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ORIGINAL ARTICLE

High-level Sp1 is Associated with Proliferation, Invasion, and Poor Prognosis in Astrocytoma

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Abstract

Astrocytoma is the most common and the most lethal primary brain tumor in adults. Grade IV glioblastoma is usually refractory to currently available surgical, chemotherapeutic, and radiotherapeutic treatments. The Specificity protein 1 (Sp1) transcription factor is known to regulate tumorigenesis in many cancers. The aim of this study was to investigate the clinicopathologic role of Sp1 protein in the carcinogenesis of astrocytoma. This study analyzed 98 astrocytoma cases treated at Kaohsiung Medical University Hospital during 2002–2012. Clinicopathologic parameters associated with Sp1 were analyzed by chi-square test, Kaplan-Meier analysis, and Cox regression analyses. In vitro proliferation, invasion, and migration were compared between non-siRNA groups and Sp1 siRNA groups. In glioblastoma cells treated with Sp1 siRNA, Western blot was also used to detect expressions of Sp1, Ki-67, VEGF, cyclin D1, E-cadherin, cleaved caspase-3 and Bax proteins. Expression of Sp1 was significantly associated with WHO grade ($p = 0.005$) and with overall survival time ($p < 0.001$). Multivariate analysis further revealed that prognosis of astrocytoma was significantly associated with Sp1 expression ($p = 0.036$) and IDH-1 expression ($p < 0.001$). *In vitro* silencing of Sp1 downregulated Sp1, Ki-67, and cyclin D1 but upregulated E-cadherin, Bax, and cleaved caspase-3. These data suggest that Sp1 is a potential prognostic marker and therapeutic target in astrocytoma.

Keywords Astrocytoma · Specificity protein 1 · WHO grade · Immunohistochemistry staining · Apoptosis

Introduction

Astrocytoma is the most common primary brain tumor in humans. The World Health Organization (WHO) classifies astrocytomas into four grades [1]. Grade I includes benign and slow-growing neoplasms such as pilocytic astrocytoma. Grade II is diffuse astrocytoma that is invasive but relatively slow-growing and can be completely removed by surgical intervention. Grades III and IV are high grade astrocytomas such as anaplastic astrocytoma and glioblastoma (GBM).

Yi-Ting Chen, Hung-Pei Tsai, Chee-Yin Chai and Aij-Lie Kwan contributed equally to this work.

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Grades III and IV are characterized by cellular atypia, mitosis and hypercellularity. Glioblastoma, the most aggressive malignant primary brain tumor in adults, is morphologically characterized by microvascular proliferation or necrosis. Although combined treatment with surgery, radiotherapy and chemotherapy can improve GBM outcomes, overall survival remains extremely poor. The median survival time is 12 months, the 1-year survival rate is less than 20%, and the 3-year survival rate is less than 3%. Glioblastoma has a low survival rate because it is often unresectable and because its recurrence rate is high (up to 90%) [2, 3]. Therefore, biomarkers of GBM and its pathogenesis are urgently needed for effective personalized treatment.

Specificity protein (Sp) transcription factors (including Sp1, Sp3 and Sp4) in the Sp/Kruppel-like factor (Sp/KLF) family are widely used markers of differentiation, embryonic and early postnatal development, and cell cycle regulation in many diseases, including cancers. One important transcription factor in the Sp/KLF family is Sp1 encoded by the *Sp1* gene on chromosome 12. The Sp1 activates a broad spectrum of cellular genes [4]. It binds directly to DNA in GC-rich elements of promoters following 3 C2H2-type zinc fingers located at the C-terminus and enhances gene transcription [5]. The Sp1 is also known to participate in cell differentiation, cell growth, apoptosis, immune responses, DNA damage response, histone modification and chromatin remodeling. Recent studies show that Sp1 has roles in cell cycle regulation, hormonal activation, apoptosis and angiogenesis [6]. Growing evidence also indicates that Sp1 has important mediating roles in pro-oncogenic factors in cell proliferation, survival, invasion and migration in tumorigenesis. Therefore, tumor cells can be effectively treated by targeting miRNA-SP1 [7, 8]. High Sp1 expression is associated with poor prognosis in many cancers, including cancers of the thyroid, pancreas, skin, stomach, breast and nasopharynx [9–14]. However, Sp1 expression in astrocytomas has not been well under discussion. Hence, the aim of this study was to evaluate Sp1 expression in astrocytoma cell lines and tissue samples at the protein level by Western blot and immunohistochemical (IHC) analysis. This study also investigated whether Sp1 and isocitrate dehydrogenase (IDH) 1 proteins expression correlates with clinicopathologic parameters of astrocytoma.

Materials and Methods

Specimen and Pathologic Evaluation

This study is performed on unlinked specimen without patients' personal identification and the study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-20140169). Ninety-eight patients who had received surgical intervention for malignant astrocytomas at Kaohsiung Medical University Hospital during 2001–2012 were enrolled. Clinical data were collected by reviewing the medical records of individual cases. All specimens analyzed in this study had been obtained by surgical resection and had been processed according to standard pathologic procedures. In all cases, slides of astrocytoma specimens stained with hematoxylin and eosin were reviewed to confirm the definitive diagnosis and pathologic characteristics, including tumor grade [15].

