SAMSN1 Is Highly Expressed and Associated with a Poor Survival in Glioblastoma Multiforme

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Abstract

Objectives: To study the expression pattern and prognostic significance of SAMSN1 in glioma.

Methods: Affymetrix and Arrystar gene microarray data in the setting of glioma was analyzed to preliminarily study the expression pattern of SAMSN1 in glioma tissues, and Hieratical clustering of gene microarray data was performed to filter out genes that have prognostic value in malignant glioma. Survival analysis by Kaplan-Meier estimates stratified by SAMSN1 expression was then made based on the data of more than 500 GBM cases provided by The Cancer Genome Atlas (TCGA) project. At last, we detected the expression of SAMSN1 in large numbers of glioma and normal brain tissue samples using Tissue Microarray (TMA). Survival analysis by Kaplan-Meier estimates in each grade of glioma was stratified by SAMSN1 expression. Multivariate survival analysis was made by Cox proportional hazards regression models in corresponding groups of glioma.

Results: With the expression data of SAMSN1 and 68 other genes, high-grade glioma could be classified into two groups with clearly different prognoses. Gene and large sample tissue microarrays showed high expression of SAMSN1 in glioma particularly in GBM. Survival analysis based on the TCGA GBM data matrix and TMA multi-grade glioma dataset found that SAMSN1 expression was closely related to the prognosis of GBM, either PFS or OS (P<0.05). Multivariate survival analysis with Cox proportional hazards regression models confirmed that high expression of SAMSN1 was a strong risk factor for PFS and OS of GBM patients.

Conclusion: SAMSN1 is over-expressed in glioma as compared with that found in normal brains, especially in GBM. High expression of SAMSN1 is a significant risk factor for the progression free and overall survival of GBM.

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Introduction

Malignant glioma is the most common and lethal form of cancer that originates from the central nervous system. Glioblastoma multiforme (GBM) also named as grade IV astrocytoma by the World Health Organization (WHO), accounts for approximately 60 to 70% of malignant glioma and is the most biologically aggressive subtype[1]. The prognosis of GBM is rather dismal and the average survival time is only 14.6 months from initial diagnosis, even when considering the current standards of treatment, which includes surgery, followed by radiotherapy and Temozolomide-based chemotherapy [2]. Since current treatment gained little benefit in the setting of GBM, greater attention has been paid to the expression of specific molecular markers with the goal of determining their possible prognostic and therapeutic significance.

SAMSN1 (SAM domain, SH3 domain, and nuclear localization signals 1), also termed HACS1/SLY2/NASH1, is a member of a family of three adapter proteins that are highly homologous and characterized by the presence of protein-protein interaction domains. Proteins with the sterile α motif (SAM) domain are able to associate with each other and can also self associate. Chimeric fusion of the SAM domain with
the βPDGF receptor [3], AML1 [4], c-Abl [5], and JAK2 [6] can promote the oncogenic transformation of the SAM domain. Src homology 3 (SH3) domains are known to mediate interactions of proteins in a number of signal transduction pathways. The presence of these domains in a protein is often indicative of adaptor or scaffolding functions.

The SAMSN1 gene localizes to a region on human chromosome 21 (21q11.2). The region is subject to frequent translocation events in hematopoietic malignancies. The transcript of SAMSN1 has been found in acute myeloid leukemia, lymphoma, and multiple myeloma cell-lines [7]. Evidence is lacking with regard to the role that SAMSN1 plays in certain solid tumors. A recent study found that SAMSN1 was positively associated with, and has predictive value in the setting of ulcerative colitis-associated colorectal cancer [8]. Another study found that the expression of SAMSN1 was reduced in lung cancer cell-lines. However, introduction of the expression vector for this gene did not result in any significant growth inhibition [9]. In central nervous system, SAMSN1 is expressed at a low level in normal brain [10], and the SAMSN1 protein might exert an influence on blood vessel formation during normal brain development [11]. The role of SAMSN1 in glioma remains unclear, and to the best of our knowledge, there has not been a prior report of its functional expression and prognostic value in glioma.

Although there has been no previous report about the action of SAMSN1 in glioma, a former study has shown that SAMSN1 expression can be increased in B lymphocyte by IL-4 stimulation through both STAT6 and PI3k/PKC/NF-κB pathways [10]. PI3K and NF-κB are important molecules participating in GBM pathogenesis. RTK/RAS/PI3K signaling pathway have been found to be one major pathway that were altered in GBM (altered in 88% human GBM)[12]. Furthermore, NF-κB is a downstream molecule of PI3K signaling, and is a major anti-apoptotic mediator that is over-expressed in glioma [13]. In the nucleus, SAMSN1 binds to Sin3-associated polypeptide 30 (SAP30) and histone deacetylase 1 (HDAC1), and forms a stable ternary complex [14]. The activity of HDAC1 was thus increased. It was reported that HDAC1 siRNA could elicit a concentration-dependent inhibition of HeLa cell proliferation [15]. In addition, HDAC1 has previously been found to be associated with many cancers, including glioma [16], gastric cancers [17], prostate cancers [18], liver cancers [19], breast cancers [20], and melanoma [21]. Thus it is reasonable to speculate that there might be some important relationships between the expression of SAMSN1 and glioma.

Searching Oncomine (www.oncomine.org), we have found two datasets that showed a significant over-expression of SAMSN1 in GBM as compared with normal brain (p = 2.55E-6 and 2.02E-11, fold change = 4.423 and 4.519, respectively), and an additional dataset showed that SAMSN1 was expressed higher in GBM than was found in other kinds of glioma (p = 3.75E-8, fold change = 2.664). These observations drew our interest to further study the possible role of SAMSN1 in the prognosis and pathogenesis of glioma.

In the current study, we analyzed Affymetrix and Arroystar gene microarray data in the setting of glioma. The objective of this analysis was to study the expression pattern of SAMSN1 in glioma tissues, and attempt to find preliminarily evidence on whether its expression is correlated with the clinical prognosis of glioma patients. We further made survival analysis stratified by SAMSN1 expression of more than 500 GBM cases based on the data of The Cancer Genome Atlas (TCGA), in order to determine SAMSN1’s prognostic significance in GBM. At last, we detected the expression of SAMSN1 in large numbers of glioma and normal brain tissue samples using Tissue Microarray (TMA); the purpose of this latter analysis was to further clarify the expression pattern of SAMSN1 and its prognostic significance in each grade of Glioma.

