SRSF1 induces glioma progression and has a potential diagnostic application in grading primary glioma

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Abstract. Glioma is the most common intracranial tumor of the central nervous system in adults; however, the diagnosis of glioma, and its grading and histological subtyping, is challenging for pathologists. The present study assessed serine and arginine rich splicing factor 1 (SRSF1) expression in 224 glioma cases in the Chinese Glioma Genome Atlas (CGGA) database, and verified its expression by immunohistochemical analysis of specimens from 70 clinical patients. In addition, the prognostic potential of SRSF1 concerning the survival status of patients was evaluated. *In vitro*, the biological role of SRSF1 was assessed using MTT, colony formation, wound healing and Transwell assays. The results revealed that SRSF1 expression was significantly associated with the grading and the histopathological subtype of glioma. As determined using a receiver operating characteristic curve analysis, the specificity of SRSF1 for glioblastoma (GBM) and World Health Organization (WHO) grade 3 astrocytoma was 40 and 48%, respectively, whereas the sensitivity was 100 and 85%. By contrast, pilocytic astrocytoma tumors exhibited negative immunoexpression of SRSF1. Additionally, Kaplan-Meier survival analysis indicated that high SRSF1 expression predicted a worse prognosis for patients with glioma in both the CGGA and clinical cohorts. *In vitro*, the results demonstrated that SRSF1 promoted the proliferation, invasion and migration of U87MG and U251 cells. These data suggested that immunohistochemical analysis of SRSF1 expression is highly sensitive and specific in the diagnosis of GBM and WHO grade 3 astrocytoma, and may have an important role in glioma grading. Furthermore, the lack of SRSF1 is a potential diagnostic biomarker for pilocytic astrocytoma. However, neither in oligodendroglioma and astrocytoma, nor in GBM was an association detected between SRSF1 expression and *IDH1* mutations or *1p/19q* co-deletion. These findings indicated that SRSF1 may serve as a prognostic factor in glioma cases and could have an active role in promoting glioma progression.

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Introduction

Glioma is the most common intracranial tumor of the central nervous system (CNS) in adults, with >300,000 new cases diagnosed worldwide each year, causing ~2.5% of all cancer-related deaths (1). Historically, glioma was considered to originate from the differentiated astrocytic and oligodendrocytic components of the CNS (2). Based on the World Health Organization (WHO) classification of CNS tumors, glioma is divided into primary and secondary glioma, graded as WHO 1-4, and includes astrocytoma, oligodendroglioma, glioblastoma (GBM) and other subtypes (3). Although a series of molecular parameters have been incorporated in the classification, including *IDH1/2* mutation status, *CDKN2A/B* homozygosity, *EGFR* amplification and +7/-10 chromosome alteration (4-6), the grading and histological subtyping of glioma is frequently challenging for pathologists. In addition, due to its aggressive behavior and high recurrence rate, the 5-year survival rate of patients with glioma is currently still low, at <5% (7). Therefore, effective biomarkers for accurate diagnosis and prognostic evaluation, and therapeutic targets are urgently required. Serine and arginine rich splicing factor 1 (SRSF1; also called SF2/ASF) was the first defined alternative splicing protein, which mainly functions in RNA splicing, and contains two N-terminal RNA recognition motifs and a short C-terminal RS domain (8-11). Previous studies have reported that SRSF1 is dysregulated in human cancer. SRSF1 has been shown to promote colon cancer development by activating DBF4B exon 6 splicing (12). Furthermore, SRSF1 can promote mammary epithelial cell transformation by specifically regulating the splicing of key targets downstream of mTOR and/or is functionally linked to MYC (13). SRSF1 can also inhibit autophagy through regulating Bcl-x splicing and interacting with PIK3C3 in lung cancer (14). In addition to regulating target genes, Das *et al* (15) demonstrated that the deubiquitinating enzymes ubiquitin-specific peptidase (USP)15 and USP4 regulate alternative splicing of SRSF1, resulting in isoform-specific functions in lung cancer cells.

The METTL3 stabilized long non-coding RNA SNHG7 has also been reported to accelerate tumor progression via the SRSF1/c-Myc axis in prostate cancer (16). However, the possible functions of SRSF1 in glioma, which may contribute to enhanced malignancy, remain largely unknown.

It has been reported that SRSF1 is highly expressed in GBM and oligodendroglioma (17). However, to the best of our knowledge, whether SRSF1 expression is associated with the histopathological subtype of glioma, WHO grade and glioma-associated molecular characteristics remains unknown. To address these issues, the present study aimed to investigate the expression of SRSF1 in pilocytic astrocytoma, astrocytoma, oligodendroglioma and GBM. The present study also sought to examine SRSF1 expression in different grades of glioma to explore whether immunohistochemistry (IHC) of this marker could be valuable as a surrogate for the distinction of WHO grade. Moreover, SRSF1 immunoreactivity was assessed in *IDH*-mutant and *1p/19q* co-deletion glioma. If the results confirmed the relevance of SRSF1 with molecular characteristics, SRSF1 IHC has the potential to enable pathologists to take advantage of the accessibility and relatively low cost of IHC without the need for molecular testing.

Materials and methods

Data acquisition and specimen collection. The RNA-sequencing data and corresponding clinical data of patients with glioma (n=224) were downloaded from the Chinese Glioma Genome Atlas (CGGA) (http://www.cgga.org.cn). In addition, paraffin-embedded glioma tissues (WHO grade 1-4; n=70) were collected from The Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital; Kunming, China). The collected clinical data included age, sex, tumor sites, grade, *IDH* mutation status, *1p/19q* codeletion status, histological type and overall survival time from CGGA and clinical patients. All 70 patients with glioma were recruited between March 2011 and December 2021, and were followed up from the date of surgery until the date of analysis, with the follow-up time ranging between 3 and 120 months. The study was approved by the Ethics Committee of The Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital; approval no. KYLX2022090) and experiments were undertaken with the understanding and written informed consent of all the patients. The study conformed with The Declaration of Helsinki).

