels of SGCB affected several immune cells in the tumor microenvironment. **Conclusion:** These data showed that SGCB was expected to serve as an independent prognosis biomarker in GBM. This identification may provide new possibilities for targeted therapies.

Key words: Gene Set Enrichment Analysis, CIBERSORT, Glioblastoma multiforme, sarcoglycan beta, single-cell sequencing

INTRODUCTION

Brain tumors are one of the common cancers. It is estimated that there were approximately 25,050 new cases and 18,280 patients died of brain tumors.[1] Gliomas, which constitute nearly 30% of primary brain tumors and 80% of malignant ones, are particularly lethal and contribute to most deaths from primary brain tumors.[2] Based on the 2021 WHO classification,[3] glioma was divided into three subtypes: (A) glioblastoma multiforme (GBM), isocitrate dehydrogenase (IDH) wild type, (B) astrocytoma, IDH mutant, and (C) oligodendroglioma, IDH mutant. GBM is the most aggressive and lethal in glioma, and the 5-year survival rate for patients with GBM is only 5.01%.[4] Traditional treatment of GBM includes surgery, radiation therapy, and chemotherapy.[5] Temozolomide (TMZ) is currently a widely used chemotherapeutic drug for GBM.[5,6] TMZ is an alkylating agent, leading to the methylation of O6- and N7-methylguanine or N3-methyladenine. DNA mismatch repair enzymes would correct the modified nucleotide, resulting in DNA breaks and inducing cell apoptosis in tumor when the mechanisms of DNA repair failure.[6] However, approximately 90% of

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patients experience early disease recurrence, causing poor prognosis.[6] Therefore, improving current treatment becomes a crucially important issue.

Sarcoglycan beta (SGCB) is a transmembrane protein discovered for its role in the dystrophin-glycoprotein complex (DGC) of muscle fibers. Disruption of the DGC, including mutations or defects in SGCB, might lead to extracellular signal-regulated kinase (ERK) 1/2 signaling alteration, which affects muscle cell survival and causes muscle degeneration.[7] Several evidence suggest that SGCB expression was altered in various cancers and might affect tumor progression. Pan-cancer transcriptome analyses have identified SGCB as highly expressed and affects prognosis in multiple malignancies.[8] High SGCB expression contributed to higher risk scores and poor survival in hepatocellular carcinoma (HCC).[9,10] In colorectal cancer, SGCB upregulation was associated with increased recurrence risk.[11] Moreover, a high level of SGCB was associated with more aggressive disease and poorer patient survival in glioma, especially the IDH-wild-type subgroup.[12] However, the mRNA of SGCB correlated with better prognosis in clear cell renal cell carcinoma (CCRCC).[13] In breast cancer, it was shown that the expression of SGCB was negatively related to the amount of cytotoxic T-cells and $CD8^+$ T-cell infiltration and positively correlated to M2 macrophage signatures.[14] The study indicated that a high level of SGCB might involve tumor growth and infiltration and enhanced inflammatory response in esophageal carcinoma.[15] These studies showed that SGCB might involve tumor progression in several cancers. However, the detailed mechanism and role of SGCB in cancers is unclear, especially glioma.

Due to TMZ resistance and increased recurrence rate of GBM, improving current treatment for GBM became a crucially important issue. The study aimed to identify a novel biomarker in GBM and investigate its role and function. We found that SGCB is a potential biomarker in the development of GBM therapeutics. The previous study indicated that high SGCB expression was positively related to the poor survival of GBM.[12] In the study, we would further investigate the role and effect of SGCB in GBM, including survival rate, cell components, and signal transduction. The discovery could offer new opportunities for targeted therapies.

MATERIALS AND METHODS

Data collection

We collected the glioma transcriptome (mRNA) expression and clinical parameters, such as age, tumor grade, histology, overall survival, and vital status from The Cancer Genome Atlas (TCGA) transcriptome dataset (https://portal.gdc.cancer.gov/).[16-19] There are 690 cases in total [Table 1], including oligodendroglioma, oligoastrocytoma, astrocytoma, and glioblastoma. Due to the utilization of an outdated 2007 WHO classification system,[20] the cases were subsequently reorganized into three distinct subgroups based on the revised 2021 WHO classification.[3] These subgroups included (A) GBM, IDH-wild type, (B) astrocytoma, IDH mutant, and (C) oligodendroglioma, IDH mutant. There were 28 cases that cannot be further classified due to lacking molecular information. The study was conducted in accordance with the Declaration of Helsinki and was approved by TSGH-IRB (A202205193). Informed written consent was obtained from all patients.

Differential expression analysis

We collected the glioma mRNA expression from the TCGA dataset (https://portal.gdc.cancer.gov/), and the bioinformatics analyses were performed using R (version 4.1.0, www.r-project.org) along with appropriate R packages. We used limma and ggplot2 R packages to collect SGCB mRNA expression, and the standard normalization and log2 transformation were used. In all the gliomas, there were 5 cases in the normal

Table 1: Summary of glioma cases from the TCGA database

2007 adult glioma classification	*n*	2021 adult glioma classification	*n*	
Grade 2 oligodendroglioma	115	Grade 2 oligodendroglioma	183	101
Grade 2 oligoastrocytoma	77	Grade 3 oligodendroglioma		82
Grade 2 astrocytoma	65	Grade 2 IDH mutant astrocytoma	247	124
Grade 3 oligodendroglioma	82	Grade 3 IDH mutant astrocytoma		101
Grade 3 oligoastrocytoma	55	Grade 4 IDH mutant astrocytoma		22
Grade 3 astrocytoma	130	Grade 2 IDH wild-type astrocytoma	232	14
Grade 4 glioblastoma	166	Grade 3 IDH wild-type astrocytoma		10
		Grade 4 IDH wild-type astrocytoma		208
Total	690	Total		662

group and 662 cases in the tumor groups, including 232 cases of IDH-wild-type astrocytoma, 247 cases of IDH-mutant astrocytoma, and 183 cases of oligodendroglioma. We compared the expression in the tumor and the normal group. Statistical significance was defined at $P < 0.05$.