### Materials and Methods

#### Acquisition of clinical specimens

Glioma specimens were obtained from archived tissue samples derived from patients with glioma who underwent surgical treatment at Changzheng Hospital, China from January, 2000 through December, 2012. Glioma was diagnosed according to the 2007 WHO Classification of Tumors of the Central Nervous System. The selection criteria were as follows: 1) the subject presented with a diagnosis of glioma and no history of other tumors; 2) the subject had complete demographic and clinical data, such as age, gender, clinical manifestations, tumor size, extent of resection, adjuvant therapy, and date of relapse and/or death; 3) the subject underwent evaluation by enhanced head MRI scanning for tumor relapse or progression after surgery at least once every six months. Normal brain tissues were obtained from severe head trauma patients for whom partial resection of normal brain was required for decompression during surgery. Written informed consent of the patients was provided by their legal surrogates to permit surgical procedures and use of resected tissues. This study was approved by the Specialty Committee on Ethics of Biomedicine Research, Second Military Medical University of China. Human tissue acquisition and use in this study complied with the National Regulations on the Use of Clinical Samples in China.

#### Collection of clinical information and follow up

Data was collected by review of the clinical history. Information was recorded including the patient’s characteristics (e.g., gender, age), relevant symptoms or history (e.g., seizure, intracranial hypertension evidence such as headache, vomiting and papilla edema; whether there was a pre-existing low-grade glioma), tumor characteristics (e.g., size, boundary, whether or not associated with a cystic change or evidence of necrosis), extent of resection, post-surgical treatment protocol (e.g., whether the patient took assistant radiotherapy or chemotherapy), overall survival time and progression-free survival time, or SAMSN1 expression status (e.g., high levels or low expression levels). For analysis, a patient’s age was stratified into ≥60 or less than 60 years. The extent of resection was classified as gross total resection, subtotal resection (i.e. greater than 95% of the enhancing tumor was resected), and partial resection. The tumor size was described by mean tumor diameter (MTD, defined as the geometric mean of 3 diameters on MRI scan), and sorted into ≥4 cm and <4 cm. The follow-up
was conducted by telephone or direct correspondence. Postsurgical treatment, including adjuvant radiotherapy and chemotherapy, was fully discussed with the patient or their relatives. The time of tumor relapse or death was verified by the patient or their relatives, by medical recording, or by the social security record. Overall survival (OS) was calculated in months from the date of diagnosis to the time of death, regardless of cause. Progression free survival (PFS) was defined as the period from the initial date of diagnosis to the time of tumor progression by MRI, or to the time of death of the patient from glioma.

Gene microarrays and data processing

We built 23 Affymetrix microarrays (Affymetrix Human U133 2.0, GEO dataset: GSE45921), and 9 Agystar microarrays (8 x 60K, Arraystar, V2.0, GEO dataset: GSE51146) in 2007 and in 2012 respectively. The sample preparation and microarray hybridization were both performed based on the manufacturer’s standard protocols. Briefly, 1 μg of total RNA from each sample was amplified and transcribed into fluorescent cRNA with the manufacturer’s “Label” protocol. The labeled cRNAs were hybridized onto the Affymetrix U133 plus 2.0 or Arraystar V2.0. After washing the slides, the arrays were scanned by the Gene array Scanner. The standardized SAMSN1 expression was obtained by dividing SAMSN1 expression of each sample by the mean SAMSN1 expression of normal brains. After standardization, both microarray datasets were integrated for analysis.

Hierarchical Clustering based on clinical prognosis

The gene expression data in the Affymetrix microarrays was used for cluster analysis. Patients who presented with malignant (WHO III to IV grade) glioma were included and grouped as “alive” or “dead” due to the status of the patients at the end of the follow-up period. Genes that were important for prognostic prediction of malignant glioma were filtered and listed.

TCGA data acquisition and processing

The TCGA project provides multimodal data of more than 500 GBM cases, which can be acquired from the TCGA website (https://tcga-data.nci.nih.gov/tcga/). The dataset was searched for GBM cases with either clinical follow-up information or level 3 gene expression data based on the Affymetrix microarrays (Human gene U133A). The expression value of SAMSN1 gene was collected for each case, and was classified as either High (expression value ≥8.0) or Low(expression value <8.0). OS was calculated in days from the date of diagnosis to the time of death. PFS was defined as the days from the initial date of diagnosis to the time of tumor progression or tumor recurrence, or death of the patient from GBM.

Tissue Microarray (TMA) and Immunohistochemistry

The tissue microarray slides (Outdo Co., Shanghai, China) were built as previously described [22–24] after tumor verification with H&E staining and relevant immunohistochemistry staining by at least two experienced pathologists. One core punch sample was taken from each specimen, and measured 1.5 mm in the greatest dimension from the center of the tumor foci.

Immunohistochemical staining using a polyclonal anti-SAMSN1 antibody (1:500, Abgent, San Diego, CA, USA) was performed by the avidin-biotin complex (ABC) method (Vector Laboratories, Burlingame, CA, USA). The expression of SAMSN1 was determined by two independent pathologists blinded to the clinicopathological conditions with previously described criteria [25]. Briefly, the staining intensity in the cytoplasm or nucleus was graded respectively using a scale from 0 to 3 (0 for no immunostaining, 1 for light brown coloration, 2 for medium brown coloration, and 3 for a dark brown color). The percentage of positively stained cells was scored as: 0, ≤10% of the entire malignant cell population; 1, >10% and ≤60% of the entire malignant cell population; 2, >60% and ≤90% of the entire malignant cell population; 3, >90% of the entire malignant cell population. The intensity score multiplied with the percentage score was used to derive the final composite score, and was classified as “high” (final score ≥5) and “low” (final score <5). Scoring discrepancies were resolved by discussion.

