Increased Expression of Sema3C Indicates a Poor Prognosis and Is Regulated by miR-142-5p in Glioma

Haidong Zhang, Hui Ma, Wenling Zhang, Deyi Duan, Guangting Zhu, Wei Cao, and Bin Liu*

Department of Neurology, Jining NO.1 People’s Hospital; Jining, Shandong 272011, China.
Received September 24, 2019; accepted January 4, 2020

Sema3C has been reported to promote glioma stem cells self-renewal and glioblastoma growth. However, the prognostic value and the regulatory mechanism for its abnormal expression in glioma remain poorly understood. In the current study, the immunohistochemistry results demonstrated that Sema3C was overexpressed in 169 of 216 (78.2%) interpretable glioma patients compared with 3 of 15 (20.0%) interpretable non-neoplastic brain cases \(p = 0.0001\). Sema3C overexpression was significantly associated with histologic type \(p = 0.0008\), high Ki67 labeling index \(p = 0.02\), tumor grade \(p = 0.002\) and wild type IDH1 \(p = 0.0001\). Importantly, its overexpression predicts the shorter overall survival of glioma patients \(p = 0.0017\), especially the ones with high grade \(p = 0.0124\). Functionally, Sema3C silencing significantly reduced the proliferation and invasion of glioma cells, indicating an oncogenic role of Sema3C in glioma in vitro. To elucidate the reason accounting for its overexpression, it is identified miR-142-5p as a tumor suppressor that directly targets Sema3C in glioma cells. miR-142-5p and Sema3C were co-regulators of epithelial–mesenchymal transition. Clinically, miR-142-5p expression was conversely related with Sema3C expression in glioma samples. Together, we identified that Sema3C could promote the progression of glioma and its expression was negatively regulated by miR-142-5p in vitro. Thus, the miR-142-5p-Sema3C axis plays importantly in glioma and holds potential to be therapeutic targets as well.

Key words Sema3C; miR-142-5p; glioma; metastasis

INTRODUCTION

As the most common malignant tumor in the central nervous system (CNS), glioma accounts for about 70% of tumors in the CNS. Although advances have been made in surgery, radiation therapy and chemotherapy, the prognosis of glioblastomas patients and the median survival time of patients has not significantly improved.1-2 The poor prognosis of glioma is largely attributed to their highly growth, invasive/migratory nature and recurrence rate.

Semaphorins are a family of secreted or membrane-bound proteins that were initially recognized as a kind of axon guidance factors in nervous system,3 and they are classified into eight classes based on their structural elements.3 Class 3 semaphorins can be secreted and its positively charged domain at the carboxyl terminus could bind to the negatively charged components of the extracellular matrix.4 To date, all class 3 semaphorins have been analyzed (i.e. Semaphorins 3A, 3B, 3D, 3E, 3F, and 3G) and could exert pro- or anti-tumor properties in a tissue specific manner.4 Regarding Sema3C, abnormal expression was reported in various tumors and it could promote the disease progression in glioma, prostate and gastric cancer.2,5-7 In glioma, increased Sema3C level is correlated with glioma malignancy and could promote the survival and tumorigenicity of glioma through activating Rac1.5,6 However, the prognostic value and the molecular mechanism leading to its abnormal expression in glioma remain unclear.

miRNAs are closely related with tumor progression through its positive or negative effects on cell invasion, proliferation, apoptosis and stem cell maintenance.9 Recently, many miRNAs, such as miR-16, miR-155, miR-21, and miR-218, have been found to be involved in gliomas deterioration.9-12 Besides, previous study showed that miR-142-3p protects against glioma by modulating the immunity activity of M2 macrophages through the transforming growth factor beta signaling pathway.13 Furthermore, miR-142 inhibited glioma cell migration and invasion by directly targeting Rac1 thus leading to the suppression of matrix metalloproteinases (MMPs).14 Clarifying the novel target gene for miR-142 would be helpful to elucidate the character of glioma.

In the current study, we have characterized the expression and prognostic significance of Sema3C. Besides, we also aimed to clarify the biological roles of Sema3C in glioma and elucidate its relationship with miR-142-5p.