IHC Staining

In each case, tissue blocks fixed in formalin and embedded in paraffin were cut into sections 3 μm thick. The sections were deparaffinized, rehydrated and autoclaved at 121 °C for 10 min in Target Retrieval solution, pH 6.0 (DAKO; S2369) to retrieve antigens. After 20 min at room temperature, 3% hydrogen peroxide was used to block endogenous peroxidase for 5 min. The sections were washed twice with Tris buffer then incubated with a 1:200 dilution of Sp1 (Thermo, Chicago, USA) and a 1:1000 dilution of IDH-1 (Thermo, Chicago, USA) for 1 h at room temperature. Next, the sections were washed twice with Tris buffer and incubated with secondary antibody conjugated with horseradish peroxidase for 30 min at room temperature. Finally, the slides were incubated in 3,3-diaminobenzidine (Dako; K5007) for 5 min, counterstained with Mayer's hematoxylin for 90 s, and mounted with Malinol. The tumor cells were then scored by percentage and intensity of SP1 IHC staining. The breast cancer tissue was used as positive control. The percentage of tumor cells with positive staining was graded as 0 (none), 1 (1–9%), 2 (10–50%), and 3 (>50%). Staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The staining index (SI) was calculated by multiplying intensity and percentage of positive tumor cells, which obtained scores of 0, 1, 2, 3, 4, 6 and 9. The cutoff was a total score of 4. High expression of SP1 was defined as a score of ≥4, and low expression was defined as a score of ≤3. [16] The tumor cells were scored by intensity of IDH-1 IHC study. Staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). High expression of IDH-1 was defined as a score of 3 [17].

Cell Culture

All cells lines were obtained from the ATCC Cell Line Bank and incubated with 5% CO_2 at 37 °C. The GBM8401 cell line was cultured with RPMI medium supplemented with 10% FBS. The U87MG and SVG cell lines were cultured with MEM medium containing 10% FBS. The G5T cell line was cultured with DMEM medium containing 10% FBS. The GBM8401, U87MG, and G5T cell lines were isolated from

GBM patients, and the SVG cell line was isolated from normal brain tissue as normal control.

Transfection

The Sp1 siRNA in glioma cells was extracted by using DharmaFECT Transfection Reagents (Dharmacon™). The sequence for human Sp1 siRNA#1 was 5'-CUACUACUACCACCAGCAAdTdT-3', and the sequence for 2 (Sigma) was 5'-UUGCUGGUGGUAGUAGUAGdTdT-3'. Transfection was performed using 5 μM Sp1 siRNA. After transfection with siRNA, cells were cultured for 3 days before use. Expression of Sp1 was detected by Western blot analysis.

Western Blot

After lysis of samples in 200 μl lysis buffer, 50 μg samples were loaded into the wells of an SDS-PAGE system at 50 V for 4 h. The proteins were then transferred from the gel to the PVDF membrane. After incubation with blocking buffer for 1 h, the membranes were incubated with primary antibody [Sp1 (1:200; Thermo; PA5–29165), Ki-67 (1:1000; Dako; M7240), cleaved caspase-3 (1:500; Cell signaling; #9664), E-cadherin (1:1000; SANTA CRUZ; sc-8426), and β-actin (1:20000; SIGMA; A5441), Bax (1:500; Cell signaling; #2772), cyclin D1 (1:1000; Thermo; RM-9104)] for 2 h at room temperature and then with secondary antibody [Goat anti-Rabbit (1:5000; Millipore; AP132P) and Goat anti-Mouse (1:5000; Millipore; AP124P)] for 90 mins. Enhanced chemiluminescense solution (Western Lightning; 205–14,621) was used to detect specific bands in a MINICHEMI (Thermo) system. Each western blot was repeated three times.

Cell Viability

The GBM8401 cell line was obtained by dissolving the cells in RPMI culture medium containing 10% serum. The cells were then transferred to six-well plates (1×10^6 cells per 2 ml RPMI per well) and incubated in 5% CO_2 at saturated humidity and 37 °C for 24 h. After 72 h co-culture with 5 μM Sp1 siRNA, the cells were counted.

Invasion Assay in vitro

Cell invasion assay was performed *in vitro* using a Transwell (CORNING; COR3452) invasion assay kit. Cells were seeded at 5×10^5 cells per insert, and the lower chamber of the Transwell was filled with 2 ml medium with nonsense siRNA (si-non) or Sp1 siRNA. After 24 h incubation, cells remaining on the upper surface of the Transwell membrane were removed with a cotton swab. Cells that had invaded through the Transwell to the bottom of the insert were fixed, stained, photographed and quantified by counting them in 6 randomly selected high-power fields.

Migration Assay in vitro

Cell invasion was analyzed by wound healing assay (ibidi; 80,209) performed in 6-well plates coated with culture-inserts (Indio: LOT150831) and then cultured at 37 °C for 12 h. The cells were then seeded at 1×10^5 per well, and siRNA was added after 24 h. After another 24 h, cells were washed twice with PBS and photographed.

Data Analysis

The SPSS 19.0 (Chicago, IL, USA) software was used for statistical analysis. Chi-square test was used to determine whether Sp1 protein expression correlated with specific clinicopathologic parameters. The survival rate was analyzed by Kaplan-Meier method with log-rank test. Multivariate Cox regression analyses were used to verify the independent effect of each variable analyzed in this study. A p value less than 0.05 was considered statistically significant.

Result

Clinicopathologic Parameters of the Study Group

Of the 98 astrocytoma patients analyzed in this study, 23 were aged >60 years, and 75 were aged ≤60 years (mean age, 49.7 years). Thirty-six patients had low grade (grade II) astrocytoma, and 62 had high grade (grades III-IV) astrocytoma according to the WHO classification system.