Statistical analysis

The expression of SAMSN1 was described as mean ± standard deviation. Independent T-test was used to calculate the difference of the data between two groups. Chi-square test was used to evaluate the difference of rates among different groups. Kaplan-Meier estimates (log-rank test) were used to study if a variable was related to the OS or PFS of glioma patients. Multivariate Cox proportional hazards regression models were used to explore the role of multiple characteristics in the prognosis of glioma patients. All calculations were performed with the SPSS 18.0 software program (SPSS Inc, Chicago, IL, USA).

Results

Gene microarray dataset analysis suggests SAMSN1 is highly expressed and has prognostic importance in high-grade glioma

Thirty-two samples were included in gene microarrays, including 5 normal brains, 2 WHO grade I glioma, 13 WHO grade II glioma, 3 WHO grade III glioma, and 9 WHO grade IV glioma. (Table 1). Because of the limited sample size, it was not possible to compare the SAMSN1 expressions of each grade glioma. For simplicity, we classified it as low grade (WHO grade I and II) glioma and high grade (WHO grade III and IV) glioma. It was found that in normal brains, SAMSN1 expressions were at a low level, whereas in low and high-grade glioma, SAMSN1 was found to be expressed at high levels (Figure 1). Compared to normal brain, the expression of SAMSN1 was 1.53 ± 1.04 (p >0.05) in low-grade glioma and 2.05 ± 1.53 (p=0.037) in high-grade glioma. (Figure 2)

To further study the gene expression profile relevant to the prognosis of malignant glioma, we made hierarchical clustering with the gene expression data of Affymetrix microarrays. Sixty-
nine genes (SAMSN1 included) were filtered out, following which the expression of SAMSN1 could be applied to the prognosis of high grade glioma and classified into two groups. In one of the groups (including case 16, 17, 20, 21, and 22), all patients died at the end of the follow-up, and the average OS was 13 months. By contrast in another group (including case 18 and 19), the patients were alive at the end of the follow-up, and the average OS was 72.5 months. So the latter group has a longer OS and better prognosis at the end of follow-up than the former one. (Table 2, Figure 2).

**TCGA dataset analysis proves SAMSN1 as a risk factor for GBM survival**

By search the dataset we have got a total of 576 GBM cases with clinical follow-up information, and 557 GBM cases with level 3 Gene expression data based on the Affymetrix microarrays (Human Gene U133A). By matching the two data matrix, we got 523 GBM cases with full data of both clinical and SAMSN1 gene expression. Based on the Kaplan-Meier estimates (log-rank test), we found SAMSN1 expression was significantly related to the prognosis of GBM in both the PFS and the OS (p<0.05). PFS of the SAMSN1 low group was 344.3±32.5 days, versus 235.6±20.2 days in the SAMSN1 high group (p=0.021). As for OS, it was 524.3±39.1 days in the SAMSN1 low group, versus 414.1±27.5 days in the SAMSN1 high group (p=0.021). (Figure 3)

**Large sample TMA confirms SAMSN1 is highly expressed in glioma**

To confirm the results in gene microarray study, we further study the SAMSN1 expressions in large sample TMA. A total of 272 (9 grade Ⅰ, 101 grade Ⅱ, 45 grade Ⅲ, and 117 grade Ⅳ) glioma specimens and 16 normal brains were included in the TMA. It was found that SAMSN1 mostly expressed in the nucleus (8.3% of all glioma cases, 4.3% in GBM), high nuclear

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**Table 1. Expression of the SAMSN1 gene as Determined by Affymetrix and Arryestar Microarrays and Relevant Clinical Data.**

| Num | Array | Pathology | WHO Grade | SAMSN1 Expression | Corrected SAMSN1 Expression | Survival | OS |
|-----|-------|-----------|-----------|------------------|-----------------------------|----------|----|
| 1   | AM    | 1.        | 1         | 979.3            | 4.13                        | alive    | 71 |
| 2   | AM    | 1.        | 1         | 242.2            | 1.02                        | alive    | 70 |
| 3   | AM    | 2.        | 2         | 278.4            | 1.17                        | alive    | 86 |
| 4   | AM    | 2.        | 2         | 289.8            | 1.22                        | alive    | 85 |
| 5   | AM    | 1.        | 2         | 366.3            | 1.54                        | alive    | 40 |
| 6   | AM    | 2.        | 2         | 324.9            | 1.37                        | alive    | 70 |
| 7   | AM    | 2.        | 2         | 241.4            | 1.02                        | dead     | 42 |
| 8   | AM    | 3.        | 2         | 159.4            | 0.67                        | dead     | 15.4 |
| 9   | AM    | 3.        | 2         | 233.2            | 0.98                        | alive    | 66 |
| 10  | AM    | 1.        | 2         | 278.8            | 1.17                        | dead     | 49 |
| 11  | AM    | 3.        | 2         | 349.6            | 1.47                        | alive    | 75 |
| 12  | AM    | 3.        | 2         | 330.2            | 1.39                        | lost     | NA |
| 13  | AM    | 2.        | 2         | 163              | 0.69                        | alive    | 79 |
| 14  | AM    | 1.        | 2         | 293.3            | 1.24                        | alive    | 73 |
| 15  | AM    | 1.        | 2         | 921              | 3.88                        | dead     | 70 |
| 16  | AM    | 1.        | 3         | 313.9            | 1.32                        | dead     | 5  |
| 17  | AM    | 1.        | 3         | 171.7            | 0.72                        | dead     | 21 |
| 18  | AM    | 1.        | 3         | 174.8            | 0.74                        | alive    | 71 |
| 19  | AM    | 1.        | 4         | 178.7            | 0.75                        | alive    | 74 |
| 20  | AM    | 1.        | 4         | 435.7            | 1.84                        | dead     | 7  |
| 21  | AM    | 1.        | 4         | 490.9            | 2.07                        | dead     | 20 |
| 22  | AM    | 1.        | 4         | 937.3            | 3.95                        | dead     | 12 |
| 23  | AM    | NB        | -         | 237.3            | 1.00                        | -        | -  |
| 24  | AS    | 1.        | 4         | 27.95            | 5.26                        | dead     | 13 |
| 25  | AS    | 1.        | 4         | 21.45            | 4.04                        | dead     | 8  |
| 26  | AS    | 1.        | 4         | 5.00             | 0.94                        | dead     | 8  |
| 27  | AS    | 1.        | 4         | 5.80             | 1.09                        | lost     | NA |
| 28  | AS    | 1.        | 4         | 9.99             | 1.88                        | dead     | 6.5 |
| 29  | AS    | NB        | -         | 5.00             | 0.94                        | -        | -  |
| 30  | AS    | NB        | -         | 5.00             | 0.94                        | -        | -  |
| 31  | AS    | NB        | -         | 6.24             | 1.18                        | -        | -  |
| 32  | AS    | NB        | -         | 5.00             | 0.94                        | -        | -  |