Survival and prognostic analysis

The survival and prognostic analysis was conducted using the survival and survminer R packages. The tumor cases were selected and divided into high and low levels of SGCB groups and the cutoff value was optimal cutoff value. Integrating lifetime and alive state of patients, the Kaplan–Meier survival curves were generated. The Cox survival analysis was used to estimate the hazard ratios (HRs) of SGCB on tumor malignancy while comparing it with the tumor malignancy and other clinical prognosis factors, including age, gender, grade, the Karnofsky score, radiotherapy, and chemotherapy. Statistical significance was defined at $P < 0.05$.

Gene Set Enrichment Analysis

We conducted Gene Set Enrichment Analysis (GSEA) analysis[21] using the R packages, involving limma. The tumor cases were selected and the SGCB mRNA expression was extracted and divided into high- and low-level groups and the cutoff was median. The difference of each gene was obtained by the high-level group compared with the low-level group. Finally, the enrichment scores and normalized enrichment scores were obtained. Gene sets with false discovery rate (FDR) $P < 0.05$ were considered significant.

Single-cell sequencing databases

We analyzed a published single-cell RNA-seq dataset, GSE131928, containing 7930 single cells from 28 patients with GBM[22] and GSE89567, containing 6341 single cells from 10 patients with IDH-mutant astrocytoma.[23] Cells were divided into four groups: tumor cells, T cells, glial cells, and myeloid cells on the Browser platform.[24] According to the SGCB mRNA expression, we could further identify which cell components have high SGCB expression. Statistical significance was defined at $P < 0.05$.

CIBERSORT analysis

CIBERSORT analysis was conducted by sorting 22 immune cells according to all gene mRNA expression from the TCGA database and specific genes of immune cells. These immune cells involved naïve B cells, memory B cells, plasma cells, CD8 T cells, naive CD4 T cells, resting memory CD4 T cells, activated memory CD4 T cells, helper follicular T cells, regulatory T cells (Tregs), gamma delta T cells, resting NK cells, activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils, and neutrophils.

Ivy Glioblastoma Atlas Project

In the Ivy Glioblastoma Atlas Project (IvyGAP) datasets, the GBM tumors were divided into five structures: leading edge, infiltrating tumor, cellular tumor, pseudopalisading cells around necrosis, and microvascular proliferation based on the result of H and E staining. The RNA-seq was conducted by selected two regions per structure, and 122 RNA samples were generated from 10 tumors. Moreover, cancer cell clusters were isolated by laser microdissection. We collected SGCB mRNA expression in five structures from IvyGAP datasets. Statistical significance was defined at $P < 0.05$.

RESULTS

Sarcoglycan beta significantly increases in many cancers, as well as glioma

To understand the expression of SGCB in cancer, we used TIMER2.0 (http://timer.cistrome.org). The result showed that SGCB is highly expressed in tumor groups compared with normal groups in the several cancers [Figure 1 and Supplementary Table 1]. A high level of SGCB was raised in the GBM tumor groups ($n = 153$) and the low-grade glioma ($n = 516$) compared with the normal groups ($n = 5$) ($P = 0.075$). In addition, similar results have been seen in other cancers, including liver HCC and pheochromocytoma and paraganglioma (PCPG). This result is consistent with previous studies,[9,10] implying that SGCB was a prognostic factor in several cancers, including glioma.

Sarcoglycan beta is highly expressed in IDH-wild-type tumors, which strongly correlate with the poor survival rate

To clarify the role of SGCB in glioma, we collected the mRNA expression and clinical characteristics of glioma patients from the TCGA transcriptome dataset (https://portal.gdc.cancer.gov/). However, due to the utilization of an outdated 2007 WHO classification system,[20] the cases were subsequently reorganized into three distinct subgroups based on the revised 2021 WHO classification.[3] These subgroups included (A) GBM, IDH wild type, (B) astrocytoma, IDH mutant, and (C) oligodendroglioma, IDH mutant. Next, we performed several bioinformatics analyses, including differential expression analysis (DEG) analysis, Kaplan–Meier survival analysis, and Cox survival analysis. In the DEG analysis [Figure 2a-d], we