Hematoxylin and eosin (H&E) and IHC. H&E staining was used to observe morphology. After incubation at 60˚C for 3 h, 4-μm paraffin-embedded slices were dewaxed in xylene and then placed successively in high to low concentrations of alcohol to hydrate. Subsequently, the tissues were stained with hematoxylin for 7 min at room temperature and rinsed with water. After differentiation in hydrochloric alcohol, until blue, the slices were stained with eosin for 1 min, dehydrated in 70, 80, 95 and 100% ethanol for 1 min each, followed by 100% ethanol I and 100% ethanol II for 2 min, and then the tissues were cleared with xylene I and II for 8 min each. After air drying, moderate neutral balsam was added quickly. Finally, slices were covered with coverslips and were observed under a light microscope (Leica Microsystems, Inc.).

IHC was performed to detect SRSF1 expression, Ki-67 index and IDH1 R132 expression in paraffin-embedded sections of human WHO grade 1-4 glioma and normal tissues from other benign lesions. After baking at 60˚C for 3 h, the slices were dewaxed in xylene, then hydrated with gradient alcohol and placed in a solution of sodium citrate at pH 6.0, followed by high-pressure heat to repair the antigens. After sealing the non-specific binding site with 10% goat serum (Fuzhou MaixinBiotech Co., Ltd.) for 30 min at room temperature, the slices were incubated with the primary rabbit polyclonal anti-SRSF1 (1:300; Santa Cruz Biotechnology, Inc.), anti-Ki-67 (ready-to-use; Fuzhou Maixin Biotech Co., Ltd.) and anti-IDH1 R132 (ready-to-use; Fuzhou Maixin Biotech Co., Ltd.) antibodies for 60 min and secondary antibody for 30 min. DAB (Fuzhou Maixin Biotech Co., Ltd.) was then added for 5 min. Finally, the samples were restained with hematoxylin for 1 min. Staining was examined under a DM1000 microscope (Leica Microsystems, Inc.) to obtain IHC scores. The brown staining of the cell nuclei was interpreted as positive SRSF1 and Ki-67 staining, and staining of the cell cytoplasm was considered positive IDH1 R132 staining. The immunoreactive score (IRS) was calculated by multiplying the staining intensity by the percentage of positive cells, as previously reported (18). The staining intensity was scored as follows: 0 (negative staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The percentage of cells that were positive was scored as follows: 0 (<5%), 1 (5-30%), 2 (31-50%), 3 (51-75%) and 4 (>75%). Low and high expression of SRSF1 were defined as IRS <6 and IRS ≥6, respectively.

Cell culture and stable transduction. The human U87MG cell line was purchased from Jinyuan Biotechnology Co., Ltd., which was glioblastoma of unknown origin and has been authenticated using STR profiling. The U251 cell line was kindly provided by Mr. Qian Yao (Yunnan Cancer Hospital). All cells were cultured in DMEM supplemented with 10% FBS (both from Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified incubator containing 5% CO_2. The negative control overexpression (LV-vector), SRSF1 overexpression (LV-SRSF1), short hairpin (sh)RNA negative control (sh-NC) and shRNA targeting SRSF1 (sh1-SRSF1, sh2-SRSF1 and sh3-SRSF1) lentiviruses were synthesized by Beijing Qingke Biotechnology Co., Ltd. The overexpression and knockdown lentiviruses were constructed using the pCDH-CMV-MCS-EF1-copGFP-T2A-Puro and pLVX-ShRNA2-Puro lentiviral vectors, respectively, and were packaged in 293T cells (puromycin resistance) by Qingke Biotechnology Co., Ltd. The 2nd generation system was used and transfected with 2.5 μg lentiviral plasmid. Lentiviral particles were collected and U87MG and U251 cells were subsequently transduced with the LV-vector, LV-SRSF1, sh-NC, sh1-SRSF1, sh2-SRSF1 and sh3 SRSF1 lentiviruses for 48 h at a multiplicity of infection of 20. After transfection for 48 h, the stably transduced cells were selected with 2.5 μg/ml puromycin for 5 days. A total of 0.5 μg/ml puromycin was used for maintenance. The cells were then collected and used for the subsequent experiments.

MTT assay. U87 and U251 cells were cultured in 96-well plates at a density of $2x10^3$ cells/well in 150 μl complete medium and incubated for 0, 1, 3 and 5 days. Subsequently,