NB, Normal Brain; NA, Not Available; Num, Number; Arrays: AM: Affymetrix; AS: Arryestar; Pathology: (1), astrocytoma; (2), Oligodendrocytoma; (3), Ependymoma; doi: 10.1371/journal.pone.0081905.t001
expression was very rare, only 0.7% of all glioma cases, compared with 60.1% glioma with a high cytoplasmic expression of SAMSN1. (Figure 4) In cytoplasm, the scores of SAMSN1 expression in normal brain and WHO grade I – IV glioma were 1.69 ± 1.30, 5.11 ± 3.33, 5.66 ± 2.93, 5.60 ± 3.12, and 5.86 ± 3.02, respectively. The level of SAMSN1 expression was higher in each grade of glioma than was found in normal brains (p<0.01), whereas the differences among grades of glioma were not significant (p>0.05). In nuclear, the scores of SAMSN1 expression in normal brain and WHO grade I - IV glioma were 0.00 ± 0.00, 0.00 ± 0.00, 0.37 ± 1.03, 0.03 ± 0.16, and 0.06 ± 0.38, respectively. Grade II glioma exhibited a pivotal elevation of nuclear SAMSN1 expression compared to all other groups (p<0.05) (p<0.05).

High SAMSN1 expression is a risk factor for GBM prognosis, either primary or secondary, but not for other grades of glioma

Survival analysis stratified by SAMSN1 expression was made by Kaplan-Meier estimates in each grade of glioma. By this analysis, we found that the cytoplasmic expression of SAMSN1 was closely associated with the prognosis of GBM, PFS and OS (p<0.05). However, it was not associated with the prognosis of WHO grade II, III or IV stage glioma (p>0.1). In the high-SAMSN1-expressing GBM, the median PFS of patients was 9 (95% CI 6.159 - 9.841) months, whereas in the low-SAMSN1-expressing group, the median PFS was 15 (95% CI 9.666 - 20.334) months (p = 0.002, Figure 5a). Similarly, in GBM with a high level of SAMSN1 expression, the median OS was 11 (95% CI 9.389 - 12.611) months, versus 15 (95% CI 8.336 - 21.664) months seen in low SAMSN1 expressing GBM (p=0.005, Figure 5b). For the high expression of SAMSN1 in the Nucleus was very rare (2/288), when Nucleus stain of SAMSN1 added to the calculation, it didn't affect the results. Further, we classified the GBM as primary and secondary, and made survival analyses by Kaplan-Meier estimates in each group. It was also found that SAMSN1 was closely associated with the prognosis of both primary and secondary GBM, either PFS or OS (p<0.05). (Figure 6)

Clinical characteristics and correlation of SAMSN1 expression with other clinical features of GBM subjects included in TMA

Among the 117 GBM patients included in the TMA, the age of the patients was 49.7 ± 16.2 years, and the male/female ratio was 1.925 (77/40). 102 patients were primary, and 15 patients were secondary tumors. Seizure was found in 12.8% of the patients and intracranial hypertension presented in 43.6% of the patients at diagnosis. MTD of the tumor was 4.4 ± 1.2 cm, and 25.6% of the tumors presented with a cystic
Hierarchal clustering of the gene expression data obtained by Affymetrix microarrays. Sixty-nine genes (including SAMSN1) were filtered out and associated with the prognosis of high-grade glioma, which could then be classified into two groups (Group 1: 16, 17, 20, 21, 22; Group 2: 18 and 19). In group 1, all patients died at the end of the follow-up, and the average overall survival (OS) time was 13 months. By contrast in group 2, the patients were alive at the end of the follow-up, and the average OS was 72.5 months. The prognosis of the two groups was obviously quite different.

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## Table 2. Expression of 69 Genes Relevant to the Prognosis of High-Grade Glioma in Affymetrix Microarrays.