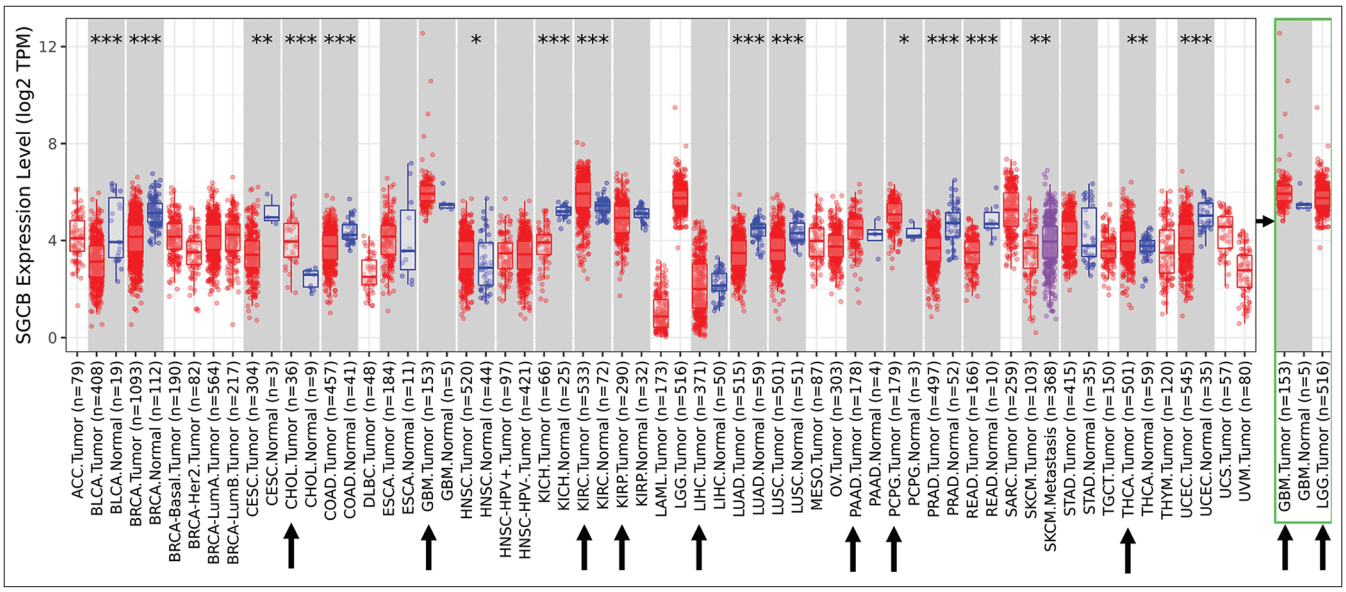


Figure 1: The higher mRNA expression of sarcoglycan beta (SGCB) in several cancer tumors compared with normal tissue. The analysis showed the relationship between SGCB expression and cancer tumors, including glioblastoma multiforme (GBM). Arrow showed the cancer types with high level of SGCB. Green frame indicated SGCB expression in GBM and low-grade glioma tumors (P = 0.075). BLCA = Bladder urothelial carcinoma; BRCA = Breast invasive carcinoma; CESC = Cervical Squamous Cell Carcinoma and endocervical adenocarcinoma; CHOL = Cholangiocarcinoma; COAD = Colon adenocarcinoma; DLBC = Diffuse large B-cell lymphoma; ESCA = Esophageal carcinoma; GBM = Glioblastoma multiforme; HNSC = Head-and-neck squamous cell carcinoma; KICH = Kidney chromophobe; KIRC = Kidney renal clear cell carcinoma; KIRP = Kidney renal papillary cell carcinoma; LAML = Acute myeloid leukemia; LGG = Brain lower grade glioma; LIHC = Liver hepatocellular carcinoma; LUAD = Lung adenocarcinoma; LUSC = Lung squamous cell carcinoma; MESO = Mesothelioma; OV = Ovarian serous cystadenocarcinoma; PAAD = Pancreatic adenocarcinoma; PCPG = Pheochromocytoma and paraganglioma; PRAD = Prostate adenocarcinoma; READ = Rectum adenocarcinoma; SARC = Sarcoma; SKCM = Skin cutaneous melanoma; STAD = Stomach adenocarcinoma; TGCT = Testicular germ cell tumors; THCA = Thyroid carcinoma; THYM = Thymoma; UCEC = Uterine corpus endometrial carcinoma; UCS = Uterine carcinosarcoma; UVM = Uveal melanoma; ACC = Adrenocortical carcinoma; HPV = Human papillomavirus; ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

found that SGCB is highly expressed in tumor groups ($n = 232$) compared with normal groups ($n = 5$) in IDH-wild-type subtype and there was a significant difference [Figure 2b, $P < 0.05$]. However, despite there were similar results in IDH-mutant subtype [Figure 2c] and oligodendroglioma [Figure 2d], there was no statistical significance. Besides, the Kaplan–Meier survival analysis [Figure 2e-h] showed that a high level of SGCB was positively correlated with a poor survival rate in IDH-wild-type [Figure 2f, $P = 0.011$] and IDH-mutant subtype [Figure 2g, $P = 0.003$]. However, there was no statistical significance in oligodendroglioma [Figure 2h, $P = 0.231$].

To identify whether SGCB is an independent prognosis factor in glioma, the Cox survival analysis was performed [Figure 2i-l]. The results showed that the HR of SGCB was higher than 1 in the IDH-wild-type subgroup [Figure 2j, HR = 1.303], IDH-mutant subtype [Figure 2k, HR = 1.787], and oligodendroglioma [Figure 2l, HR = 1.091], indicating that the expression of SGCB is positively correlated with tumor malignancy in glioma. Furthermore, compared with other prognosis factors, including age, gender, grade, the Karnofsky score, radiotherapy, and chemotherapy, the P value of SGCB was 0.034, showing that SGCB is independent of other prognosis factors in the IDH-wild-type subgroup. Similar results were observed in IDH-mutant and oligodendroglioma subtypes, but there was no statistical significance [Figure 2k and l]. These data suggest that SGCB has a high impact on tumor progression and can serve as an independent prognostic factor in IDH-wild-type glioma.

Sarcoglycan beta correlated with cell cycle and DNA duplication in glioma

To clarify the role and function of SGCB in glioma, we used GSEA analysis to understand the signaling transduction of SGCB. In our results, it was shown that a high level of SGCB was positively related to G2M_checkpoint, E2F_targets, mitotic_spindle, epithelial _mesenchymal_transition, myc_targets_v1, hypoxia, androgen_response, UV_response_dn, mTORc1_signaling, TNFa_signaling_via_NFkb, TGF_beta_ signaling, protein_secretion, and hedgehog_signaling in the IDH-wild-type group [Figure 3a and Table 2]. However, a high level of SGCB was negatively related to KRAS_signaling_dn, bile_acid_metabolism, and interferon_alpha_response in the IDH-wild-type group. After further analyzing to investigate the intersection of three subgroups, we found that there were five common pathways in three subtypes:

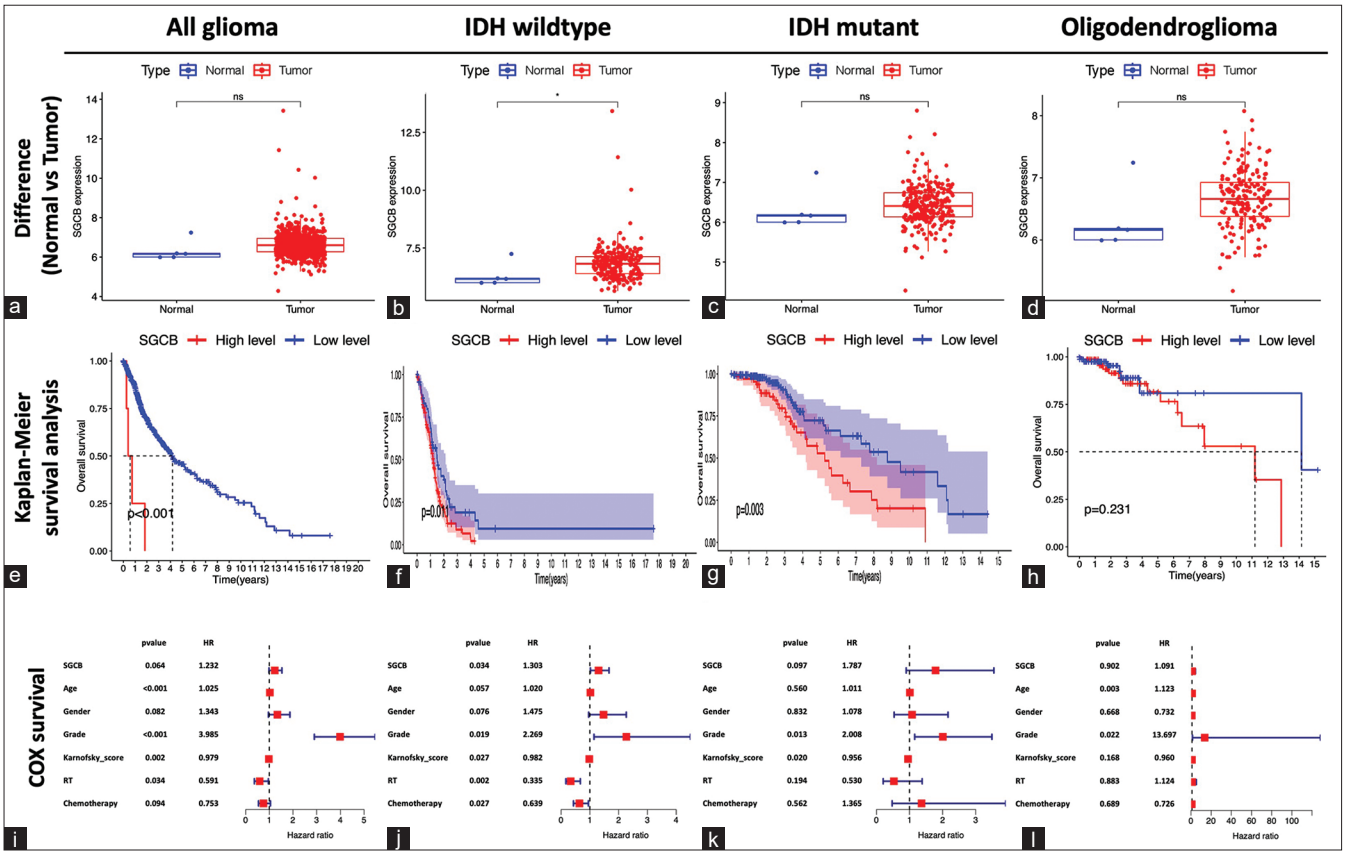


Figure 2: Sarcoglycan beta (SGCB) is highly expressed in IDH-wild-type tumors, which strongly correlates with the poor survival rate. The bioinformatics information from The Cancer Genome Atlas and analyses were conducted using R packages. (a-d) The DEG analysis of SGCB in all gliomas and three subtypes. The red dots represented the tumor group, and the blue dots represented the normal group. In all the gliomas, there were 5 cases in the normal group and 662 cases in the tumor groups, including 232 cases of IDH-wild-type astrocytoma, 247 cases of IDH-mutant astrocytoma, and 183 cases of oligodendroglioma. (e-h) The Kaplan–Meier survival analyses of SGCB. The red line represented the high level of SGCB group, and the blue line represented the low level of SGCB group. The cutoff value was optimal cutoff value. (i-l) The multiple Cox survival analyses of SGCB. The Cox survival analysis was used to estimate the hazard ratios of SGCB on tumor malignancy while comparing it with the tumor malignancy and other clinical prognosis factors, including age, gender, grade, the Karnofsky score, radiotherapy, and chemotherapy. SGCB = Sarcoglycan beta; HR = Hazard ratio; IDH = Isocitrate dehydrogenase; RT = Radiation therapy

G2M_checkpoint, E2F_targets, mitotic_spindle, and protein_secretion [Figure 3b]. The results indicated that SGCB correlated with cell cycle, DNA duplication, and the regulated release of protein in glioma and a high level of SGCB might induce cell division and cell proliferation and change the tumor microenvironment by secreting a large amount of proteins, further promoting the development and progression of glioma.

Sarcoglycan beta was mainly expressed in the tumor components in glioblastoma multiforme

To further identify the role of SGCB in GBM, we used IvyGAP datasets. We collected SGCB mRNA expression in five structures, including leading edge, infiltrating tumor, cellular tumor, pseudopalisading cells around necrosis, and microvascular proliferation. In our analysis, it was shown that SGCB was mainly expressed in the infiltrating tumor, cellular tumor, and pseudopalisading cells in GBM. However, we found that in the leading edge and the microvascular proliferation region, SGCB was lowly expressed compared with other regions and there was a significant difference [Figure 4a and b]. From the results of the H and E staining and RNA-seq [Figure 4c and d], we also found a similar result. The result showed that SGCB was mainly expressed in the tumor component rather than normal cells and the vascular region. Moreover, SGCB might promote tumor infiltration, tumor proliferation, and tumor progression and cause tissue necrosis in GBM.

Sarcoglycan beta is mainly expressed in the tumor and glial cell components in single-cell sequencing datasets

To further investigate whether SGCB is mainly expressed in the tumor cells, we analyzed the single-cell sequencing datasets in IDH-wild-type [Figure 5a-c] and IDH-mutant subgroup [Figure 5d-f]. In our analysis, cells were divided into four groups: tumor cells, T cells, glial cells, and myeloid