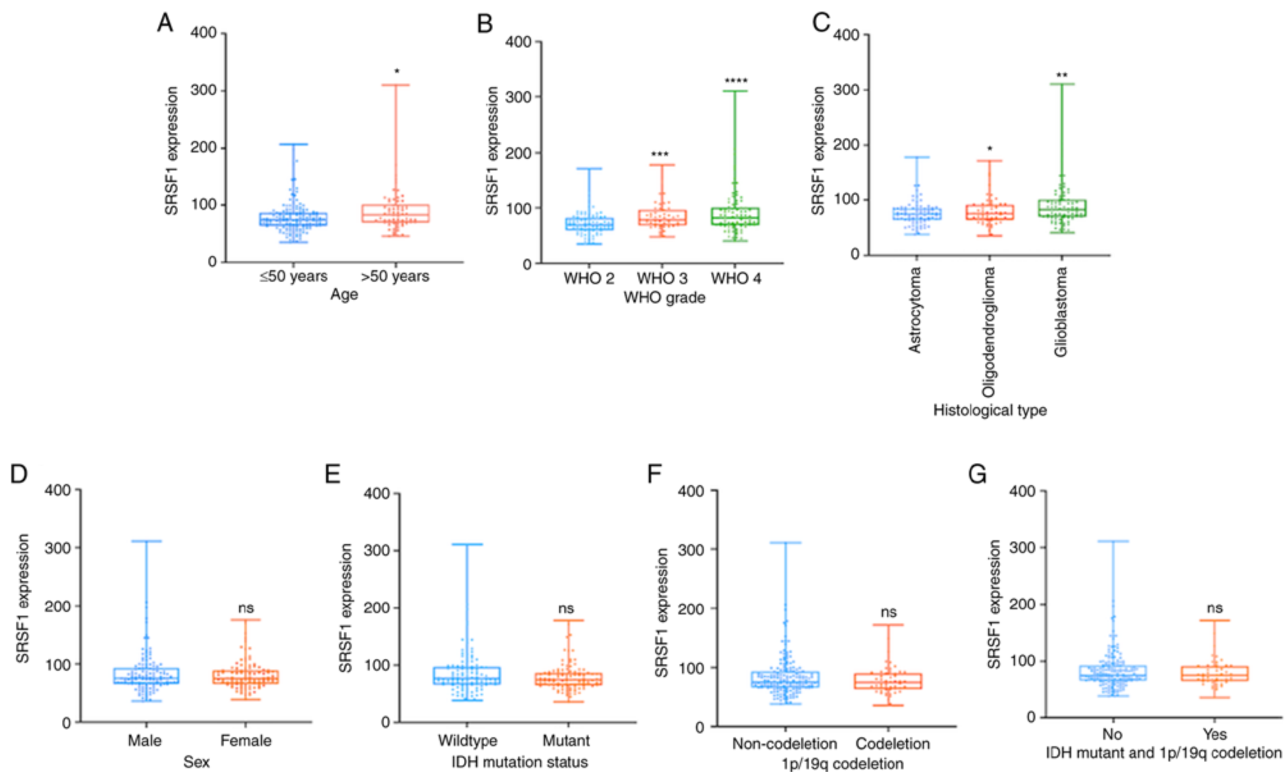

Figure 1. Association between SRSF1 mRNA expression and clinicopathological features in the Chinese Glioma Genome Atlas dataset. (A) SRSF1 expression was increased in older patients. (B) SRSF1 expression increased with WHO grade. (C) Statistical association between SRSF1 expression and histological types. No association was detected between SRSF1 expression and (D) sex, (E) IDH mutation status, (F) 1p/19q co-deletion status, and (G) both IDH mutation and 1p/19q co-deletion status. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. SRSF1, serine and arginine rich splicing factor 1; WHO, World Health Organization.

20 µl MTT reagent (5 mg/ml; BestBio) was added to each well for 4 h at 37˚C, then dissolved in 150 µl DMSO with agitation for 10 min at room temperature. Absorbance was measured at 490 nm.

Colony formation assay. SRSF1 overexpression and knockdown cells were seeded in 6-well plates at 0.5×10^3 cells/well and cultured for 2 weeks. Colonies were fixed with 4% paraformaldehyde (Fuzhou Maixin Biotech Co., Ltd.) at room temperature and stained with 2 ml 0.1% crystal violet (MilliporeSigma) at room temperature for ≥2 h and images were captured. A colony was defined as a group of >50 cells. The number of colonies was first counted manually under a light microscope and then reconfirmed using ImageJ analysis software (National Institutes of Health).

Wound healing assay. Transduced cells (8×10^4/well) were seeded in a 6-well plate and scratched using a sterile pipette tip once confluence reached >90%. The cell culture was replaced in serum-free medium at room temperature. Cells were observed under a light microscope (Olympus Corporation) at 0, 24 and 48 h, with images captured under a light microscope (Olympus Corporation) at 0 and 48 h after scratching and quantified using ImageJ analysis software (National Institutes of Health).

Transwell assay. Transduced U87 and U251 cells were seeded in at 5×10^4 cells/well in 200 µl serum-free medium into the upper chamber (Corning Inc.) of a Transwell plate coated with Matrigel (MilliporeSigma) on ice at 37˚C for 2 h, whereas 800 µl medium containing 20% FBS was added to the bottom chamber. After 48 h of incubation at 37˚C, the invasive cells were stained with 1 ml 0.1% crystal violet for 4 h at room temperature. The images were captured under a light microscope (Olympus Corporation) for data analysis.

Western blot analysis. U87 and U251 cells were collected by centrifugation at 800 x g for 10 min at 4˚C, and lysed in RIPA buffer (Beijing Solarbio Science & Technology Co., Ltd.) at 4˚C for 30 min. Samples were centrifugation at 12,000 x g for 5 min at 4˚C and the supernatants were collected. Total protein was extracted from transduced U87 and U251 cells and detected using the BCA protein assay (BestBio). Proteins (30 µg) were separated by SDS-PAGE on 10% gels and were transferred onto PVDF membranes. After blocking with 5% non-fat milk for 1 h at room temperature, the membranes were incubated with rabbit anti-SRSF1 (1:1,000; cat. no. SC33652; Santa Cruz Biotechnology, Inc.) overnight at 4˚C followed by incubation with anti-Rabbit IgG-HRP (1:10,000; cat. no. SA00001-2; ProteinTech) for 1 h at room temperature. Rabbit anti-β-tubulin (1:1,000; cat. no. SC5274; Santa Cruz Biotechnology, Inc.) was used as the loading control. The protein bands were visualized using ECL (cat. no. WBKLS0100; MilliporeSigma). The bands in western blot images were semi-quantified using ImageJ analysis software (version 1.8.0; National Institutes of Health).

Statistical analysis. All experiments were repeated three times and the data are presented as the mean ± SD. Data were analyzed using SPSS 26.0 software (IBM Corp.). Unpaired

Table I. Association between SRSF1 expression and the clinicopathological characteristics of patients with glioma in the Chinese Glioma Genome Atlas cohort.

	SRSF1 expression, n (%)				
Variable	Low (≤82.7)	High (>82.7)	Total	χ^2	P-value
Age, years				5.190	0.022[a]
≤50	105 (75.5)	52 (61.2)	157		
>50	34 (24.5)	33 (38.8)	67		
Sex				0.606	0.436
Male	50 (36.0)	35 (41.2)	85		
Female	89 (64.0)	50 (58.8)	139		
Histological type				8.004	0.018[a]
Astrocytoma	59 (42.4)	24 (28.2)	83		
Oligodendroglioma	37 (26.6)	19 (22.4)	56		
Glioblastoma	43 (31.0)	42 (49.4)	85		
WHO grade				15.88	<0.001[b]
WHO 2	70 (50.4)	20 (23.5)	90		
WHO 3	26 (18.7)	23 (27.1)	49		
WHO 4	43 (30.9)	42 (49.4)	85		
IDH				1.989	0.158
Mutant	74 (53.2)	37 (43.5)	111		
Wildtype	65 (46.8)	48 (56.5)	113		
1p/19q				0.512	0.474
Codeletion	37 (26.6)	19 (22.4)	56		
Non-codeletion	102 (73.4)	66 (77.6)	168		
IDH mutant and 1p/19q codeletion				2.382	0.123
Yes	37 (26.6)	15 (17.6)	52		
No	102 (73.4)	70 (82.4)	172		

[a]P<0.05, [c]P<0.001. SRSF1, serine and arginine rich splicing factor 1; WHO, World Health Organization.