| Patients' ID | 16  | 17  | 20  | 21  | 22  | 18  | 19  |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| ACTR2        | 0.9 | 0.4 | 0.6 | 0.7 | 0.6 | -0.3| -0.2|
| AGPAT5       | 0.6 | 0.1 | 0.8 | 0.6 | 0.8 | 2.1 | 2.3 |
| AP3B1        | 0.6 | 1   | 0.7 | 0.8 | 0.9 | -0.1| 0   |
| ARL3         | -0.8| -0.8| -1  | -1.4| -1  | 0.2 | 0.3 |
| AUH          | -1.7| -2.7| -2.6| -2.3| -2.1| 0.6 | 0.4 |
| C14orf126    | -0.5| -0.7| -0.6| -0.5| -0.8| 0.5 | 0.4 |
| C20orf23     | 2.1 | 1.4 | 2.3 | 1.6 | 2.3 | -0.7| -0.5|
| CP           | 2   | 0.3 | 2.2 | 2.2 | 1.9 | 0   | -1.9|
| DTNB1        | 0.5 | -0.2| -0.3| 0   | -0.8| 0   | 2.7 |
| ELOVL1       | 0.6 | 0.8 | 0.9 | 1   | 1   | 0.1 | 0   |
| FAM13A1OS    | 0.1 | -1.3| -1  | -1.4| -1.1| 1.5 | 1.8 |
| FLJ13811     | 0.3 | -0.2| 0.3 | -0.2| 0.1 | 1.2 | 1.1 |
| FLJ21963     | -0.4| -0.7| 0.1 | 0.4 | 1   | -2  | -1.8|
| FTSJ2        | 1   | 0.6 | 0.8 | 0.8 | 0.8 | 0   | 0   |
| GAD1         | -0.3| 1.9 | 2   | -0.8| 0.1 | -5.4| -5.4|
| GOLT1A       | -2  | -2.3| -1.9| -2.2| -1.8| 0   | 0   |
| GPR126       | 0.1 | 0.6 | 0.4 | -1.1| -0.4| -3.3| -3.1|
| GS1          | 1.3 | 2.3 | 2.9 | 2.3 | 1.6 | -0.7| -0.8|
| GTF21        | 1.2 | 0.9 | 1.3 | 0.8 | 0.7 | 0   | -0.1|
| HAVCR2       | 1.2 | 1.2 | 1.8 | 2.7 | 2.2 | -1.2| -1.4|
| JMY          | -0.2| -0.4| -0.2| -0.5| -0.6| 0.6 | 0.5 |
| KLHL24       | 0.9 | 0.5 | 0.7 | 0.4 | 0.6 | 1.6 | 1.5 |
| LIG3         | 3.1 | 1.8 | 2.6 | 2   | 2.5 | -0.7| -0.9|
| LILRB2       | 3   | 3.3 | 2.4 | 3.8 | 3.3 | -1.1| -0.8|
| LOC254559    | -0.1| 0.5 | -0.6| 0.3 | -0.7| 2.2 | 2.1 |
| LOC399818    | -1.6| -1.1| -1.5| -1.3| -1.1| 0.2 | 0.1 |
| LOC400960    | -0.3| -0.9| -0.3| -1.2| 0.4 | 2.2 | 2   |
| LOC653461    | -0.9| -0.9| -0.6| -0.7| -0.7| 0   | 0   |
| LOC92249     | -0.4| -0.3| 0.1 | -0.5| -0.1| 0.8 | 0.7 |
| LRPI1        | -0.4| -1  | -0.9| -0.9| -1.3| 0.8 | 1   |
| MMP19        | -1.3| -1.1| -1.2| -1.2| -1.7| 0.1 | 0   |
| MSH5         | -0.4| -0.1| -0.2| -0.2| -0.7| 0.8 | 0.8 |
| MTTPN        | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | -0.2| -0.2|
| NAV1         | -0.6| 0.2 | -0.2| 0.1 | -0.3| 1.3 | 1.3 |
| NDFIP1       | 0.2 | 0.2 | 0.5 | 0.3 | 0.5 | -0.8| -0.7|
| NF1          | 0.1 | 0.5 | 0.7 | 0.5 | 1   | -0.7| -0.8|
| NFATC2IP     | -0.1| -0.2| -0.1| 0   | -0.1| 0.6 | 0.6 |
| NPR3         | -1.4| -1.9| -1.6| -0.3| -1.2| 2.3 | 2.6 |
| NUBPL        | -0.1| 0.1 | 0.2 | -0.1| 0.3 | 0.9 | 0.9 |
| PARD6B       | -0.5| -0.6| -0.7| -0.8| -0.8| 0.9 | 1   |
| PIGK         | 0.3 | 0   | -0.1| -0.2| 0.2 | -1.1| -1.1|
| PODNL1       | 0.3 | 0.4 | 0.6 | 0.2 | 0.2 | 1.3 | 1.2 |
| PPP4R2       | 1.5 | -0.2| 0.6 | 0   | 0   | -2.8| -3.2|
| PRR8         | 1.1 | 0   | -0.4| 0.5 | -0.4| -3.3| -3.3|
| PTEN         | 0.9 | -0.2| 0.1 | 0.4 | 0.3 | 2   | 1.9 |
| PVR          | -0.7| 0   | -0.3| 0.3 | 0   | -3.1| -2.8|
| QRTTD1       | -1.4| -1.5| -1.3| -1.9| -0.2| 1.7 | 2.1 |
| RBBP8        | 1.6 | 1.3 | 1.1 | 1.3 | 1   | 0.1 | 0.1 |
| RNF14        | 0.7 | 1   | 1   | 1.2 | 1.3 | -0.1| 0   |
| RNF146       | -0.6| -0.9| -0.8| -0.8| -0.9| 0.1 | 0.1 |
| SAMS1        | 0.2 | -0.5| 0   | 1   | 2   | -0.2| -0.3|
| SEH1L        | 0.6 | 0.6 | 0.4 | 1.1 | 0.7 | -0.8| -0.9|
| SETD5        | 0.8 | 1   | 0.9 | 0.8 | 0.5 | 2   | 1.9 |
| SIGLEC10     | 0.8 | 0.3 | 1.2 | 0.5 | 0.5 | -0.7| -0.8|
change, 18.8% with necrosis, and 61.5% of tumors had a non-clear boundary. Among the tumors, 77.8% were totally resected, 19.7% were sub-totally resected, and 2.6% was subjected to partial resection. Adjuvant radiotherapy was undertaken by 74.4% of the patients and chemotherapy was undertaken by 76.1%. The PFS of the total GBM patients was 13.97 ± 14.03 months (with a median of 10 months), and the OS was 16.30 ± 14.92 months (with a median of 11 months).

GBM patients with a high SAMSN1 expression were more rarely had a long survival (OS ≥ 36 months) as compared with those with a low level of SAMSN1 expression (3.9% vs. 22.2%, p=0.002). The MTD of GBM with a high level of SAMSN1 expression was more likely to be over 4 cm (67.1% vs. 50%.

The expression data of genes was treated with log2 function.

| Patients' ID | 16  | 17  | 20  | 21  | 22  | 18  | 19  |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| SLC24A1      | -1  | -1.6| -1.3| -1.2| -0.7| 0.9 | 1   |
| SLC31A2      | -0.8| 0.1 | 0.4 | 0   | 0.1 | -2.5| -2.6|
| ST18         | -1.4| -1.1| -1.1| -1.9| -0.7| 0.7 | 0.9 |
| SUZ12P       | -1  | -0.7| -1  | -0.7| -0.5| 0.1 | 0.2 |
| SYNJ2        | -1.8| -1  | -0.3| -1.5| 0.6 | -5.7| -6.1|
| TAF6         | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | -0.9| -0.8|
| TFCP2        | -1.1| -0.7| -1.1| -1.2| -0.6| 0.4 | 0.4 |
| TNP01        | 0.5 | 0.2 | 0.4 | 0.2 | 0.5 | 1.3 | 1.4 |
| TNPO1        | 0.6 | 0.6 | 0.4 | 0.3 | 0.4 | 1.4 | 1.5 |
| TNRCS6B      | 0.1 | -0.3| -0.1| -0.4| -0.3| 1.2 | 1.2 |
| TUBGCP2      | -0.2| -0.3| -0.4| -0.8| -0.8| 0.6 | 0.7 |
| WDR39        | -0.4| -0.2| -0.2| -0.5| -0.5| 0.5 | 0.5 |
| WHDC1L1      | -1.4| -1.6| -1.2| -1  | -0.9| 0.1 | 0.2 |
| ZC3H12A      | 0.6 | 0.8 | 0.7 | 1.3 | 0.5 | -1.7| -1.6|
| ZCWPW1       | -1.1| 0.3 | -0.8| -1.3| -1  | 2.2 | 2   |

The expression data of genes was treated with log2 function.