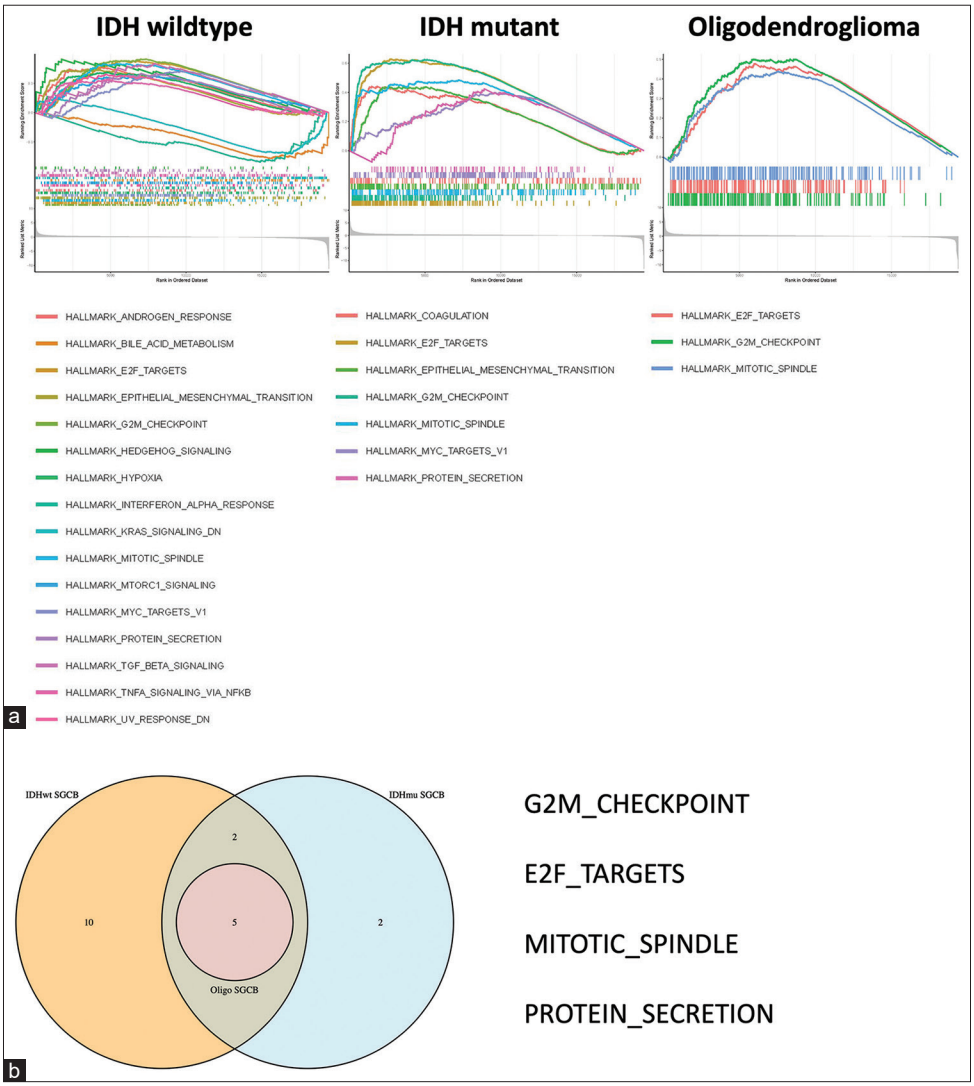


Figure 3: Sarcoglycan beta (SGCB) correlated with cell cycle and DNA duplication in glioma. (a) We downloaded the transcriptome data, separated the higher and lower SGCB expression groups, and performed Gene Set Enrichment Analysis (GSEA) analysis. We conducted GSEA analysis[21] using the R packages, involving limma. The tumor cases were selected and the SGCB mRNA expression was extracted and divided into high- and low-level groups and the cutoff was median. The difference of each gene was obtained by the high-level group compared with the low-level group. (b) After further analyzing to investigate the intersection of three subgroups, we found that there were five common pathways in three subtypes. SGCB = Sarcoglycan beta; IDH = Isocitrate dehydrogenase

cells [Figure 5a and d]. According to the SGCB mRNA expression, we found that SGCB is expressed in all cell types but mainly expressed in the tumor and glial cell components [Figure 5b and e]. Similar results were obtained in the statistical chart [Figure 5c and f]. The data showed SGCB mainly expressed in the tumor cells and glial cells rather than T cells and myeloid cells.

Sarcoglycan beta affected tumor microenvironment in the IDH-wild-type subgroup

According to the analysis of single-cell sequencing datasets, we found that SGCB was highly expressed in the glial cell component, indicating that SGCB might affect the tumor environment by upregulating in the glial cells. To further validate the hypothesis about the effect of SGCB in the glioma microenvironment, we used CIBERSORT analysis. In our analysis, we found that a high level of SGCB positively correlated with Treg cells, neutrophils, and M0 macrophages in the IDH-wild-type subgroup [Figure 6]. Besides, high levels of SGCB negatively correlated with resting dendritic cells, M1 macrophages, M2 macrophages, and CD8 T cells in the IDH-wild-type group. However, no similar results were found in the IDH-mutant group. According to the positive correlation of the M0 macrophages and negative correlation of M1 macrophages and M2 macrophages in IDH-wild-type GBM,