Student's t-test was used to compare the means of two groups of data. The associations between SRSF1 expression and pathological characteristics were analyzed by the χ^2 test and Fisher's exact test. IHC scores were compared between two groups using the Mann-Whitney U test, and among multiple groups using the Kruskal-Wallis and Dunn's test. The statistical significance among three or more groups was analyzed by one-way analysis of variance, and the LSD (equal variances) or Tamhane's T2 (unequal variances) test were used for post-hoc pairwise comparisons after the homogeneity of variance test. Spearman correlation analysis was used to detect the correlation between two variables. Receiver operating characteristic (ROC) curves were used to estimate the sensitivity and specificity of SRSF1 for glioma grading, and the area under the curve (AUC) was calculated. Survival analysis was analyzed by the Kaplan-Meier method, with the log-rank test applied for comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Association between SRSF1 and clinicopathological characteristics in the CGGA database. To preliminarily probe the relationship between the expression of SRSF1 and primary glioma, data (mRNAseq_325) from the CGGA database were assessed. The clinicopathological and molecular features are summarized in Table I. A total of 224 primary glioma cases were analyzed, including 83 astrocytoma, 56 oligodendroglioma and 85 GBM cases. Significant differences between SRSF1 expression (by PCR) and age were determined (Fig. 1A); however, no statistically significant association between SRSF1 expression and sex was observed (Fig. 1B). Notably, the expression levels of SRSF1 were highly associated with WHO grade. A total of 70/90 WHO 2 glioma cases (77.8%) showed low expression of SRSF1, and the remaining 20/90 cases (22.2%) showed high expression of SRSF1, which indicated that decreased expression of SRSF1 was more frequent in WHO 2 glioma. The percentage of patients with high SRSF1 level was higher in GBM (49.4%) than that in WHO grade 3 (27.1%) and 2 (23.5%) glioma (Fig. 1C), as verified in clinical specimens in Table II. In terms of histological subtype, SRSF1 was diffusely strongly expressed in GBM cases (49.4%), astrocytoma cases (28.2%) and oligodendroglioma cases (22.4%), which indicated that the immunopositivity of SRSF1 was associated with GBM (Fig. 1D). Additionally, the present study further investigated the association between SRSF1 expression and molecular

Table II. Association between SRSF1 expression and clinicopathological characteristics of the 70 patients with glioma.

	SRSF1 IRS, n (%)				
Variable	Low	High	Total	χ^2	P-value
Age, years				8.750	0.003[a]
≤50	12 (40.0)	30 (75.0)	42		
>50	18 (60.0)	10 (25.0)	28		
Sex				0.491	0.484
Male	19 (63.3)	22 (55.0)	41		
Female	11 (36.7)	18 (45.0)	29		
Predominant side				2.619	0.269
Left	12 (40.0)	23 (57.5)	35		
Right	13 (43.3)	14 (35.0)	27		
Middle	5 (16.7)	3 (7.5)	8		
Predominant site				5.417	0.168
Frontal lobe	16 (53.3)	23 (57.5)	39		
Temporal lobe	4 (13.3)	9 (22.5)	13		
Parietal lobe	2 (6.7)	4 (10.0)	6		
Others	8 (26.7)	4 (10.0)	12		
WHO grade				41.864	<0.001[b]
WHO 1	9 (30.0)	1 (2.5)	10		
WHO 2	18 (60.0)	2 (5.0)	20		
WHO 3	3 (10.0)	17 (42.5)	20		
WHO 4	0 (0.0)	20 (50.0)	20		
Ki-67 index				24.083	<0.001[b]
≤10%	26 (86.7)	11 (27.5)	37		
>10%	4 (13.3)	29 (72.5)	33		
IDH1 R132				0.049	0.824
Mutant	21 (70.0)	27 (67.5)	48		
Wildtype	9 (30.0)	13 (32.5)	22		

[a]P<0.01, [b]P<0.001. IRS, immunoreactive score; SRSF1, serine and arginine rich splicing factor 1; WHO, World Health Organization.

features in the CGGA database, which showed that neither 1*p*/19*q* co-deletion or *IDH* mutations were associated with SRSF1 expression (Fig. 1E and F). The clinicopathological and molecular features are summarized in Table I.

Association between SRSF1 and clinicopathological characteristics in clinical cases. Consistent with the before mentioned findings, the present study further analyzed the relationship between the immunohistochemical expression of SRSF1 and the clinical features of 70 primary glioma specimens (Table II). The cohort included 41 men and 29 women with a median age of 45 years (range, 2-81 years). High SRSF1 expression was more frequently observed in younger patients (75%), and low expression was more frequent in older patients (60%) (Fig. 2C). Regarding the predominant side, 50% of the cases were on the left, 39% were on the right, and the remaining cases were in the middle. The tumors were mainly located in the frontal lobe in 39/70 cases (56%), in the temporal lobe in 13/70 cases (19%) and the fewest cases were in the parietal lobe. Notably, there were no significant associations between SRSF1 expression and the following clinicopathological variables: Sex, predominant side and site. Furthermore, the present study revealed that SRSF1 exhibited diffusely strong immunoreactivity in WHO grade 3 and 4 glioma (HGG) samples compared with that in WHO grade 1 and 2 glioma (LGG) and normal tissue samples (Fig. 2A). Among the 30 WHO 1 and WHO 2 (LGG) cases, 27 (90%) exhibited low IHC levels of SRSF1, and only three (10%) exhibited high levels, whereas no detectable immunostaining was observed in all pilocytic astrocytoma cases. Consistently, all GBM samples presented strong and diffuse immunostaining, and 85% of WHO grade 3 astrocytoma cases were moderately immunostained, showing a weak immunopositivity for SRSF1 in 15% of HGG cases (Fig. 2B). These data are consistent with those of the CGGA cohort. Notably, Spearman analysis showed a positive correlation between SRSF1 immunoexpression and WHO grade (data not shown), indicating that its expression gradually increased as WHO grade progressed. In addition, 29 (72.5%) cases with an increased Ki-67 index exhibited significantly higher SRSF1 expression levels than cases with a low Ki-67 index, whereas