MATERIALS AND METHODS

Cell Culture and Sample Collection The human glioma cell lines, U251 and U87, were purchased from the Chinese Academy of Sciences (Shanghai, China) and cultured according to the manufacture’s protocols. A total of 216 paraffin-embedded glioma and 15 non-neoplastic brain (NB) tissues samples were obtained from the Renmin Hospital of Jining, Shandong, China. These cases were from 138 males and 78 females with old age ranging from 35 to 69 years (median, 51 years). According to WHO classification in 2007, post-operative samples obtained from 216 patients diagnosed with different malignancy grade gliomas: 15 grade I astrocytomas (pilocytic), 72 grade II astrocytomas (diffuse), 83 grade III astrocytomas (anaplastic) and 46 grade IV astrocytomas (glioblastomas). The patients did not receive any chemotherapy or radiotherapy before surgery. The clinicopathologic parameters of all the patients are summarized in Table 1. Non-neoplastic brain tissues \(n = 15\) were obtained from the ones undergo-
ing decompressive craniectomy after brain injuries and serve as controls. The diagnosis in each tissue was confirmed by histological staining with hematoxylin and eosin (H&E). The reliable follow-up times ranging from 1 to 68 months (mean 22 months). Prior consent was obtained from patients and our study was approved by the Ethics Committees of The first people’s Hospital of Jining.

RNA Isolation and Real-Time Quantitative PCR (RT-qPCR) Total RNA was extracted from adherent cultures using Trizol (Invitrogen, U.S.A.) and reversely transcribed into cDNA using First Strand cDNA Synthesis Kit (TOYOBO, Japan). qPCR was carried out using the SYBR green PCR master mix (Applied Biosystems, Carlsbad, CA, U.S.A.) on an LightCycler 480 (Roche, Switzerland) as previously described.15) The miRNA levels of Sema3C were quantified relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the 2\(^{-\Delta\DeltaCT}\) method. The sequence of primers used in this study was listed in Supplementary Table 1.

To isolate miRNA from formalin-fixed paraffin-embedded tissues, samples were examined by hematoxylin and microdissected under a dissecting microscope (Leica ASLMD, Witts Baden, Germany). Then, miRNA easy FFPE kit (Qiagen, Hilden, Germany) was applied to achieve the co-purification of total RNA, including miRNA. The expression of miR-142-5p was measured using SYBR Primescr ipt miRNA RT-PCR kit by applying RNA U6 as its endogenous control and the melting curve analysis of each product was performed to confirm its specificity.

Protein Isolation and Western Blotting Proteins isolation and Western blotting were performed as previously described.16,17) The primary antibodies were as followings: Sema3C and GAPDH (Abcam, Cambridge, Cambridgeshire, U.K.), as well as E-cadherin, Vimentin and Snail (Cell Signaling Technology, Danvers, MA, U.S.A.). The signals were visualized by an enhanced chemiluminescence kit (Amersham, Buckinghamshire, U.K.).

MTS (Methyl Thiazolyltetrazelium) Assay, BrdU Assay, Invasion Assays and Flow Cytometry Analysis Cell viability was measured by MTS assays at 490 nm.18) BrdU incorporation (Merck Millipore) was measured at absorbance 450 nm. Invasive cells were transferred into 8\(\mu\)m-pore-size Matrigel Invasion Chambers (BD, Franklin Lakes, NJ, U.S.A.) and cells adhering to the lower surface were fixed with methanol, stained with Giemsa solution and counted in five randomly selected microscopic fields (>200). For cell cycle analysis, cells were treated with 5\(\mu\)g/mL of aphidicolin (Sigma-Aldrich, Munich, Germany) and subsequent 1mL propidium iodide (0.1 mg/mL with 0.1% TritonX-100). Then, the samples were analyzed by flow cytometry (FACS Calibur, BD).

Immunohistochemistry (IHC) IHC was performed by the standardized labeled streptavidin biotin (LSAB) kit (Dako Cytomation, Carpinteria, CA, U.S.A.).19) The slides were incubated with anti-human Sema3C antibody (1:100 dilution, Abcam, Cambridge, MA, U.S.A.), anti-human Ki67 antibody (1:100 dilution, Dako, Carpinteria, CA, U.S.A.) and anti-human IDH1-R132H antibody (at 1:60 dilution, Dianova, Hamburg, Germany). The slides were evaluated blindly by two independent observers (HD and WF) and the following scoring system was utilized to validate Sema3C expression and Ki67 labeling index.20) Standard of IDH1-R132H staining: (1) a strong cytoplasmic immunoreaction product was scored positive; (2) a weak diffuse staining and staining were not scored positive.