Correlation Between Sp1 and IDH-1 Expression and Clinicopathologic Parameters

Table 1 presents Sp1 and IDH-1 expression in relation to characteristics of the astrocytoma patients. Figure 1 compares nuclear IHC staining results between high and low expressions of Sp1 and IDH-1. Expressions of Sp1 showed no significant correlations with age ($p = 0.945$), gender ($p = 0.409$), Karnofsky Performance Scale (KPS) ($p = 0.103$), or tumor size ($p = 0.235$). However, Sp1 expression was significantly associated with WHO grade ($p = 0.005$). That is, high Sp1 expression was significantly associated with high grade astrocytoma in the WHO classification system.

There is no significant correlations with age ($p = 0.154$), gender ($p = 0.159$), tumor size ($p = 0.681$), or KPS ($p = 0.279$) but IDH-1 expression was significantly associated with WHO grade ($p = 0.003$). That is, high IDH1 expression was significantly associated with low grade astrocytoma in the WHO classification system.

Table 1 Expression of Sp1 and IDH-1 correlated with clinicopathologic parameters in astrocytomas

	No.	Sp1 expression (n, %)		*p*-value	IDH-1 expression (n, %)		*p*-value
		Low	High		Low	High	
Age				0.945			0.154
≦60	75	32 (32.7%)	43 (43.9%)		35 (35.7%)	40 (40.8%)	
>60	23	10 (10.2%)	13 (13.3%)		15 (15.3%)	8 (8.2%)	
Gender				0.409			0.159
Male	56	26 (26.5%)	30 (30.6%)		25 (25.5%)	31 (31.6%)	
Female	42	16 (16.3%)	26 (26.5%)		25 (25.5%)	17 (17.3%)	
Grade				0.005*			0.003
II	36	22 (22.4%)	14 (14.3%)		11 (11.2%)	25 (25.5%)	
III/ IV	62	20 (20.4%)	42 (42.9%)		39 (39.8%)	23 (23.5%)	
Size				0.235			0.681
≦3 cm	58	22 (22.4%)	36 (36.7%)		31 (31.6%)	27 (27.6%)	
>3 cm	40	20 (20.4%)	20 (20.4%)		19 (19.4%)	21 (21.4%)	
KPS				0.103			0.279
≦70	67	25 (25.5%)	42 (42.9%)		37 (37.8%)	30 (30.6%)	
>70	31	17 (17.3%)	14 (14.3%)		13 (13.3%)	18 (18.4%)	

*Statistically significant ($p < 0.05$)

Survival Analysis

After log-rank test, factors associated with Sp1 expression in the astrocytoma cases were analyzed by Kaplan-Meier analysis. High Sp1 expression was significantly associated with low overall survival ($p < 0.001$) (Fig. 2). Mean survival time was significantly longer in patients with low Sp1 expression (33.61 months, 95% confidence interval: 27.07–40.16 months) compared to those with high Sp1 expression (15.45 months, 95% confidence interval: 11.44–19.46 months). However, Table 2 shows that, according to multivariate analysis after Cox regression, the survival time was significantly associated with Sp1 (HR, 0.594; 95% confidence interval, 0.360–0.981; $p = 0.042$) and IDH-1 expression (HR, 7.732; 95% CI, 3.559–16.798; $p = <0.001$).