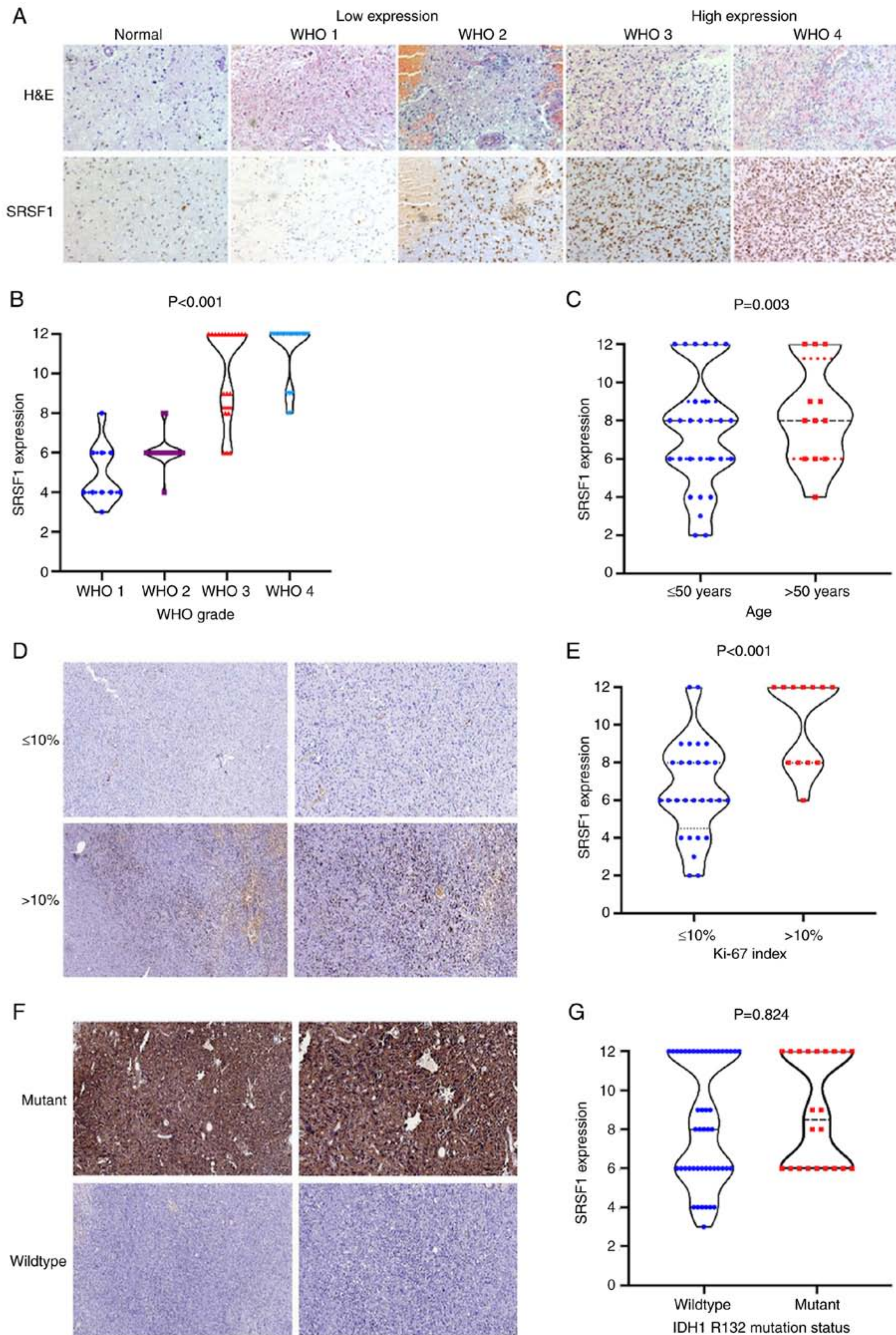

Figure 2. Association between SRSF1 immunoexpression and clinicopathological features in primary glioma cases. (A) Representative H&E and IHC images of SRSF1 protein in glioma samples and normal brain tissues (original magnification, x200). (B) Violin plot of the IRS of SRSF1 in WHO 1, WHO 2, WHO 3 and WHO 4 glioma samples. (C) Distribution of SRSF1 expression in patients with primary glioma grouped according to age. (D) Representative images of IHC staining of Ki-67 index in glioma samples (original magnifications, x10 and x20). (E) Violin plot of the IHC scores of SRSF1 in different Ki-67 index groups. (F) Representative images of IHC staining of IDH1 R132 mutations in glioma samples (original magnifications, x10 and x20). (G) Violin plot of the IHC scores of SRSF1 in IDH1 R132 mutation status. HE, hematoxylin and eosin; IHC, immunohistochemistry; SRSF1, serine and arginine rich splicing factor 1; WHO, World Health Organization.