Cell Transfection Cell transfection was performed using Lipofectamine 2000 (Invitrogen). The Sema3C-specific small interfering RNAs (siRNAs), nonspecific negative control siRNAs, Hsa-miR-142-5p mimics and Hsa-miR-142-5p inhibitor were designed and synthesized by Gene-Pharma (China). The sequences of siRNA targeting Sema3C are as following: siSema3C#1 (sense strand: 5’-GCA GAU GAU UA A CCA AU AU UU-3’; anti-sense strand: 5’-UAU AACC A AA UUA GUAGACG-3’); siSema3C#2 (sense strand: 5’-GGCCUU GUAUUGUCGUAU-UU-3’; anti-sense strand: 5’-TATGTTTCTCGGAA-3’); Non-specific negative control siRNAs (NC) were also used (sense strand: 5’-UCUCGAGCA GUGACG-3’; anti-sense strand: 5’- UAGAUCUUCUGAGACCUU-3’). Negative control and Normal control stand for non-target siRNA and untransfected control, respectively.

Plasmid Construction and Luciferase Reporter Assay The wild-type reporter plasmid of Sema3C 3’-UTR was generated on the miR-142-5p target recognition sites (seed sequences) as shown in Supplementary Table 1. The wild-type 3’-UTR of Sema3C gene was cloned into the pmirGLO dual luciferase reporter vector using SalI and XbaI restriction sites. The mutant 3’UTR that interferes with seed sequence recognition was also cloned to validate its specificity. The luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, U.S.A.). The sequence of primers was listed in Supplementary Table 1.

Statistical and Survival Analysis Statistical Package for Social Sciences, version 19.0 (SPSS) was used for statistical analyses. All results were presented as mean ± standard deviation (S.D.) from at least three separate experiments. Difference between groups was analyzed by one-way ANOVA on ranks tests. p-Values < 0.05 were considered as statistically significant.

RESULTS

Expression of Sema3C Gene in Glioma and NB Tissues Using immunohistochemical staining method, the expression of Sema3C protein was firstly detected in 216 archived paraffin-embedded glioma samples and 15 NB tissues (Fig. 1A). Cytoplasm location of Sema3C protein was predominantly found in the glioma. Furthermore, it was highly expressed in 78.2% (169/216) of glioma samples, while a significantly lower frequency (20.0%, 3/15) in NB samples (p < 0.001) (Table 1). Based on the above data, we further evaluated the overall survival between patients with or without Sema3C overexpression. Patients with low levels of Sema3C expression had better survival than those with high expression (Fig. 1B) (p = 0.017). We further analyzed the survival curve by glioma grade and although the differences of survivals between higher and lower Sema3C expression in the low grade did

| Table 1. SEMA3C Expression in Different Brain Tissues |
|----------------|----------------|----------------|----------------|
| Group           | Number of cases | Glioma | Normal brain | p-Value |
| Sema3C positive | 172             | 169    | 3             | 0.0001 |
| Sema3C negative | 59              | 47     | 12            |        |

Biol. Pharm. Bull.  Vol. 43, No. 4 (2020)
not reach significant level (Fig. 1C) ($p = 0.0899$), it showed a significant difference in the high grade glioma category (Fig. 1D) ($p = 0.0124$).

**The Clinicopathologic Characteristics of Sema3C Expression in Glioma Patients** To better understand the clinical significance of Sema3C expression, we analyzed the relationship between its expression levels and clinicopathologic characteristics in individuals with glioma (Table 2). We found that the expression level of Sema3C was positively correlated with the status of histological subtypes ($p = 0.008$) and pathology classification (WHO I-II vs. WHO III-IV) ($p = 0.002$), but not age or sex in glioma patients.

Next, we analyzed the relation between Sema3C with other molecular markers. In our study, mutation of IDH1 was present in 40.7% (88/216) of glioma cases. As shown in Table 2, IDH1 mutation occurred less frequently in patients with Sema3C overexpression (36.4%, 32/88) than those patients without Sema3C overexpression (63.6%, 56/88; $p = 0.0001$). Furthermore, high Ki67 labeling index ($>10\%$ nuclei staining) was observed in 74.1% (160/216) of glioma cases. Notably, Ki67 positivity and Sema3C overexpression was significantly correlated ($p = 0.02$, Table 2).