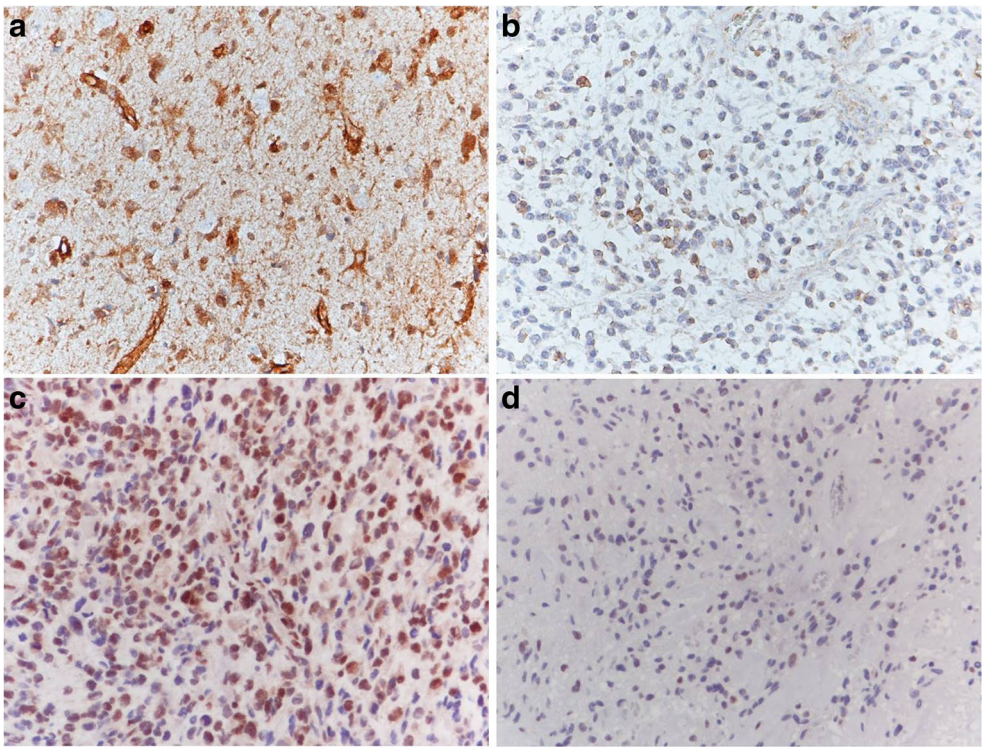

Fig. 1 Representative immunohistochemical staining results for IDH-1 and Sp1 in astrocytoma samples from patients with different staining scores. **a** Astrocytoma with high IDH-1 expression (score: 2–3). **b** Astrocytoma with low IDH-1 expression (score: 0–1). (100X) (**c**) Astrocytoma with high Sp1 expression (score: 4–9). **d** Astrocytoma with low Sp1 expression (score: 1–3). (100X)

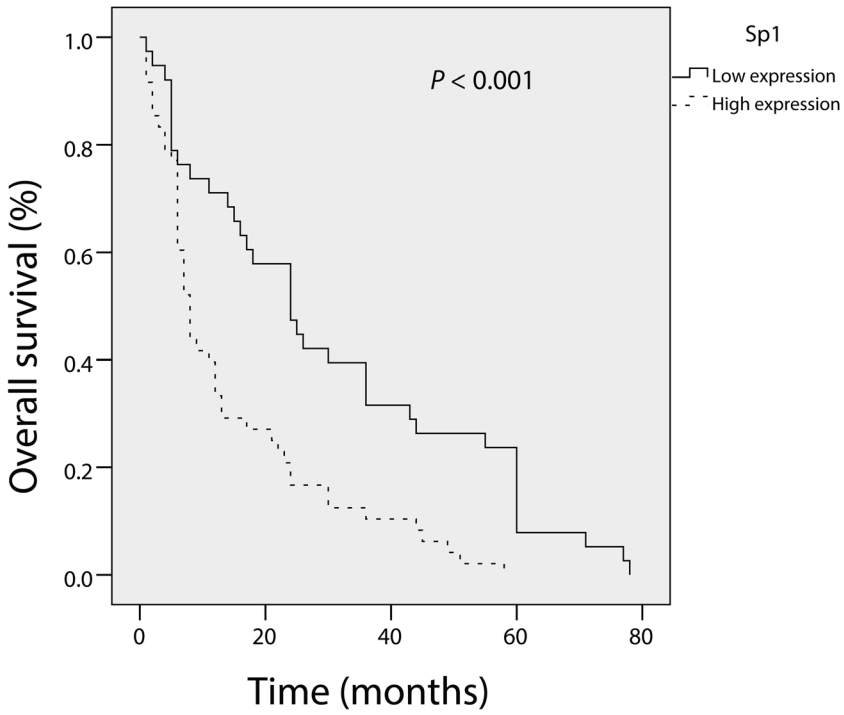

Fig. 2 Analysis of Sp1 expression by Kaplan-Meier method with log-rank test

Upregulation of Sp1 Protein Expression in GBM Cell Lines

Expression of Sp1 protein was investigated by Western blot analysis in the normal glial cell line SVG and in GBM cell lines GBM8401, U87MG, and G5T. The Western blot results showed that expression of Sp1 protein was significantly higher in GBM8401 ($p < 0.0001$), U87MG ($p = 0.019$) and G5T ($p = 0.041$) compared to SVG. Figure 3 shows that, of all cell lines, Sp1 expression was highest in GBM8401. Therefore, GBM8401 was further treated with siRNA of Sp1. After 72 h incubation with Sp1 siRNA (si-Sp1 group) or nonsense siRNA (si-non group), expression of Sp1 protein was compared between the si-Sp1 group and the si-non group (control group). The knockdown Sp1 down-regulated Sp1 protein expression by approximately 62.56%. Figure 4 shows that Sp1 protein expression was significantly lower in the si-Sp1 group compared to the control group ($p = 0.002$) group and compared to the si-non group ($p = 0.001$) .

Silencing Sp1 Inhibited GBM8401 Proliferation

To determine the regulatory effects of Sp1 protein on GBM proliferation, cell numbers were compared between GBM8401 incubated with Sp1 siRNA and GBM8401 with nonsense siRNA. Figure 5 shows that, after 72 h incubation with siRNA, assays of cell viability by trypan blue showed significantly lower Sp1 viability in the siRNA group compared to the control group ($p < 0.001$) and the si-non group ($p < 0.001$). However, the control group and si-non group showed no significant difference. These data implied that the Sp1 knockdown decreased the viability of GBM cells (Fig. 5).

Table 2 Multivariate analysis of prognostic indicators of astrocytoma in Cox regression model

	Univariate analysis			Multivariate analysis		
	Relative risk	95% CI	*P*	Relative risk	95% CI	*P*
Age	0.385	0.226–0.655	<0.001*	0.505	0.292-0.874	0.015*
Gender	0.856	0.555-1.322	0.484			
WHO grade	0.342	0.212–0.550	<0.001*	0.399	0.232-0.685	0.001*
Tumor size	1.072	0.691–1.665	0.755			
KPS	1.713	0.086–2.703	0.21			
Sp1 expression	0.415	0.258–0.669	<0.00*1	0.575	0.343-0.964	0.036*
IDH-1 expression	3.960	2.459–6.378	<0.001*	4.274	2.512-7.272	<0.001*

* Statistically significant ($p < 0.05$)