Table 2: The signaling pathways of SGCB in glioma from GSEA analysis

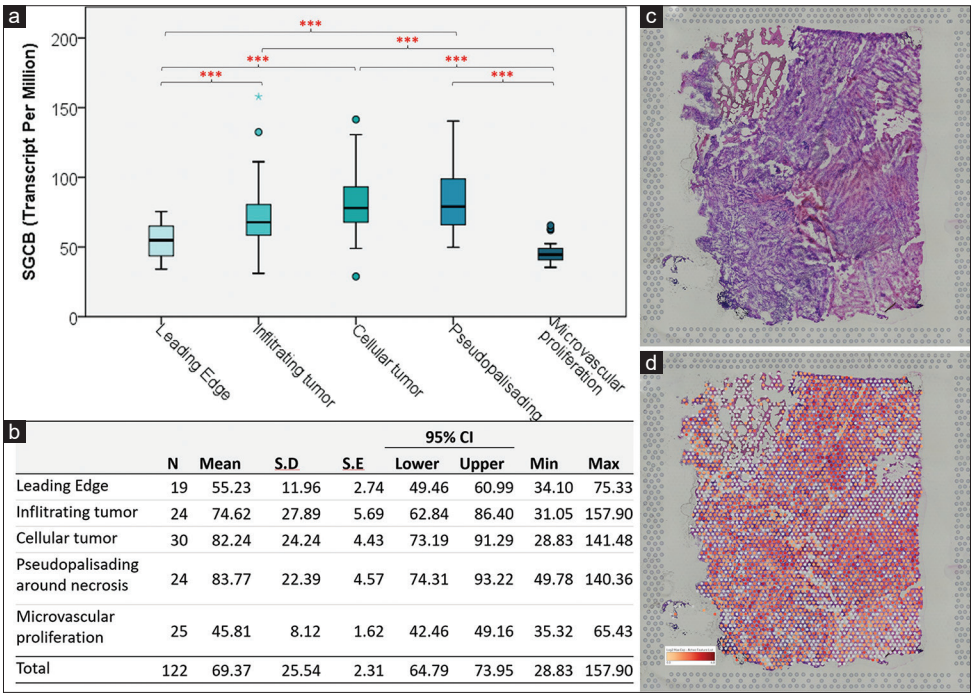
ID	Enrichment score	NES	*P*	*q*
All glioma				
E2F_TARGETS	0.613099194	2.384643413	1.00E-10	9.82E-10
EPITHELIAL_MESENCHYMAL_TRANSITION	0.585968041	2.279117058	1.00E-10	9.82E-10
G2M_CHECKPOINT	0.58582002	2.278670169	1.00E-10	9.82E-10
HYPOXIA	0.476027507	1.851609097	4.11E-06	3.03E-05
MITOTIC_SPINDLE	0.456456142	1.77548216	3.34E-05	0.000196909
MTORC1_SIGNALING	0.447123288	1.739180059	7.99E-05	0.00039262
GLYCOLYSIS	0.432153681	1.681458554	0.000186133	0.000783718
KRAS_SIGNALING_UP	0.426749844	1.659839412	0.000390026	0.001436936
COAGULATION	0.455929148	1.713004396	0.000680107	0.002188045
MYC_TARGETS_V1	0.41945503	1.631466302	0.000742373	0.002188045
APOPTOSIS	0.441264526	1.682791343	0.000830093	0.002224171
CHOLESTEROL_HOMEOSTASIS	0.505916731	1.743406666	0.001889016	0.004639688
TNFA_SIGNALING_VIA_NFKB	0.404010651	1.571395537	0.002201998	0.004992383
ANGIOGENESIS	0.570897495	1.7584191	0.00462939	0.009746084
UV_RESPONSE_DN	0.403157777	1.52394472	0.010600113	0.020828292
PANCREAS_BETA_CELLS	0.512022417	1.602176815	0.01687523	0.031085951
IL6_JAK_STAT3_SIGNALING	0.435312522	1.538586566	0.018816284	0.03262266
ALLOGRAFT_REJECTION	0.355799895	1.383880266	0.028312571	0.041326419
INFLAMMATORY_RESPONSE	0.355248882	1.381815235	0.028312571	0.041326419
P53_PATHWAY	0.362596117	1.410818348	0.028402493	0.041326419
INTERFERON_GAMMA_RESPONSE	0.353258809	1.373996737	0.029445074	0.041326419
HEDGEHOG_SIGNALING	0.478479145	1.473761708	0.04144385	0.055522862
IDH-wild type				
G2M_CHECKPOINT	0.547061744	2.228356479	5.02E-08	1.80E-06
E2F_TARGETS	0.530542305	2.158140405	1.76E-07	3.14E-06
MITOTIC_SPINDLE	0.500622672	2.039195368	3.37E-06	4.02E-05
EPITHELIAL_MESENCHYMAL_TRANSITION	0.439595773	1.788188032	0.00026236	0.00234743
MYC_TARGETS_V1	0.423304528	1.721918493	0.00068761	0.004921843
HYPOXIA	0.411998793	1.67820212	0.00109845	0.006552158
ANDROGEN_RESPONSE	0.462010197	1.672751983	0.005737102	0.02933255
INTERFERON_ALPHA_RESPONSE	-0.506067085	-1.637758301	0.007943571	0.035537028
UV_RESPONSE_DN	0.392027016	1.513685157	0.00983938	0.036304262
MTORC1_SIGNALING	0.374129539	1.523948605	0.010143838	0.036304262
BILE_ACID_METABOLISM	-0.470200175	-1.556299482	0.01315595	0.041869325
KRAS_SIGNALING_DN	-0.423255013	-1.483283272	0.014038538	0.041869325
TNFA_SIGNALING_VIA_NFKB	0.33761227	1.373339459	0.027533006	0.074366354
TGF_BETA_SIGNALING	0.5007219	1.644031239	0.029090368	0.074366354
PROTEIN_SECRETION	0.400958695	1.443374746	0.039096768	0.093283517
HEDGEHOG_SIGNALING	0.545517854	1.641208426	0.044840333	0.100300746

Contd...

Table 2: Contd...

ID	Enrichment score	NES	*P*	*q*
IDH-mutant				
E2F_TARGETS	0.628120779	2.281228029	1.00E-10	2.21E-09
G2M_CHECKPOINT	0.625750054	2.272928622	1.00E-10	2.21E-09
MITOTIC_SPINDLE	0.485123049	1.762125398	0.000170216	0.002508442
EPITHELIAL_MESENCHYMAL_TRANSITION	0.43868982	1.59324695	0.001421367	0.015709842
COAGULATION	0.442338142	1.566971527	0.007801714	0.068983574
MYC_TARGETS_V1	0.399386024	1.450502233	0.01054396	0.077692337
INTERFERON_GAMMA_RESPONSE	0.370361677	1.345090732	0.039955605	0.240840617
PROTEIN_SECRETION	0.424525145	1.425850137	0.043580683	0.240840617
Oligodendroglioma				
G2M_CHECKPOINT	0.501429414	1.854980794	4.61E-05	0.002234064
E2F_TARGETS	0.478255523	1.77253955	0.000134817	0.003263983
MITOTIC_SPINDLE	0.439137275	1.624538147	0.002772281	0.044745592
PROTEIN_SECRETION	0.420956095	1.415018596	0.048338369	0.585148672

NES=Normalized enrichment scores



	N	Mean	S.D	S.E	95% CI Lower	95% CI Upper	Min	Max
Leading Edge	19	55.23	11.96	2.74	49.46	60.99	34.10	75.33
Inflitrating tumor	24	74.62	27.89	5.69	62.84	86.40	31.05	157.90
Cellular tumor	30	82.24	24.24	4.43	73.19	91.29	28.83	141.48
Pseudopalisading around necrosis	24	83.77	22.39	4.57	74.31	93.22	49.78	140.36
Microvascular proliferation	25	45.81	8.12	1.62	42.46	49.16	35.32	65.43
Total	122	69.37	25.54	2.31	64.79	73.95	28.83	157.90

Figure 4: Sarcoglycan beta (SGCB) is mainly expressed in the tumor components of glioblastoma multiforme (GBM) from the Ivy Glioblastoma Atlas Project (IvyGAP) datasets. (a) In the IvyGAP datasets, GBM tumors were categorized into five structures: leading edge, infiltrating tumor, cellular tumor, pseudopalisading cells around necrosis, and microvascular proliferation based on the results of H and E staining. (c-d) RNA sequencing was conducted by selecting two regions per structure, and 122 RNA samples were generated from 10 tumors. Additionally, cancer cell clusters were isolated using laser microdissection. (b) We collected SGCB mRNA expression for the five structures from IvyGAP datasets. Statistical significance was determined at $P < 0.05$, *** = $P < 0.001$. SGCB = Sarcoglycan beta; SD = Standard deviation; SE = Standard error; CI = Confidence interval

we considered that SGCB might recruit or retain macrophages in an undifferentiated M0 state, preventing them from becoming pro-inflammatory (M1) or anti-inflammatory (M2) subsets in GBM.