**Sema3C Knockdown Reduces Glioma Cell Proliferation and Invasion** Based on the above results, we aimed to determine the biological activities of Sema3C in glioma cells. To achieve this, siRNA targeting Sema3C was transfected in

### Table 2. Clinicopathological Character of Sema3C in Human Glioma

| Clinicopathological parameters | Number of cases | Sema3C protein expression (%) | $p$-Value |
|--------------------------------|-----------------|------------------------------|----------|
| Age (years)                    |                 |                              |          |
| $\leq65$                       | 105             | 30 (28.6%)                   | 75 (71.4%)| 0.538    |
| $>65$                         | 111             | 36 (32.4%)                   | 75 (67.6%)|          |
| Gender                        |                 |                              |          |
| Male                          | 138             | 42 (30.4%)                   | 96 (69.6%)| 0.959    |
| Female                        | 78              | 24 (30.8%)                   | 54 (69.2%)|          |
| Histologic type               |                 |                              |          |
| Pilocytic astrocytomas        | 15              | 8 (53.3%)                    | 7 (46.7%)| 0.008    |
| Diffuse astrocytomas          | 72              | 29 (40.3%)                   | 43 (59.7%)|          |
| Anaplastic astrocytomas       | 83              | 21 (25.3%)                   | 62 (74.7%)|          |
| Glioblastomas                 | 46              | 8 (17.4%)                    | 38 (82.6%)|          |
| $K_i-67$ expression           |                 |                              |          |
| Low                            | 56              | 24 (42.9%)                   | 32 (57.1%)| 0.02     |
| High                          | 160             | 42 (26.3%)                   | 118 (73.7%)|          |
| IDH1 mutation                 |                 |                              |          |
| No                             | 128             | 10 (7.8%)                    | 118 (92.2%)| 0.0001   |
| Yes                            | 88              | 56 (63.6%)                   | 32 (36.4%)|          |
| WHO grade                     |                 |                              |          |
| I + II                        | 87              | 37 (40.2%)                   | 50 (59.8%)| 0.002    |
| III + IV                      | 129             | 29 (24.0%)                   | 100 (76.0%)|          |
U87 and U251 (Fig. 2A). We found that silencing Sema3C expression in U87 (Fig. 2B) and U251 (Fig. 2C) inhibited the cell growth rate relative to its negative controls.

Furthermore, silencing Sema3C could significantly suppress the DNA synthesis levels as evidenced by BrdU incorporation rates (Fig. 2D) when compared with its control. We also performed cell cycle distribution analysis. The data showed that nearly 60% of cells were arrested in the G0/G1 phase in response to aphidicolin. However, after withdrawal of aphidicolin, silencing Sema3C arrested more U87 (Fig. 2E, upper) and U251 cells (Fig. 2F, upper) in the S phase. Consistently, silencing Sema3C reduced expressions of Cyclin A2 and CDK2 (key proteins in the S to G2 phase transition), but not Cyclin B1 and CDK1 (key proteins in the G2 to M phase transition) (Figs. 2E, F, lower). These results suggest that silencing Sema3C inhibits cell-cycle progression from S to G2/M phase by decreasing expression of Cyclin A2 and Cdk2 in human glioma cells. Moreover, the transwell assay showed that transfection of Sema3C-specific siRNA could significantly reduce the invasion when compared with its controls (Figs. 2G, H). In all, Sema3C could regulate the cellular proliferation and invasion of glioma cells.

**Inhibition of Sema3C Expression Suppresses Epithelial–Mesenchymal Transition (EMT) in Glioma Cells**

EMT is a dynamic process characterized by the increase of cell motility and invasion but the decrease of adhesive ability, and thus contributes greatly to cancer metastasis.\(^\text{20}\) In addition, transforming growth factor-\(\beta\)1 (TGF-\(\beta\)1) was validated as a prominent EMT inducing factor in cancer development.\(^\text{19}\) Here, we further characterized the potential role of Sema3C.
on TGF-β1-induced EMT in glioma cells. After TGF-β1 treatment at different concentrations for 72 h, the expression of Sema3C was significantly up-regulated at mRNA (Figs. 3A, B) and protein (Figs. 3C, D) levels in U87 and U251. Importantly, silencing Sema3C inhibited the TGF-β1-induced EMT, as evidenced by the increase of Vimentin and Snail, but the decline of E-cadherin (Figs. 3E, F). These results suggest Sema3C may be required for the EMT process to promote the progression of glioma.

Sema3C Is Negatively Regulated by miR-142-5p in Glioma Cells To explain the reason why Sema3C is overexpressed in glioma cells, TargetScan, PicTar and miRanda were utilized and miR-142-5p was selected a candidate miRNA that may regulate Sema3C expression (Fig. 4A). Firstly, the RT-qPCR and Western blot results showed that miR-142-5p mimic-transfected U87 and U251 decreased the expression of Sema3C at mRNA (Figs. 4B, C) and protein levels (Figs. 4D, E), whereas miR-142-5p inhibitor increase it.