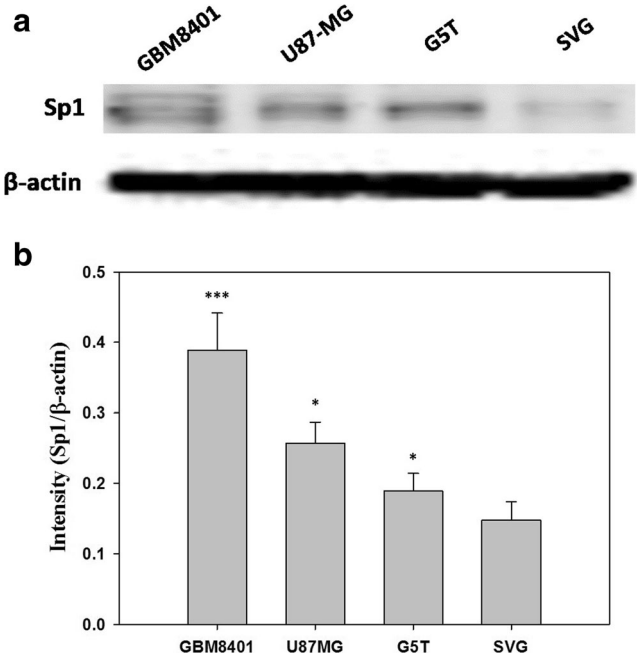

Fig. 3 **a** Western blot results for Sp1 in all cell lines. **b** Relative expression of Sp1 in all cell lines. (*$p < 0.05$, ***$p < 0.001$ in comparison with SVG)

Silencing Sp1 Inhibited Invasion and Migration in GBM Cells

Invasion and migration of GBM cells were studied by Matrigel invasion assay *in vitro*. A knock-down of Sp1 expression with specific Sp1 siRNA significantly inhibited both the invasion (Fig. 6; $p < 0.001$) and migration (Fig. 7; $p < 0.001$) of GBM8401 cells. These data indicate the important role of Sp1 in the invasion and migration of GBM cells.

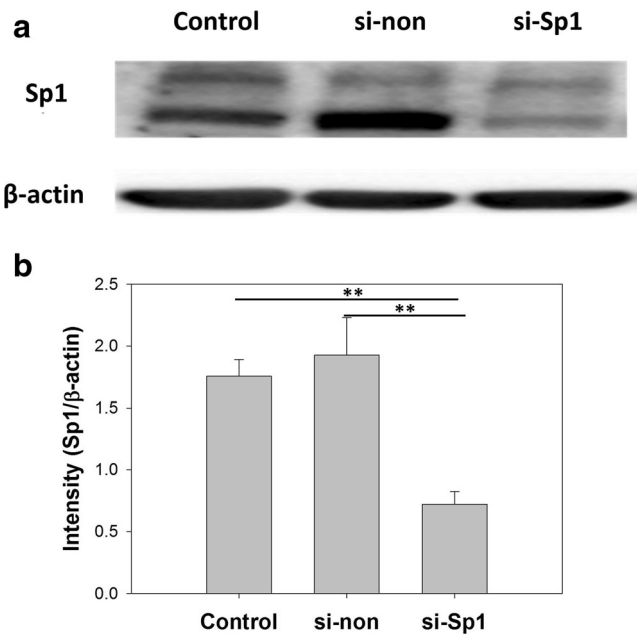

Fig. 4 Comparison of Sp1 expression between non-siRNA and Sp1 siRNA. **a** Western blot results for Sp1 in all cell lines. **b** Relative expression of Sp1 in all cell lines. (**$p < 0.01$)

Effects of Sp1 Silencing on Expression of E-cadherin, Ki-67, and Cyclin D1

To identify the effects of representative protein expressions on cell proliferation and migration, E-cadherin, Ki-67 and cyclin D1 were analyzed by Western blot. Both Ki-67 and cyclin D1 are markers of cell proliferation. E-cadherin is an epithelial-mesenchymal transformation marker. Significant effects of knock-down Sp1 expression included downregulation of Ki-67 ($p = 0.008$), downregulation of cyclin D1 ($p < 0.001$), and upregulation of E-cadherin ($p = 0.002$). (Fig. 8).

Silencing Sp1 Enhanced Apoptosis of GBM Cells

Apoptosis of GBM cells was studied by Western blot analysis of apoptosis-related proteins. Expressions of cleaved caspase-3 and Bax protein had pro-apoptotic effects. Knock-down Sp1 significantly upregulated expressions of cleaved caspase-3 ($p = 0.041$) and Bax ($p = 0.002$) (Fig. 9). These data indicated that silencing Sp1 induced apoptosis of GBM8401 cells.

Discussion

Glioblastoma is the most aggressive malignant glial tumor in adults. Despite improved surgical, chemotherapeutic, radiotherapeutic, and targeted drug treatments for GBM, median survival time remains poor. Glioblastoma is highly refractory to therapy due to its rapid growth and potential for local invasion. Therefore, a clear understanding of the pathogenesis of GBM and useful markers are needed for effective treatment of GBM.

Low and high grade astrocytomas with mutated IDH-1, which can be detected by IHC study, have been established to improve prognosis compared to ones with IDH-1 murarion [18, 19]. In our study, IDH-1 expression was associated with low tumor grade ($p = 0.003$) and significantly associated with the prognosis of astrocytoma patients ($p < 0.001$). The result is line with previous studies [20].