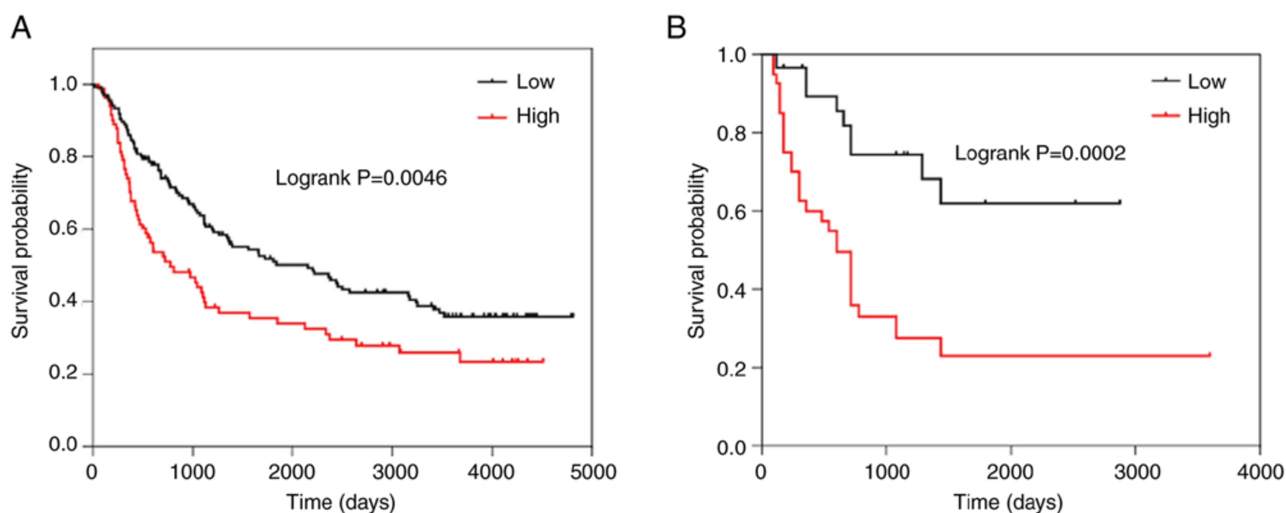

Figure 3. Kaplan-Meier overall survival analysis. Kaplan-Meier overall survival curves comparing patients with high and low expression of SRSF1 in the (A) Chinese Glioma Genome Atlas dataset and the (B) clinical cohort containing 70 patients with glioma. SRSF1, serine and arginine rich splicing factor 1.

26 (86.7%) cases with a low Ki-67 index showed low SRSF1 expression (Fig. 2D). As revealed in Fig. 2E, high expression of SRSF1 was closely asociated with Ki-67 index. Among the 10 pilocytic astrocytoma cases, immunoexpression of IDH1 R132 was completely negative. With regard to *IDH* mutations, IDH1 R132 showed strong staining, but negative immunoexpression for the *IDH* wild-type (Fig. 2F). Furthermore, no statistically significant association was detected between SRSF1 expression and IDH1 R132 immunoreactivity (Fig. 2G). The clinicopathological and molecular features of the clinical cohort are summarized in Table II

Potential use of SRSF1 for glioma grading. From the ROC curve, the specificity of SRSF1 for GBM was revealed to be 40%, the sensitivity was 100%, and the mean AUC value was 0.8 (95% CI 0.7-0.9). The specificity of SRSF1 for WHO grade 3 astrocytoma was 48%, the sensitivity was 85%, and the mean AUC value was 0.701 (95% CI 0.57-0.831) (data not shown). These findings indicated that SRSF1 performed well in distinguishing GBM and WHO grade 3 astrocytoma from WHO grade 2 astrocytoma.

Association between SRSF1 expression and survival. The Kaplan-Meier survival analysis revealed that patients with HGG and high expression of SRSF1 had shorter OS times than those with low expression of SRSF1 in the CGGA dataset (Fig. 3A), which was further confirmed in the 70 clinical glioma specimens (Fig. 3B). These findings indicated that SRSF1 may be a detrimental biomarker for the survival of gliomas.

Biological role of SRSF1 in vitro. To investigate the role of SRSF1 in the biological processes of glioma, the present study knocked down SRSF1 using individual-specific shRNAs (sh1-SRSF1, sh2-SRSF1 or sh3-SRSF1), with sh-NC as the negative control (Fig. 4A and B), and overexpressed SRSF1 in U87MG and U251 cells using LV-SRSF1 lentivirus, with LV-vector as the negative control (Fig. 4C). Western blot analysis confirmed efficient knockdown of SRSF1; SRSF1 expression was decreased by >70% in sh3-SRSF1-transduced cells compared with that in the sh-NC group, which indicated that sh3-SRSF1 had a higher interference efficiency compared with sh1-SRSF1 and sh2-SRSF1. Consequently, only sh3-SRSF1 was used in the subsequent experiments. Compared with LV-vector, overexpression of SRSF1 significantly promoted cell proliferation, as determined by MTT assay (Fig. 4D), whereas the knockdown of SRSF1 severely inhibited the proliferation of cells, with the largest difference detected after 3 days of culture (Fig. 4E). Moreover, overexpression of SRSF1 enhanced colony formation in comparison with the LV-vector group (Fig. 4F), whereas knockdown of SRSF1 reduced colony formation (Fig. 4G). In the U87MG and U251 cell lines, the wound healing assay revealed that overexpression of SRSF1 significantly enhanced cell migration, which was markedly suppressed by the knockdown of SRSF1 (Fig. 4H-J). Furthermore, overexpression of SRSF1 significantly enhanced cell invasion, as determined by Transwell assay (Fig. 4K). Conversely, knockdown of SRSF1 significantly suppressed the invasion of U87MG and U251 cells (Fig. 4L). These results indicated that SRSF1 promoted glioma progression and may act as an inducer of glioma.

Discussion

Accurate grading and histopathological subtyping are critical for the treatment and prognosis of primary glioma; however, no specific hallmarks are available to assist in the grading and prognostic evaluation of glioma. The present study hypothesized that it may be helpful to explore IHC biomarkers for the solution of these issues.