Next, we applied a luciferase reporter assay to assess the regulation of miR-142-5p towards Sema3C in glioma cells. Of note, miR-142-5p mimics reduced the luciferase activity for the wild type Sema3C genes when compared with mimic control (Fig. 4F). However, such inhibition disappeared upon mutation of the binding sites of miR-142-5p in the 3’UTR of Sema3C (Fig. 4F). Then, to test the functional relation between miR-142-5p and Sema3C in glioma, wild type and mutant type of plasmid expressing HA-tagged Sema3C were constructed. Of them, the mutant type is the one that the seeding sites of miR-142-5p are mutated. The data showed that, miR-142-5p mimics transfection could suppress the HA expression in the presence of the wild type plasmid expressing Sema3C. However, the suppression could be attenuated when co-transfecting the mutant type of Sema3C plasmid (Fig. 4G). Together, miR-142-5p suppressed Sema3C expression by directly targeting its 3’UTR in glioma cells.

miR-142-5p Inhibits the Proliferation and Invasion of Glioma Cells To further elucidate the biological role of miR-142-5p in glioma cells, cell vitality was firstly analyzed by MTS and miR-142-5p mimics inhibit the proliferation in U87 (Fig. 5A) and U251 (Fig. 5B) cells when compared with mimic control. In contrast, inhibition of miR-142-5p activated the cell vitality in both cells (Figs. 5C, D). Next,
the BrdU incorporation assay demonstrated that overexpressing miR-142-5p in U87 and U251 cells could suppress the BrdU incorporation rates (Fig. 5E), whereas transfection with miR-142-5p inhibitor exhibited the opposite tendency (Fig. 5F). Consistent with the effect of silencing Sema3C, overexpression of miR-142-5p resulted in a significant arrest in the S phase of U87 (Fig. 2G, left) and U251 (Fig. 2H, left). Consistently, miR-142-5p mimics transfection reduced expressions of Cyclin A2 and CDK2, but not Cyclin B1 and CDK1 (Figs. 2G, H, right). The invasive ability was also detected by Transwell apparatus and overexpressing miR-142-5p could inhibit the cell invasion (Figs. 5I, J), but suppressing miR-142-5p showed the opposite tendency (Figs. 5I, J). Overall, miR-142-5p may act as a tumor suppressor in glioma.

**miR-142-5p Inhibits the Invasive Capacity of Glioma Cells by Regulating EMT Process** Interestingly, our RT-qPCR results also showed that TGF-β1 treatment led to a steadily decrease of miR-142-5p in U87 and U251 cells, indicating its potential role in EMT. Detailed analysis demonstrated that trasfection with miR-142-5p mimics restored the decline of E-cadherin, while suppressed the induction of vimentin and Snail in response to TGF-β1, supporting its likely role to inhibit EMT process in glioma (Figs. 6C, D). Then, glioma cells were transfected with miR-142-5p mimics in the presence or absence of TGF-β1. Importantly, the increase of Sema3C induced by TGF-β1 stimulation was attenuated by the transfection with miR-142 mimics in U87 (Fig. 6E) and U251 (Fig. 6F). These data suggests that TGF-β1 could regulate Sema3C expression indirectly through miR-142-5p.

**Clinical Significance of miR-142-5p Expression in Human Glioma** Then, we examined miR-142-5p expression in glioma and NB tissues. The quantitative analysis showed that the expression of miR-142-5p was down-regulated in glioma samples compared with NB ones (Fig. 7A). Furthermore, its expression was progressively lost with the tumor grade increased (Fig. 7B). Significantly, the expression of miR-142-5p were inversely correlated with that of Sema3C (Fig. 7C). These data further supported the negative regulation of miR-142-5p towards Sema3C.
DISCUSSION

Previous studies found that Sema3C was elevated in various tumors and its overexpression was positively associated with progression and poor prognosis in glioma, prostate cancer, gastric cancer, breast cancer and oral neoplasia. All these data supported the likely involvement of Sema3C in tumor progression. In this study, we evaluated Sema3C expression in glioma tissues and found that Sema3C was overexpressed in a cohort of glioma tissues. Moreover, its expression level was positively associated with tumor grading of tumor patients, which was consistent with the previous study. Importantly, the multivariate analyses showed that overexpression of Sema3C predicted poor prognosis for glioma patients, especially with high grade. These data indicated a potential prognostic role in Sema3C in glioma, although more samples would be still needed in further study.