The Sp transcription factors are members of the Sp/KLF family associated with differentiation, embryonic and early postnatal development, and cell cycle regulation in multiple diseases. The Sp/KLF transcription factors activate target genes by interacting with their cognate cis-elements and multiple nuclear proteins. The Sp1 is a well-known DNA-binding protein that triggers transcriptional machinery for regulating cellular and viral expressions of genes that contain GC boxes in their promoters [8]. Knockout mouse models of Sp1 have demonstrated their critical role in early development of GC [21–23]. The roles of Sp1 in oncogenesis include cell

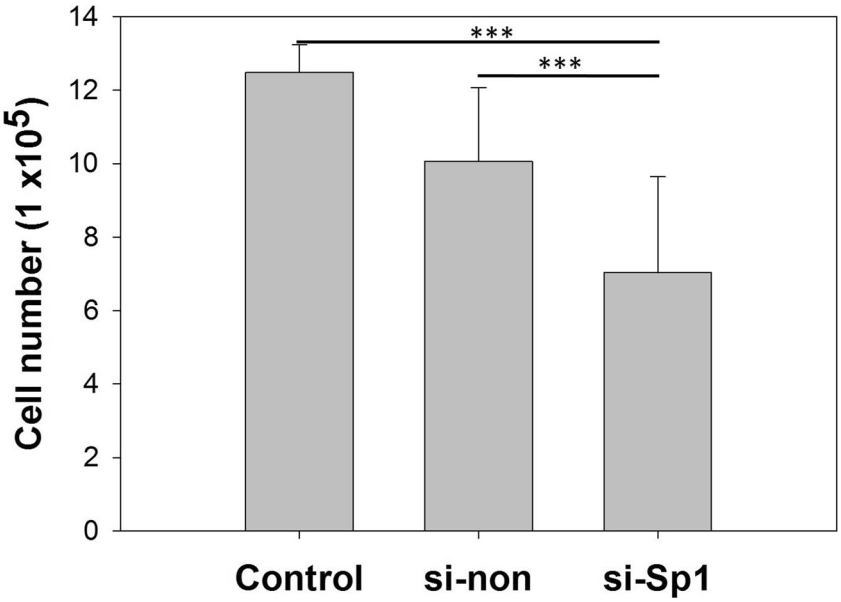

Fig. 5 Growth curve for GBM8401 cells cultured in 6-well plates for 3 days after transfection with Sp1 or with nonsense siRNA. (***$p < 0.001$)

proliferation and resistance to apoptosis, cell invasion and angiogenesis. The Sp1 protein is overexpressed in several cancer types and is reportedly an independent predictor of survival in patients with these tumors [9–14]. The chi-square analysis in our study showed that high Sp1 expression was significantly associated with advanced WHO grade ($p = 0.005$), which is in line with previous studies [16, 24]. Kaplan-Meier analysis of the astrocytoma patients revealed significantly longer survival time in those with low Sp1 expression compared to those with high Sp1 expression ($p < 0.001$). Multivariate analysis further showed that Sp1 protein expression was significantly associated with the prognosis of astrocytoma patients ($p = 0.036$). That is, Sp1 is an independent biomarker for the prognosis of astrocytoma.

Studies suggest that the roles of Sp1 in tumor development and progression are regulated by genetic and epigenetic pathways [25, 26]. For example, the Sp1 gene can self-regulate because Sp1 protein regulates its own promoter [27]. However, Sp1 can also be regulated by interaction with other proteins such as prominent oncogenes and tumor suppressors.

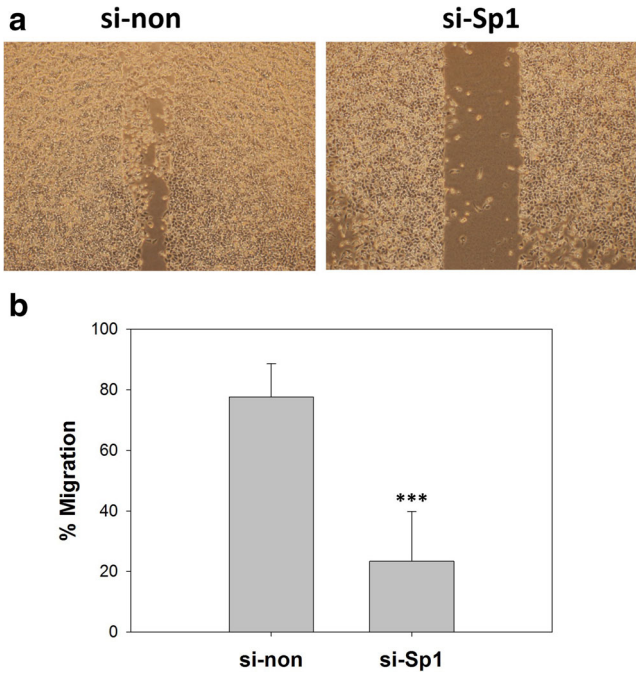

Fig. 6 Wound healing assay of GBM8401 cells 1 day after transfection with Sp1 siRNA. **a** Wound healing assay. **b** Percentage of migrated cells. (***$p < 0.001$)