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Figure 3. Univariate survival analysis in GBM stratified by SAMSN1 expression based on the TCGA data as determined by Kaplan-Meier estimates. 523 GBM cases with full data of both clinical and SAMSN1 gene expression was downloaded from the TCGA website. Kaplan-Meier estimates (log-rank test) were made and found SAMSN1 expression was significantly affect the prognosis of GBM in both PFS and OS (p<0.05).

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Figure 4. Immunochemical staining of SAMSN1 in normal brains and glioma tissues. A, normal brain, with light staining in both nucleus and cytoplasm; B, grade II glioma, light staining in the cytoplasm and deep staining in the nucleus; C, grade II glioma, cytoplasm deep staining, and scattered deep staining of the nucleus; D, primary GBM, with deep staining in both the cytoplasm and nucleus; E, primary GBM, cytoplasm deep staining, nucleus light staining; F, secondary GBM, cytoplasm deep staining, nucleus light staining.

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Figure 5. Univariate survival analysis in GBM stratified by SAMSN1 expression based on the TMA data as determined by Kaplan-Meier estimates. The expression of SAMSN1 was significantly associated with PFS (A) and OS (B) of GBM.

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p=0.075), and the boundary of the tumor was more likely to be unclear (67.1% vs. 50%, p=0.075) There were no significant correlations found between SAMSN1 expression levels and other clinical characteristics (e.g., gender, age, history of seizure and intracranial hypertension, tumor necrosis, extent of resection, and adjuvant radiotherapy or chemotherapy) (p>0.1, Table 3).

High expression of SAMSN1 was an independent risk for GBM patient survival

To study other clinical factors that might affect the prognosis of GBM, we made univariate survival analysis that was stratified by each of the clinical factors (including gender, age, seizure, intracranial hypertension, tumor size, boundary, cystic change and necrosis, extent of resection, postsurgical radiotherapy or chemotherapy, and SAMSN1 expression) with Kaplan-Meier estimates in GBM. It was found that age ≥60

Figure 6. Univariate survival analysis in subtype of GBM stratified by SAMSN1 expression based on the TMA data as determined by Kaplan-Meier estimates. The expression of SAMSN1 was significantly associated with PFS (A) and OS (B) of primary GBM, and with PFS (C) and OS (D) of secondary GBM as well.

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years, partial resection, lack of post-surgical radiotherapy, and high SAMSN1 expression were significant risk factor for PFS of GBM patients (p<0.05). An age ≥ 60 years, cystic change of the tumor, partial resection, no post-surgical radiotherapy, and high SAMSN1 expression were significant risk factor for OS of GBM patients (p<0.05, Table 4).

Variables that might have contributed to the prognosis of GBM (p<0.2 in Kaplan-Meier estimates) were filtered for multivariate survival analysis, using Cox proportional hazards regression models. It was found that high SAMSN1 expression levels were a strong risk factor for PFS of GBM patients (HR=2.119, 95% CI 1.338-3.356, p=0.001), and post-surgical radiotherapy was a strong protective factor for PFS of GBM patients (HR=0.580, 95% CI 0.374-0.901, p=0.015). As for OS of GBM patients, we obtained similar results, i.e., high levels of SAMSN1 expression were a strong risk factor (HR=2.036, 95% CI 1.279-3.238, p=0.003), and post-surgical radiotherapy was a strong protective factor (HR=0.573, 95% CI 0.370-0.888, p=0.013, Table 5).

Discussion

On the basis of classical pathologic classification, GBM is a subtype of glioma and pathognomonically the characteristic

| Clinical features | total [n(%)] | SAMSN1 low [n(%)] | SAMSN1 high [n(%)] | P |
|------------------|-------------|-------------------|-------------------|---|
| GBM              | 117         | 38                | 79                | 0.607 |
| primary          | 102(87.2)   | 34(89.5)          | 68(86.1)          |   |
| secondary        | 15(12.8)    | 4(10.5)           | 11(13.9)          |   |
| Gender           |             |                   |                   | 0.211 |
| Male             | 77(65.8)    | 22(57.9)          | 55(69.6)          |   |
| female           | 40(34.2)    | 16(42.1)          | 24(30.4)          |   |
| Age              |             |                   |                   | 0.215 |
| <60 years        | 87(74.4)    | 31(81.6)          | 56(70.9)          |   |
| ≥60 years        | 30(25.6)    | 7(18.4)           | 23(28.1)          |   |
| Seizure          |             |                   |                   | 0.209 |
| No               | 102(87.2)   | 31(81.6)          | 71(89.9)          |   |
| Yes              | 15(12.8)    | 7(18.4)           | 8(10.1)           |   |
| Increased ICP    |             |                   |                   | 0.822 |
| No               | 66(56.4)    | 22(57.9)          | 44(55.7)          |   |
| Yes              | 51(43.6)    | 18(42.1)          | 33(44.3)          |   |
| Cystic change    |             |                   |                   | 0.141 |
| No               | 87(74.4)    | 25(65.8)          | 62(78.5)          |   |
| Yes              | 30(25.6)    | 13(34.2)          | 17(21.5)          |   |
| Tumor necrosis   |             |                   |                   | 0.149 |
| No               | 95(81.2)    | 28(73.7)          | 67(84.8)          |   |
| Yes              | 22(18.8)    | 10(26.3)          | 12(15.2)          |   |
| Tumor boundary   |             |                   |                   | 0.075 |
| not clear        | 72(61.5)    | 19(50.0)          | 53(67.1)          |   |
| Clear            | 45(38.5)    | 19(50.0)          | 26(32.9)          |   |
| MTD              |             |                   |                   | 0.075 |
| <4 cm            | 45(38.5)    | 19(50.0)          | 26(32.9)          |   |
| ≥4 cm            | 72(61.5)    | 19(50.0)          | 53(67.1)          |   |
| Resection        |             |                   |                   | 0.450 |
| Total            | 91(77.8)    | 27(71.1)          | 64(81.0)          |   |
| subtotal          | 23(19.7)    | 10(26.3)          | 13(16.5)          |   |
| Partial           | 3(2.6)      | 1(2.6)            | 2(2.5)            |   |
| Chemotherapy     |             |                   |                   | 0.675 |
| No               | 28(23.9)    | 10(26.3)          | 18(22.8)          |   |
| Yes              | 89(76.1)    | 28(73.7)          | 61(77.2)          |   |
| Radiotherapy     |             |                   |                   | 0.908 |
| No               | 30(25.6)    | 10(26.3)          | 20(25.3)          |   |
| Yes              | 87(74.4)    | 28(73.7)          | 59(74.7)          |   |
| Overall survival |             |                   |                   | 0.002 |
| <36 months       | 102(90.3)   | 28(77.6)          | 74(96.1)          |   |
| ≥36 months       | 11(9.7)     | 8(22.2)           | 3(3.9)            |   |