Previous studies demonstrated that Sema3C could regulate the survival of glioma stem cells in a Rac1-dependent manner. Of note, Sema3C overexpression was significantly associated with Ki67 in clinical samples. Consistently, our studies extend the function of Sema3C in glioma to the cell proliferation and invasion in vitro. Furthermore, silencing Semaphorins family members, such as Sema3F, resulted in a marked arrest in cell cycle at S phase and similar effect was also duplicated in Sema3C-silencing glioma cells. These data suggested that Sema3C may also regulate the biological activity of glioma cells through monitoring S phase checkpoint.

IDH1 is critical gene in glioma tumors and its mutation is characterized to be correlated with low-grade glioma. Besides, a significant correlation ($p = 0.0001$) was observed between wild-type IDH1 and Sema3C overexpression, which indicates that Sema3C overexpression may be involved in the disease progression of glioma independent on IDH1 muta-
Fig. 6. miR-142-5p Regulates the EMT Process in Glioma

The relative expression of miR-142-5p was detected in U87 (A) and U251 (B) after TGF-β1 treatment at the indicated concentration. E-Cadherin, Vimentin and Snail were detected by Western blot in miR-142-5p mimics- or its control-transfected U87 (C) and U251 (D) cells following TGF-β1 (5 ng/mL) stimulation. The effect of miR-142-5p on TGF-β1 (5 ng/mL) induced Sema3C in U87 and U251 (F) was analyzed by Western blot. Vehicle stands for the group supplemented with PBS. *p < 0.01 vs. Vehicle.

Fig. 7. The Relative Expression of miR-142-5p in Clinical Glioma Tissues

(A) miR-142-5p expression was detected by RT-qPCR in glioma and NB tissues. (B) The expression of miR-142-5p in glioma tissues. (C) Sema3C mRNA level was inversely correlated with miR-142-5p level in glioma paraffin tissue. The data is shown as mean ± S.D. All relative expressions of miR-142-5p were normalized to U6. *p < 0.05. (D) A chart illustrating the existence of miR-142-5p/Sema3C axis during the pathological progression of glioma.
tion in Chinese glioma patients. Furthermore, EMT is closely related with cell migration and invasion and contributed to the progression of glioma. 24) The loss of E-cadherin and over-expression of mesenchymal cell markers such as N-cadherin, Vimentin are hallmarks of EMT. 24) Of note, our data indicate that knockdown of Sema3C inhibit the EMT process induced by TGF-β1 and Sema3C could promote glioma progression via the EMT process.

Recently, miRNAs have emerged as important regulators to control the patho-physiological processes in glioma. 25) Also, the family members of Semaphorin were also reported to be under the regulation of miRNA, such as miR-27a/b and miR-192. 26,27) Depressed expression of miR-142-5p has been detected in colorectal carcinoma, 28) acute myeloid leukemia 29) and gastric cancer 30) and its deregulation contributed to the abnormal proliferation and invasion in various tumors. 8,25) In our study, it’s observed that miR-142-5p negatively regulated the Sema3C expression by direct binding to its 3’UTR, which could partly explain the abnormal expression of Sema3C in glioma.

Here, we also identify the tumor suppressor role of miR-142-5p in glioma cells, as over-expression of miR-142-5p mimics the effects of silencing Sema3C, but a display of opposite tendency in miR-142-5p inhibitor-transfected cells. Of note, miR-142-5p could negatively regulate the EMT process in glioma cells, which further supports the likely existence of miR-142-5p-Sema3C axis in the pathological progression of glioma. The negative relation between miR-142-5p and Sema3C was further supported by the fact that the tissues with lower miR-142-5p showed higher Sema3C expression. Furthermore, miR-142-5p mediates the regulation of TGF-β1 on the Sema3C expression. More specifically, miR-142 has been reported as a potential metastasis-associated tumor suppressor in glioma by inhibiting Rac1. 31) These facts further support the miR-142-5p-Sema3C axis may be functional in glioma and in vivo experiments are warranted in future study.

Thus far, oncogenic and tumor suppressor transcription factors, such as p53, AP-1, c-Myc, nuclear factor-kappaB (NF-kB), E2F and signal transducer and activator of transcription (STAT), have been demonstrated to be able to regulate miRNAs expression in glioma cells. 31) Besides, the epigenetic deregulation, such as DNA methylation, are observed and involved in the regulation of miRNA expression. 31) More importantly, most of these factors are involved in TGF-β1-driven EMT process. 31) Further study would still be warranted to clarify the regulatory mechanism of miR-142-5p expression.