Given prior evidence of SRSF1 expression, which is expected to be highly expressed in GBM but at low levels in normal tissues, it was hypothesized that IHC for SRSF1 may be useful for distinguishing GBM and WHO grade 3 astrocytoma (HGG) from LGG. Based on the CGGA database and immunohistochemical analysis of clinical tissues, it was revealed that SRSF1 was upregulated in glioma specimens, particularly in most GBM and WHO grade 3 astrocytoma cases. Notably, the present study further confirmed that

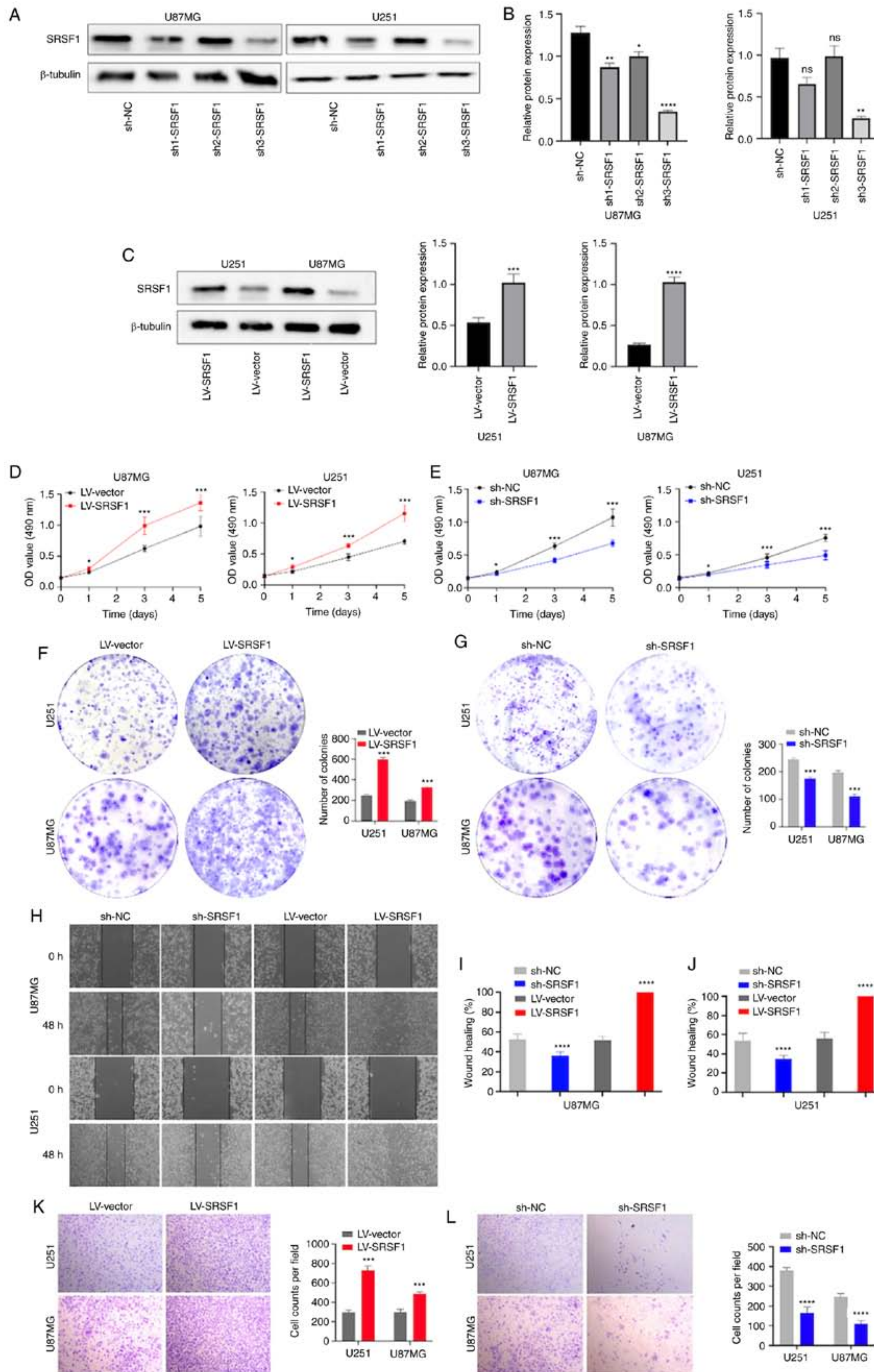

Figure 4. SRSF1 increases the proliferation, invasion and migration of U87MG and U251 cells. (A) Knockdown and overexpression efficiency post-transduction with sh-NC, sh1-SRSF1, sh2-SRSF1 and sh3-SRSF1, or LV-SRSF1 and LV-vector, as determined by western blot analysis. Semi-quantification of SRSF1 protein expression in (B) U87MG and (C) U251 cells. OD value at 490 nm of the MTT assay in U251 and U87MG cells with stable (D) overexpression or (E) knockdown of SRSF1. Results of the colony formation assay in U251 and U87MG cells with stable (F) overexpression or (G) knockdown of SRSF1. (H) Images of the wound healing assay in U251 and U87MG cells with stable overexpression or knockdown of SRSF1 (magnification, x100). Semi-quantification of wound healing assay in (I) U87MG and (J) U251 cells. Results of the Transwell invasion assay in U251 and U87MG cells with stable (K) overexpression (magnification, x200) or (L) knockdown of SRSF1 (magnification, x200). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. LV, lentivirus; NC, negative control; ns, not significant; sh, short hairpin; SRSF1, serine and arginine rich splicing factor 1.

SRSF1 was immunostained with an increasing gradient in WHO 2-4 astrocytic tumors, showing homogeneously strong immunopositivity for HGG and moderate immunopositivity for WHO grade 2 astrocytoma. In GBM and WHO grade 3 astrocytoma, the present study confirmed that immunohistochemical testing for SRSF1 provided the best diagnostic values for distinguishing them from WHO grade 2 astrocytoma (40 and 48% specificity; 100 and 85% sensitivity, respectively). In addition, SRSF1 expression was revealed to be associated with the Ki-67 index, which is a significant parameter for the increased grade of glioma (19). The present study revealed that the increased ratio of the Ki-67 index paralleled the high levels of SRSF1 expression. These results suggested a promising diagnostic ability for the SRSF1 protein in distinguishing HGG from LGG, the diagnosis of which may be challenging when tumors present no typical histological features or, in particular, molecular parameters are unavailable (20). Immunohistochemical analysis of SRSF1 may serve as an objective hallmark of glioma grading.