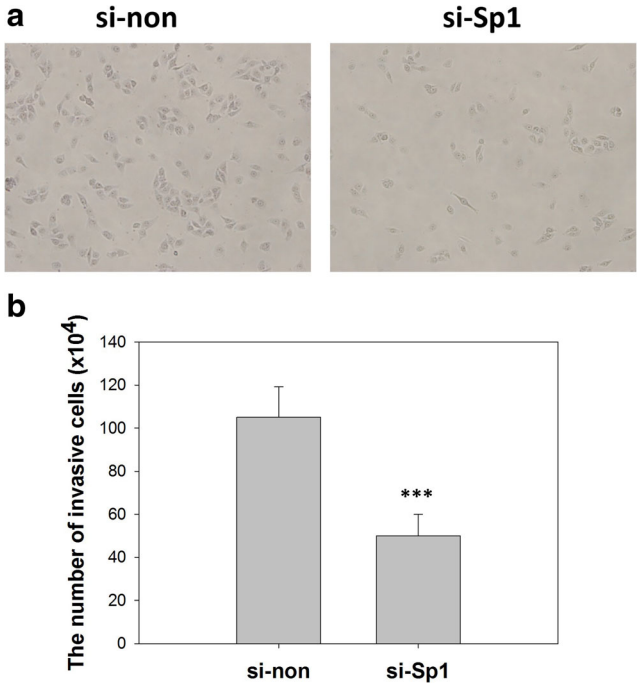

Fig. 7 Transwell invasion assay of GBM8401 cells 1 day after transfection with Sp1 siRNA. **a** Invasion assay. **b** Number of invaded cells. (***$p < 0.001$)

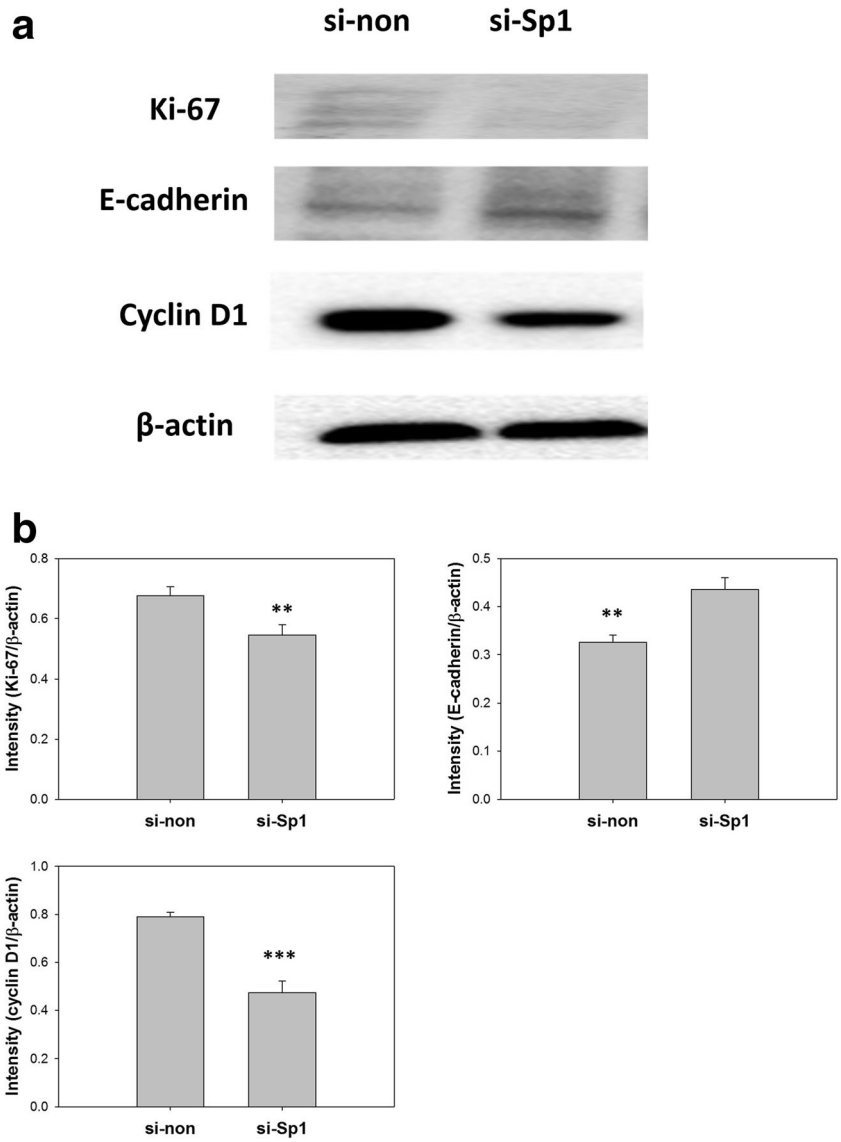

Fig. 8 Expressions of Ki-67, E-cadherin, and cyclin D1 proteins after treatment with non-siRNA and Sp1 siRNA in GBM cells. **a** Western blot results for Ki-67, E-cadherin, and cyclin D1. **b** Comparison of expressions of Ki-67, E-cadherin, and cyclin D1. (**$p < 0.01$, ***$p < 0.001$)