DISCUSSION

In our study, we found that SGCB is highly expressed in tumors compared with the normal group in IDH-wild-type glioma and

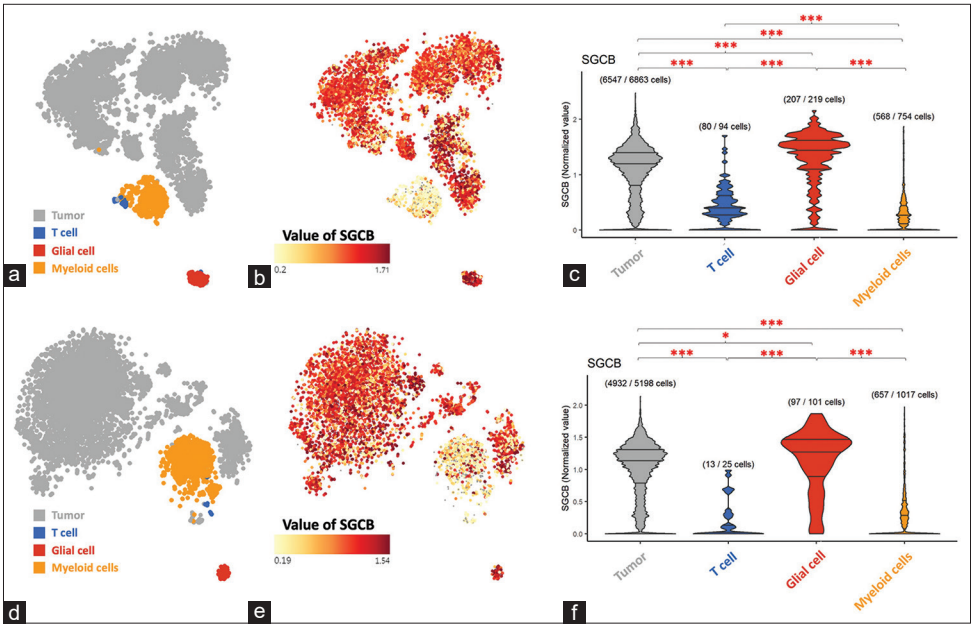


Figure 5: Sarcoglycan beta (SGCB) mainly expressed in the tumor components in glioblastoma multiforme (GBM) from the single-cell sequencing analyses. We analyzed a published single-cell RNA-seq dataset, GSE131928, containing 7930 single cells from 28 patients with GBM[22] and GSE89567, containing 6341 single cells from 10 patients with IDH-mutant astrocytoma.[23] (a) Cells were categorized into four groups: tumor cells, T cells, glial cells, and myeloid cells in GSE131928. (b) The heatmap of SGCB expression from the single-cell sequencing analyses in GSE131928. (c) The heatmap of SGCB was quantified in GSE131928. (d) Cells were categorized into four groups: tumor cells, T cells, glial cells, and myeloid cells in GSE89567. (e) The heatmap of SGCB expression from the single-cell sequencing analyses in GSE89567. (f) The heatmap of SGCB in GSE89567 was quantified. SGCB = Sarcoglycan beta; **P*<0.05; ****P*<0.001

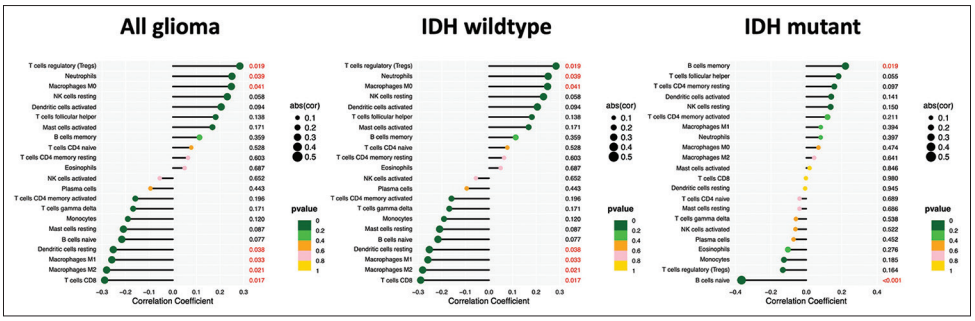


Figure 6: Sarcoglycan beta (SGCB) affected tumor microenvironment in the IDH-wild-type subgroup. We analyzed the correlation between the SGCB with 22 immune cell components in the mRNA level by the CIBERSORT analysis. CIBERSORT analysis was conducted by sorting 22 immune cells according to all gene mRNA expressions from The Cancer Genome Atlas database and specific genes of immune cells. These immune cells involved naïve B cells, memory B cells, plasma cells, CD8 T cells, naive CD4 T cells, resting memory CD4 T cells, activated memory CD4 T cells, helper follicular T cells, regulatory T cells (Tregs), gamma delta T cells, resting NK cells, activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated mast cells, eosinophils, and neutrophils. IDH = Isocitrate dehydrogenase

is associated with poor survival. It was consistent with previous studies.[12] Moreover, it was shown that SGCB was expected to serve as an independent prognosis biomarker in GBM rather than IDH-mutant astrocytoma and oligodendroglioma. Similar results have been seen in other cancers,[10,11] suggesting that SGCB might be broadly a biomarker of tumor aggressiveness in several cancers. However, SGCB is associated with a better prognosis in CCRCC,[13] suggesting that SGCB may have different effects on tumors due to the tumor microenvironment, different cellular composition, or interactions of different pathways.