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features of GBM are vascular proliferation and/or necrosis [2]. Although this classification has been an extremely valuable approach for the diagnosis, treatment plan designs, and prognosis estimates in GBM, its shortcoming has become evident as a result of accumulating knowledge and appreciation of the roles played by genetic and clinical studies. Even belonging to the same pathologic grade, the survival time and response to treatment for patients presenting with GBM might be quite different. Therefore, it has been an important topic to build a new classification scheme for prognosis prediction, or to find new therapeutic targets for molecular targeting.

Table 4. Univariate Survival Analysis of GBM Stratified by Clinical Factors as Determined by Kaplan-Meier estimate.

| Factors                  | n  | PFS (months) | OS (months) |
|--------------------------|----|--------------|-------------|
|                          |    | Mean         | median  P   | Mean        | median  P   |
| Gender                   |    |              |            |             |            |
| male                     | 76 | 15.004       | 9  0.201   | 18.080      | 11  0.413  |
| female                   | 37 | 16.671       | 12 17.980  |             |            |
| Age                      |    |              |            |             |            |
| <60 years                | 84 | 17.163       | 11  0.031  | 19.888      | 12  0.050  |
| ≥60 years                | 29 | 10.259       | 6  4.47    | 12.466      | 8           |
| Primary/secondary        |    |              |            |             |            |
| primary                  | 96 | 16.580       | 10  0.447  | 18.053      | 11  0.730  |
| secondary                | 15 | 12.067       | 10 18.000  | 14           |
| Seizure                  |    |              |            |             |            |
| yes                      | 15 | 13.352       | 10  0.861  | 18.000      | 16  0.755  |
| no                       | 98 | 16.278       | 10 18.043  | 12           |
| Increased ICP            |    |              |            |             |            |
| yes                      | 50 | 18.007       | 10  0.326  | 19.632      | 12  0.488  |
| no                       | 63 | 13.096       | 8  16.144  | 11           |
| Cystic change            |    |              |            |             |            |
| yes                      | 29 | 20.903       | 12  0.084  | 25.319      | 15  0.050  |
| no                       | 83 | 12.903       | 8  14.712  | 11           |
| Tumor necrosis           |    |              |            |             |            |
| yes                      | 20 | 12.483       | 6  0.607   | 17.950      | 11  0.816  |
| no                       | 93 | 16.394       | 10 17.926  | 12           |
| Tumor boundary           |    |              |            |             |            |
| clear                    | 43 | 16.525       | 10  0.617  | 20.120      | 15  0.433  |
| unclear                  | 70 | 13.861       | 8  17.722  | 11           |
| Size                     |    |              |            |             |            |
| MTD≥4cm                  | 65 | 13.003       | 9  0.209   | 16.265      | 12  0.656  |
| MTD<4cm                  | 48 | 17.517       | 11 18.774  | 12           |
| Resection                |    |              |            |             |            |
| Total                    | 87 | 16.340       | 9  0.038   | 19.914      | 12  0.010  |
| subtotal                 | 23 | 15.826       | 13 17.435  | 15           |
| partial                  | 3  | 4.000        | 1  4.333   | 2           |
| Radiotherapy             |    |              |            |             |            |
| yes                      | 83 | 17.332       | 11  0.049  | 20.318      | 12  0.035  |
| no                       | 30 | 10.383       | 8  11.917  | 9            |
| Chemotherapy             |    |              |            |             |            |
| yes                      | 86 | 16.978       | 10  0.158  | 20.076      | 14  0.051  |
| no                       | 27 | 10.981       | 8  11.907  | 9            |
| SAMSN1                   |    |              |            |             |            |
| high                     | 77 | 11.482       | 8  0.002   | 13.744      | 11  0.005  |
| low                      | 36 | 25.280       | 15 28.028  | 15           |

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Table 5. Multivariate Survival Analysis of GBM with Cox Proportional Hazards Regression Models.

| Factors                  | PFS HR 95% CI | OS HR 95% CI |
|--------------------------|---------------|--------------|
| Radiotherapy             | 0.580 0.374 - 0.901 0.014 0.573 0.370 - 0.888 0.013 |
| SAMSN1 Expression        | 2.119 1.338 - 3.356 0.001 2.036 1.279 - 3.238 0.003 |

HR, Hazard Ratio; HR 95% CI, 95% confidential interval of the Hazards Ratio.
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Exciting developments had been gained in the research of GBM and other types of glioma in recent years. By analyzing the DNA, mRNA and microRNA levels of more than 500 cases of GBM, the TCGA project had successfully subdivided GBM into four subtypes, labeled as classical, mesenchymal, proneural, and neural. Comparing the gene expression patterns of these 4 GBM subtypes with those of astrocytes, oligodendrocytes, neurons, and microglia suggests that the subtypes may reflect different cells of origin. Another breakthrough was the finding of the role of isocitrate dehydrogenase 1 (IDH1) mutation in glioma. Lower grade gliomas and a subset of glioblastomas with an IDH1 R132 mutation was strongly related to a good prognosis. It was
subsequently clarified that the mutation might have an indirect oncogenic effect through the activation of the hypoxia-inducible factor pathway, thus increased the metabolic adaptation of tumors to anaerobic growth. These findings give new hopes for the research and treatment of malignant glioma[2].