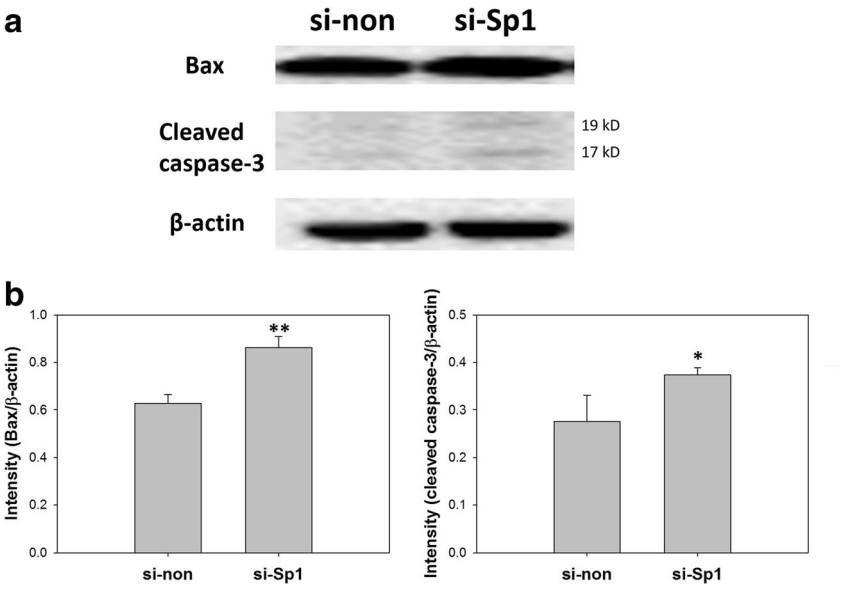

Fig. 9 Comparison of expressions of cleaved caspase-3 and Bax proteins between non-siRNA and Sp1 siRNA in GBM cells. **a** Western blot results for cleaved caspase-3 and Bax. **b** Comparison of expressions of cleaved caspase-3 and Bax. (*$p < 0.05$, **$p < 0.01$)

Binding of mouse double minute 2 to the C-terminal domain of Sp1 suppresses the DNA-binding capability of Sp1 [28]. However, pRb abolishes this suppressive effect by releasing Sp1 from the MDM2-Sp1 complex, which restores its DNA binding capability. The DNA-binding activity and transactivation potential of Sp1 are regulated through several post-translational modifications, including acetylation, ubiquitylation, sumoylation, phosphorylation, glycosylation and poly(ADP-ribosyl)ation [29]. Therefore, increased gene expression or posttranslational modification can over-activate Sp1, which can then cause tumorigenesis.

Our *in vitro* study of GBM cells showed that Sp1-siRNA inhibits upregulation of Sp1 caused by tumor invasion and proliferation. Other studies have also shown that Sp1 induced by Bcl-w affects GBM invasion and migration by activating MMP-2 [24]. Direct targeting Sp1 by Mir-128 and Mir-377 can alleviate the cell cycle, proliferation and invasion [30, 31]. Sp1 can also up-regulate Midkine expression directly and promote proliferation of glioma cells [32]. Additionally, many cancers are associated with high expressions of cyclin D1 and Ki-67, which have important roles in cell cycle regulation [33–37]. Our experiments showed that downregulation of Ki-67 and cyclin D1 was significantly higher in the si-Sp1 group compared to the si-non group. Silencing Sp1 attenuated the growth curve in GBM8401. These data show that Sp1 regulates cell proliferation. In a previous study, Tetra-O-methyl Nordihydroguaiaretic acid, a transcriptional inhibitor of Sp1-dependent genes, had a strong inhibiting effect on cell growth in GBM cell lines [38]. Additonally, high levels of Sp1 protein reportedly correlate with cancer cell migration and invasion in many malignant tumors, including gastric [13], pancreatic [39], and breast cancer [40] tumors. In many neoplasms, E-cadherin is a biomarker for migration, and loss of E-cadherin is often associated with metastasis [37, 41]. Our experiments showed that silencing Sp1 attenuated GBM cell migration and invasion and significantly down-regulated E-cadherin. A previous study reported that Sp1 binds the MMP-2 promoter for constitutive activity of invasion in astroglioma cell lines [42].

Apoptosis is classified as an intrinsic mitochondria-mediated and extrinsic death receptor-induced pathway. In the intrinsic mitochondria-mediated pathway, Bax activates caspase-3 (34kD) cleavage of subunits 17kD and 19kD [43]. The Sp1 regulates many pro- and anti-apoptotic factors, including Bcl-2 [44], XIAP [44, 45], Fas ligand (FasL) [46], BCL-2 antagonist/killer 1 (BAK) [47], TRAIL [48], DR4/5 [49], and FLIP [50]. Tumor cell resistance to extrinsic apoptotic signals is increased when expressions of cell-surface TRAIL receptors DR4/5 and TRAIL-specific inhibitor FLIP decrease [51, 52]. Therefore, Sp1 promotes tumor cell resistance to apoptosis through immune pathways and the escape immune response. Our experiments showed that expression of knock-down Sp1 protein enhanced expressions of Bax and cleaved caspase-3 protein, which suggests that silencing Sp1 induces apoptosis of astrocytoma cells through an intrinsic mitochondria-mediated pathway.

In conclusion, molecular changes in Sp1 are significantly associated with WHO grade and are an independent prognostic marker in astrocytoma. Additionally, knock-down Sp1 inhibited the proliferation, invasion, and migration of astrocytoma cells and induced apoptosis. Therefore, Sp1 is a potential prognostic marker for astrocytoma and may be applicable in novel therapeutic strategies for astrocytoma.

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Compliance with Ethical Standards

Ethical Approval This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital, Taiwan (KMUH-IRB-20140169). The study is performed on unlinked specimen without patients' personal identification and in accordance with the ethical standards laid down in the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest We declare that we have no conflict of interest.

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