In conclusion, our findings demonstrate that Sema3C over-expression was a poor prognostic factor for glioma patients, especially the ones with high grades. Furthermore, the results show that Sema3C acts as an oncogene by orchestrating EMT and was directly regulated by miR-142-5p in glioma cells, indicating the existence of miR-142-5p/Sema3C signaling during the pathological progression of glioma (Fig. 7D). Totally, our study contributes to identify new prognostic bio-markers and therapeutic targets for glioma treatment.

**Acknowledgments**

This research was supported by Grants from the National Natural Science Foundation of China (Grant No. 81302239).

**Conflict of Interest**

The authors declare no conflict of interest.

**Supplementary Materials**

The online version of this article contains supplementary materials.

**REFERENCES**

1) Wen PY, Kesari S. Malignant gliomas in adults. N. Engl. J. Med., 359, 492–507 (2008).
2) Blanc V, Naricielam J, Munson P, Freeman A, Klocker H, Masters J, Williamson M. A role for class 3 semaphorins in prostate cancer. *Prostate*, 71, 649–658 (2011).
3) Flannery E, Duman-Scheel M. Semaphorins at the interface of development and cancer. *Curr. Drug Targets*, 10, 611–619 (2009).
4) Gaur P, Bledenbery DR, Samuel S, Bose D, Zhou Y, Gray MJ, Dallas NA, Fan F, Xia L, Lu J, Ellis MJ. Role of class 3 semaphorins and their receptors in tumor growth and angiogenesis. *Clin. Cancer Res.*, 15, 6763–6770 (2009).
5) Vaiktiene P, Skirute D, Stepinaitis G, Skauminas K, Tamasauskas A, Kazlauskas A. High level of Sema3C is associated with glioma malignancy. *Diagn. Pathol.*, 10, 8 (2015).
6) Man J, Shoemake J, Zhou W, Fang X, Wu Q, Rizzo A, Prayson R, Bao S, Rich JN, Yu JS. Sema3C promotes the survival and tumorigenicity of glioma stem cells through Rac1 activation. *Cell Reports*, 9, 1812–1826 (2014).
7) Miyato H, Tsuno NH, Kitayama J. Semaphorin 3C is involved in the progression of gastric cancer. *Cancer Sci.*, 103, 1961–1966 (2012).
8) Zhang Y, Dutta A, Abounader R. The role of microRNAs in glioma initiation and progression. *Front. Biosci. (Landmark Ed.),* 17, 700–712 (2012).
9) Yang CH, Yue J, Pfeffer SR, Fan M, Paulus E, Hosni-Ahmed A, Sims M, Qayyum S, Davidoff AM, Handorf CR, Pfeffer LM. MicroRNA-21 promotes glioblastoma tumorigenesis by down-regulating insulin-like growth factor-binding protein-3 (*IgfBp3*). *J. Biol. Chem.*, 289, 25079–25087 (2014).
10) Tan Z, Che S, Wang J, Jiao Y, Wang C, Meng Q. miR-155 contributes to the progression of glioma by enhancing Wnt/beta-catenin pathway. *Tumour Biol.*, 36, 5499–5511 (2015).
11) Wang Q, Li X, Zhu Y, Yang P. MicroRNA-16 suppresses epithelial-mesenchymal transition-related gene expression in human glioma. *Molecular Medicine Reports*, 10, 3310–3314 (2014).
12) Tu Y, Gao X, Li G, Fu H, Cui D, Liu H, Jin W, Zhang Y. MicroRNA-218 inhibits glioma invasion, migration, proliferation, and stem-cell-like cell self-renewal by targeting the polycomb group gene Bmi1. *Cancer Res.*, 73, 6046–6055 (2013).
13) Xu S, Wei J, Wang F, Kong LY, Ling XY, Nduom E, Gabrusiewicz K, Doucette T, Yang Y, Yaghi NK, Fajt V, Levine JM, Qiao W, Li XG, Lang FF, Rao G, Fuller GN, Calin GA, Heimberger AB. Effect of miR-142-3p on the M2 macrophage and therapeutic efficacy against murine glioblastoma. *J. Natl. Cancer Inst.*, 106, dju162 (2014).
14) Qin W, Rong X, Dong J, Yu C, Yang J. miR-142 inhibits the migration and invasion of glioma by targeting Rac1. *Oncol. Rep.*, 38, 1543–1550 (2017).
15) Wang L, Song G, Chang X, Tan W, Pan J, Zhu X, Liu Z, Qi M, Ju J, Han B. The role of TXNDC5 in castration-resistant prostate cancer-involvement of androgen receptor signaling pathway. *OncoGene*, 34, 4735–4745 (2015).
16) Wang L, Song G, Zheng Y, Wang D, Dong H, Pan J, Chang X. miR-573 is a negative regulator in the pathogenesis of rheumatoid arthritis. *Cell. Mol. Immunol.*, 13, 839–849 (2016).
17) Wang L, Song G, Zheng Y, Tan W, Pan J, Zhao Y, Chang X. Expression of Semaphorin 4A and its potential role in rheumatoid arthritis. *Arthritis Res. Ther.*, 17, 227 (2015).
18) Wang L, Zheng Y, Xu H, Yan X, Chang X. Investigate pathogenic arthritis.
mechanism of TXNDC5 in rheumatoid arthritis. *PLOS ONE*, **8**, e53301 (2013).