In the present study, by analyzing the gene chip, the TCGA dataset, and the tissue microarray data, we have found a gene named SAMSN1 that is highly expressed in glioma, and indicates prognostic significance in GBM. We first analyzed the expression of SAMSN1 in glioma and normal brain tissues by our gene chip data. The results indicated that the expression of SAMSN1 was higher in glioma than that of the normal brain group. It was also shown that the expression of SAMSN1 might be a prognostic factor in higher grade glioma. Limited by the sample size, this was only a preliminary result which needed high-throughput testing to confirm. Subsequently, we made survival analysis based on the TCGA data which included more than 500 cases of GBM, and the result showed the SAMSN1 expression was significantly associated with GBM prognosis.

Next, we analyzed the expression of SAMSN1 by tissue microarray with a larger sample size of 288 glioma specimens and normal brain tissues, with detailed and reliable clinical follow-up data. For the expansion of the sample size, it was possible to analyze the expression of SAMSN1 and make survival analysis in glioma of each grade. The results showed that the expression of SAMSN1 was increased in all grades of glioma compared with that in normal brain tissues. We also found that a GBM with a MTD >4 cm, and non-clear boundary was more often to be seen in the high SAMSN1 group than in the low SAMSN1 group (p<0.1), possibly suggesting increasing proliferative and infiltrating ability of tumor cells with a higher level of SAMSN1 expression. In the subsequent survival analysis, the high expression levels of SAMSN1 have been shown to be independently associated with a poor prognosis of GBM and both in PFS and OS (HR = 2.119 and 2.036, respectively). Long survival (OS ≥ 36 months) of patients was also less in the high-SAMSN1 group than in the low-SAMSN1 group (p<0.01). Another factor that we found to be independently related with the PFS and OS of GBM was radiotherapy (HR = 0.580 and 0.573, respectively), which has been found to be highly protective in GBM [26]. However, interestingly, the SAMSN1 expression was not related to the prognosis of other types of glioma except for the GBM.

In our study, we found that the SAMSN1 protein was distributed mainly in the cytoplasm of both normal brain and glioma cell. Although the SAMSN1 protein could also be found in the nucleus sometimes, high expression of it was rarely seen. Previous reports concerning the subcellular localization of SAMSN1 were contradictory. Some described it as a protein mainly localized in the cytoplasm [7], whereas others reported it as a nuclear protein [27]. Brandt et al. [14] reported SAMSN1 could shuttle between the cytoplasm and the nucleus, and the 14-3-3 proteins could interact with the phosphorylated SAMSN1 and retain it in the cytosol, thus preventing its nuclear localization. This might be an explanation of SAMSN1’s subcellular locations. A recent study reported that 14-3-3 could be highly expressed in glioblastoma and correlates with the poor prognosis of the disease[28]. This finding suggested a potential linkage between SAMSN1 and the 14-3-3 proteins in glioma. Nevertheless, the results of our study didn’t show there was any relationship between the cytoplasmic and nuclear expression level of SAMSN1. Furthermore, survival analysis also didn’t find any correlations between the nuclear expression of SAMSN1 and the prognosis of any subtype of glioma. Therefore, the meaning for the SAMSN1’s subcellular distributions in glioma remains unclear.

In the gene and tissue microarrays, we found SAMSN1 was also highly expressed in some low-grade glioma specimens compared to the normal brains (for example, in table 1: case 1 and 15). We were interested in this phenomenon and wondered if the prognosis would be worse when these cases of the low grade glioma developed into the secondary GBM. We made hieratical cluster analysis on the gene expression profiles of these cases. The data showed that these cases presented similar gene expression pattern as those of the high grade glioma with poor prognosis (Figure S1). We further made survival analysis stratified on the TMA data. The result showed that SAMSN1 remained a significant risk factor for the prognosis of the subgroup of GBM (Figure 6C and D). Whether a low grade glioma which highly expressed SAMSN1 is more likely to progress into secondary GBM? And what change might occur in gene expression profile during the transformation from the low grade glioma to GBM? These are interesting questions and need further studies.

Another intriguing question is that SAMSN1 expressed highly in all grades of glioma, but why was it only related to the prognosis of GBM? We presume that there might be some key molecules only activated in GBM. Without these molecules, the cancer promoting actions of SAMSN1 could not be fully activated. In fact, SAMSN1 is an adaptor protein, which is characterized by the presence of protein-protein interaction domains. So it is very possible that SAMSN1 could interact with some key molecules in GBM and promote the cancer progression. However, these presumed key molecules still could not be identified in the current study, and may need further research to find out.

**Conclusion**

In this study, we found that the SAMSN1 gene was over-expressed in glioma as compared with that found in normal brains. High expression of SAMSN1 was found to be a strong risk factor for the progression free survival and overall survival of glioblastoma multiforme. Therefore, SAMSN1 is a valuable molecular index for prediction of GBM prognosis, and thus might represent a latent target for gene therapy in the setting of glioma.

**Supporting Information**

Figure S1. Hierarchal clustering of the gene expression data obtained by Affymetrix microarrays (case 1 and 15 added). The low-grade glioma with high levels of SAMSN1 expression (case 1 and 15) showed similar gene expression patterns as those of the high grade glioma with poor prognosis.
Performed the experiments: YY LZ TX CC. Analyzed the data:

YY LZ DF TX JXZ RQ YXZ. Contributed reagents/materials/analysis tools: YY DF TX RQ CC. Wrote the manuscript: YY LZ.

Author Contributions

Conceived and designed the experiments: YY JXC GHH YCL. Performed the experiments: YY LZ TX CC. Analyzed the data:

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