As part of the DGC, SGCB may affect the cell-matrix adhesion of tumor cells, thus contributing to tumor migration in brain tissue. Furthermore, co-amplification of SGCB and PDGFRA in some GBM cases suggests that SGCB high expression may occur with amplification of pro-cancer signaling at the same time.[12] In the GSEA analysis, we also found that a high level of SGCB was correlated with the epithelial-mesenchymal transition (EMT) in IDH-wild-type and IDH-mutant glioma rather than oligodendroglioma. The results confirmed the previous hypothesis. However,

functional validations would be performed to further validate the relationship of SGCB and EMT in glioma.

Several studies showed that SGCB and its associated complex affected the ERK1 and ERK2 signaling pathway, which is important for regulating various cellular processes, including proliferation, differentiation, and survival. The mutations or deficiencies in SGCB caused disruption of the sarcoglycan complex, which is involved in transducing mechanical signals in skeletal muscle and altered ERK1/2 phosphorylation patterns in response to the mechanical stress.[7] It was shown that activation of the ERK1/2 pathway could induce a fast-to-slow muscle fiber-type switch, through the influence of sarcoglycan complexes, including SGCB, and enhance oxidative capacity and fatigue resistance.[25] Furthermore, γ-sarcoglycan has been shown to associate with archvillin, a protein that interacted with ERK1/2. Although the direct interaction between SGCB and ERK1/2 has not been fully clarified, the integrity of the sarcoglycan complex appears to be essential for ERK1/2 signaling.[26] In GBM, the interaction between SGCB and ERK1/2 still needs to be investigated.

In the single-cell sequencing datasets, we observed that SGCB was expressed not only in the tumor component but also in other immune cells, especially glial cells in the IDH-wild-type tumor. From the CIBERSORT analysis, it was shown that SGCB positively correlated with M0 macrophages and negatively correlated with M1 macrophages and M2 macrophages in GBM, indicating that a high level of SGCB might recruit or retain macrophages in an undifferentiated M0 state, preventing them from becoming pro-inflammatory (M1) or anti-inflammatory (M2) subsets. Moreover, SGCB also correlated with Treg cells, neutrophils, resting dendritic cells, and CD8 T cells in the IDH-wild-type group. However, the detailed mechanism of SGCB on tumor microenvironment is unclear currently. We had to further validate the effect of SGCB on the tumor microenvironment.

Although cancer therapies targeting SGCB have not been explored, it was shown that SGCB might be a potential biomarker and provide new possibilities for targeted therapies in GBM according to our analyses. Due to high expression of SGCB in the tumor group than the normal group, the inhibitor of SGCB would be used to improve current therapies for GBM. Through an inhibitor of SGCB, SGCB was downregulated in the tumor and did not affect the normal tissue. Furthermore, our previous studies decreased GBM cell progression by combining TMZ and drugs, including metformin[27] and diosmin.[28] Combining TMZ and SGCB inhibitors might effectively decrease tumor progression and improve TMZ resistance in GBM. Our data also provide new possibilities for immunotherapies in GBM due to the effect of SGCB on the glial cells and macrophages.

CONCLUSION

This study demonstrates that SGCB is highly expressed in tumor and might be broadly a biomarker of tumor aggressiveness in IDH wild-type GBM. Moreover, SGCB is significantly associated with M0 macrophages, M1 macrophages, and M2 macrophages in the tumor microenvironment. Furthermore, our findings also provide new avenues for the development of targeted therapies for SGCB. In conclusion, SGCB plays a crucial role in GBM, acting both as a prognostic biomarker and a promising therapeutic target. These findings hold the potential to significantly improve clinical outcomes for patients suffering from this aggressive form of glioma, offering new hope for future treatments.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: The *P*-value of SGCB in cancers from The Cancer Genome Atlas databases

Tumor	Normal	*P*
BLCA.Tumor (*n*=408)	BLCA.Normal (*n*=19)	6.42572451216329E-05
BRCA.Tumor (*n*=1093)	BRCA.Normal (*n*=112)	9.79684326595173E-38
CESC.Tumor (*n*=304)	CESC.Normal (*n*=3)	0.00804004067716473
CHOL.Tumor (*n*=36)	CHOL.Normal (*n*=9)	5.17060552287588E-06
COAD.Tumor (*n*=457)	COAD.Normal (*n*=41)	8.37261846200503E-09
ESCA.Tumor (*n*=184)	ESCA.Normal (*n*=11)	0.539716048776163
GBM.Tumor (*n*=153)	GBM.Normal (*n*=5)	0.0754155431817728
HNSC-HPV+.Tumor (*n*=97)	HNSC-HPV-.Tumor (*n*=421)	0.759988129720756
HNSC.Tumor (*n*=520)	HNSC.Normal (*n*=44)	0.0250552075075941
KICH.Tumor (*n*=66)	KICH.Normal (*n*=25)	1.1342871745397E-11
KIRC.Tumor (*n*=533)	KIRC.Normal (*n*=72)	1.02509916351957E-09
KIRP.Tumor (*n*=290)	KIRP.Normal (*n*=32)	0.106594976174466
LIHC.Tumor (*n*=371)	LIHC.Normal (*n*=50)	0.209989017336061
LUAD.Tumor (*n*=515)	LUAD.Normal (*n*=59)	1.14322737522921E-20
LUSC.Tumor (*n*=501)	LUSC.Normal (*n*=51)	3.37579509043667E-13
PAAD.Tumor (*n*=178)	PAAD.Normal (*n*=4)	0.422940462391923
PCPG.Tumor (*n*=179)	PCPG.Normal (*n*=3)	0.0398428851876857
PRAD.Tumor (*n*=497)	PRAD.Normal (*n*=52)	1.35199282468294E-16
READ.Tumor (*n*=166)	READ.Normal (*n*=10)	3.65527482154916E-06
SKCM.Tumor (*n*=103)	SKCM.Metastasis (*n*=368)	0.00412419825094402
STAD.Tumor (*n*=415)	STAD.Normal (*n*=35)	0.226282709524997
THCA.Tumor (*n*=501)	THCA.Normal (*n*=59)	0.00241725403998696
UCEC.Tumor (*n*=545)	UCEC.Normal (*n*=35)	3.07396642556205E-11