19) Wang L, Zhang J, Yang X, Chang YW, Qi M, Zhou Z, Zhang J, Han B. SOX4 is associated with poor prognosis in prostate cancer and promotes epithelial–mesenchymal transition in vitro. *Prostate Cancer Prostatic Dis.*, **16**, 301–307 (2013).

20) Wang L, Li Y, Yang X, Yuan H, Li X, Qi M, Chang YW, Wang C, Fu W, Yang M, Zhang J, Han B. ERG-SOX4 interaction promotes epithelial–mesenchymal transition in prostate cancer cells. *Prostate*, **74**, 647–658 (2014).

21) Herman JG, Meadows GG. Increased class 3 semaphorin expression modulates the invasive and adhesive properties of prostate cancer cells. *Int. J. Oncol.*, **30**, 1231–1238 (2007).

22) Cole-Healy Z, Vergani P, Hunter K, Brown NJ, Reed MW, Staton CA. The relationship between semaphorin 3C and microvessel density in the progression of breast and oral neoplasia. *Exp. Mol. Pathol.*, **99**, 19–24 (2015).

23) Olar A, Sulman EP. Molecular markers in low-grade glioma-toward tumor reclassification. *Semin. Radiat. Oncol.*, **25**, 155–163 (2015).

24) Kahlert UD, Nikkhah G, Maciaczyk J. Epithelial-to-mesenchymal(-like) transition as a relevant molecular event in malignant gliomas. *Cancer Lett.*, **331**, 131–138 (2013).

25) Wong JW. MicroRNA-induced silencing of glioma progression. *J. Neurosci.*, **30**, 3868–3869 (2010).

26) Urbich C, Kaluza D, Fromel T, Knau A, Bennwitz K, Boon RA, Bonauer A, Doebele C, Boeckel JN, Hergenreider E, Zeiher AM, Kroll J, Fleming J, Dimmel M. MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A. *Blood*, **119**, 1607–1616 (2012).

27) Yan-Chun L, Hong-Mei Y, Zhi-Hong C, Qing H, Yan-Hong Z, Ji-Fang W. MicroRNA-192-5p Promote the proliferation and metastasis of hepatocellular carcinoma cell by targeting SEMA3A. *Appl. Immunohistochem. Mol. Morphol.*, **25**, 251–260 (2017).

28) Ghanbari R, Mosakhani N, Asadi J, Nourace N, Mowla SJ, Yazdani Y, Mohamadkhanh A, Poustchi H, Knuttila S, Malekzadeh R. Downregulation of plasma MiR-142-3p and MiR-26a-5p in patients with colorectal carcinoma. *Iranian Journal of Cancer Prevention*, **8**, e2329 (2015).

29) Chen FF, Lou YJ, Chen J, Jin J, Zhou JN, Yin XF, Zhu JZ, Hu C, Yu MX, Wang HP, Jin J. Absence of miR-142 mutation in Chinese patients with acute myeloid leukemia. *Leuk. Lymphoma*, **55**, 2961–2962 (2014).

30) Zhang X, Yan Z, Zhang J, Gong L, Li W, Cui J, Liu Y, Gao Z, Li J, Shen L, Lu Y. Combination of hsa-miR-375 and hsa-miR-142-5p as a predictor for recurrence risk in gastric cancer patients following surgical resection. *Ann. Oncol.*, **22**, 2257–2266 (2011).

31) Ouyang Q, Xu L, Cui H, Xu M, Yi L. MicroRNAs and cell cycle of malignant glioma. *Int. J. Neurosci.*, **126**, 1–9 (2016).