The present study also demonstrated that SRSF1 levels were associated with histopathological subtypes in the CGGA cohort, consistent with the findings of Broggi *et al* (21), which reported that SRSF1 was lower in astrocytoma and more frequently expressed in anaplastic oligodendroglioma. Similarly, the present study identified a high frequency of SRSF1 expression in GBM and WHO grade 3 astrocytoma; however, the frequency of expression was low in WHO grade 2 astrocytoma and oligodendroglioma. In this regard, it is reasonable to propose that the potential application of SRSF1 in the distinction of GBM from low-grade astrocytoma and oligodendroglioma. However, SRSF1 expression is not expected to be useful in the differential diagnosis of astrocytoma and oligodendroglioma. The association between SRSF1 and oligodendroglioma remains to be determined. In contrast to immunostains for SRSF1 in astrocytoma and oligodendroglioma, pilocytic astrocytoma lacked SRSF1-positive cells; therefore, for practical diagnostic purposes, in cases where the microscopic features, clinical setting and imaging data are unclear, pure SRSF1 negativity may be considered supportive evidence for pilocytic astrocytoma. The diagnostic use of the SRSF1 protein in distinguishing adult diffuse astrocytoma from ependymoma and pilocytic astrocytoma has also been reported (21). However, SRSF1 is not a reliable marker for distinguishing sub-ependymal giant cell astrocytoma from pleomorphic xanthoastrocytoma (21). On the basis of preliminary observations comparing SRSF1 expression in limited histopathological subtypes, larger-scale subtypes of CNS tumors are required to elucidate the clinical utility of SRSF1 immunoreactivity for diagnosis.

Given the high frequency of SRSF1 expression in GBM and WHO grade 3 astrocytoma, it was hypothesized that SRSF1 IHC may be associated with glioma-related molecular markers, such as *IDH* mutations and 1*p*/19*q* co-deletion status. However, SRSF1 IHC expression was not revealed to be associated with 1*p*/19*q* co-deletion and *IDH* mutations in the present study. Since SRSF1 regulates pivotal alternative splicing events of some tumor-related genes, further prospective studies incorporating all clinically relevant molecular markers are needed to evaluate for these molecular subgroups.

Beyond the significance for auxiliary diagnosis, Kaplan-Meier survival analysis demonstrated that patients with HGG and high SRSF1 expression had shorter OS times than those with low SRSF1 expression in the CGGA datasets and among the clinical cases. A preliminary study previously reported that SRSF1 is a predictive factor for basal cell carcinoma recurrence (18). It has also been demonstrated that SRSF1 is associated with various factors affecting the prognosis of ovarian cancer (22), hepatocellular carcinoma (23) and hematological malignancies (24). Moreover, the Ki-67 index, which was revealed to be positively associated with SRSF1, predicted a poorer prognosis in HGG cases, as confirmed in the present study (25). Thus, it was hypothesized that SRSF1 may serve as a prognostic factor for survival in glioma. Close follow-up is essential for tumors with high levels of this protein.

Based on the present clinical findings, SRSF1 appears to serve a crucial tumor-promoting role in primary glioma. It has been reported that SRSF1 is downregulated by the circ-PABPN1/microRNA-638 axis to suppress colorectal cancer development *in vitro* and *in vivo* (26). Barbagallo *et al* (27) also reported that CircSMARCA5 regulates VEGFA mRNA splicing through SRSF1 in GBM. However, the role of SRSF1 in CNS tumors has not been sufficiently elucidated. Therefore, the present study investigated the biological functions of SRSF1 in the U87MG and U251 cell lines. MTT and colony formation assays showed that overexpression of SRSF1 promoted glioma cell proliferation, which was suppressed after knockdown of SRSF1, indicating that SRSF1 may be associated with the malignant proliferation of glioma. Moreover, wound healing data confirmed that overexpression of SRSF1 significantly enhanced cell migration, suggesting that SRSF1 may promote glioma progression. The results from a Transwell assay showed that cells stably overexpressing SRSF1 exhibited markedly increased invasion, whereas invasion was suppressed in cells with SRSF1 knockdown. Consistent with the present study, Zhou *et al* (17) verified that the knockdown of SRSF1 impaired cell survival and invasion in GBM cell lines (17). These results demonstrated that SRSF1 could have a role in glioma development, further confirming the pro-tumor activity of SRSF1.

In conclusion, the present study revealed that SRSF1 is diffusely expressed in GBM and WHO grade 3 astrocytoma. As strong and diffuse SRSF1 expression is rare in WHO grade 2 astrocytoma, immunohistochemical testing for high SRSF1 has potential clinical value as an auxiliary approach for the distinction of HGG from WHO grade 2 astrocytoma. Since pilocytic astrocytoma exhibited absent immunoreactivity for SRSF1 compared with astrocytoma and oligodendroglioma, the detection of negative SRSF1 expression may be used as an auxiliary biomarker for pilocytic astrocytoma. Furthermore, SRSF1 could be considered a prognostic indicator and the present study indicated that SRSF1 serves an active role in promoting glioma progression. Some limitations in the present study need to be fully considered. First, upstream and downstream mechanisms have not been sufficiently investigated. Second, the utility of a single protein can be limited; therefore, it must be incorporated into a series of biomarkers, and more clinical

evidence is needed to confirm the conclusions of this study in neuro-oncology practice.

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Availability of data and materials

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

Authors' contributions

LY conceived the study. LY, GB, FR JY and KX interpreted and analyzed the data. KX and JY prepared the manuscript. KX and GB revised the manuscript for important intellectual content. LY and JY supervised the study. LY, GB, FR, JY and KX confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in line with the principles of The Declaration of Helsinki. Approval was granted by the Ethics Committee of The Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital) (Date 2022; approval no. KYLX2022090). Written informed consent was obtained from all individual participants or their parents/guardians included in the study.

Patient consent for publication

The authors affirm that human research participants provided informed oral consent for publication.

Competing interests

The authors declare that they have no competing interests.

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