JBUON 2021; 26(5): 2074-2083
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Serpin peptidase inhibitor, clade E nexin group 1 promotes cellular proliferative capacities and malignant behaviors in glioblastoma through upregulating hairy and enhancer of split-1

Tao Ma[1], Kai Qian[1], Tuoye Xu[1], Xi Wang[2], Tengda Qian[1], Jing Shi[1], Lixin Li[1]

[1]Department of Neurosurgery, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, Nanjing, Jiangsu, China [2]Department of Intensive Care Unit, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, Nanjing, Jiangsu, China.

Summary

Purpose: *Glioblastoma (GBM) remains one of the most fatal malignancy with limited available treatment. Serpin peptidase inhibitor, clade E nexin group 1 (SERPINE1) was found up-regulated in multiple cancers and play crucial roles in facilitating tumor progression and metastasis respectively. However, the role of SERPINE1 in glioblastoma was poorly understood.*

Methods: *We tested the hypothesis that SERPINE1 mediated malignant behaviors in GBM via regulating hairy and enhancer of split-1 (HES1).*

Results: *First, SERPINE1 is confirmed to be up-regulated in GBM, while further functional analysis demonstrated that SERPINE1 promoted cell proliferation, migration and invasion in GBM by performing the CCK-8 assay, colony formation assay, wound healing assay and transwell assay. Finally, it was proved that SERPINE1 achieved its pro-tumor functions in GBM via regulating the expression of HES1.*

Conclusions: *Collectively, our results highlight the critical contribution of SERPINE1 in a series of malignant characteristics of GBM via regulating the expression of HES1, which shed new light on a new direction to develop a more effective therapeutic management of malignant tumors like GBM.*

Key words: *SERPINE1- HES1-Glioblastoma-proliferation-migration-invasion*

Introduction

Glioblastoma (GBM) is one of the most common tumors of the central nervous system, which accounts for 80% of all malignant tumors in the US [1-3]. Despite recent progress in chemotherapy and immunotherapy, the median survival of GBM patients is less than one year [4]. One of the reasons could be that the expression pattern of oncogenes and tumor suppressor genes involved in GBM development is still barely understood. Understanding the molecular mechanisms is helpful in improving diagnosis rate, providing therapeutic strategies, and prolonging patient survival.

GBM is characteristic by high permeability of tumor blood vessels and fast proliferative endothelial cells [5], suggesting that there might be a relevance between extracellular matrix (ECM) dysregulation and glioma invasion. Serpin peptidase inhibitor, clade E nexin group 1 (SERPINE1), or plasminogen activator inhibitor 1 (PAI-1), is a secreted protein that inhibits the urokinase type plasminogen activator receptor (uPAR) and tissue plasminogen activator (PLAT) , which is associated with ECM proteolysis and intracellular cell signaling activation, leading to enhanced tumor metas-

Corresponding author: Lixin Li, PhD. Department of Neurosurgery, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, 300 Guangzhou Rd, Nanjing 210029, Jiangsu, China
Tel: +86 13851678682; Email: lilixin129159@163.com
Received: 15/06/2021; Accepted: 29/07/2021

tasis [6-8]. As one of the main regulators in the PA system, SERPINE1 plays important roles in tumor proliferation, migration and invasion [9]. In several tumor types, especially in head and neck carcinoma [1,10], the SERPINE1 expression is up-regulated, and validated as a poor prognostic marker [11,12]. Furthermore, it is verified as a predictor of therapeutic efficacy on different therapeutic methods in node-negative breast cancer patients [13,14]. Previous publications demonstrated that overexpression of PAI-1 in GBM is significantly correlated with shorter survival [15]. However, inside the molecular basis of the interplay between SERPINE1 and GBM remains unclear.

In this study, we confirmed that the up-regulation of SERPINE1 is related in glioma tumor and cell lines, thereby aiming at defining its impact on the malignant phenotype of glioma cells via mediating the expression of Hes1, which helps provide insight into molecular pathways of GBM development, and a more effective treatment at eliminating GBM cells.

Methods

Bioinformatic exploration

GBM datasets were retrieved from the Gliovis (http://gliovis.bioinfo.cnio.es/) including Rembrandt, Gravendeel, Phillips, Gill, Freije, Reifenberger, Ducray, Walsh, Nutt, Grzmil, Murat and TCGA website (http://tcgadata.nci.nih.gov/docs/publications/coadread_2012/), CGGA website (http://www.cgga.org.cn/) and GEO database (https://www.ncbi.nlm.nih.gov/geo/) for Cancer Sample. These four sets of databases were applied to compare the SERPINE1 expression profile between the GBM cancer and normal tissues.

Cell culture and patient sample

Glioma cell lines LN229, U87, GBM8401 and U251 and NHA cell line (normal human astrocyte) were purchased directly from American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and humidified with 5% CO_2 at 37°C. By reaching 80% confluence, cell lines were subcultured.

Immunoblot analysis

Total cell lysates were extracted by lysis buffer. The proteins were quantified and equal amounts of protein were electrophoresed, transferred onto a polyvinylidene difluoride membrane and blocked with 5% skimmed milk at 4°C. Then all membranes were incubated with Anti-SERPINE1 (1:1000), anti-E-cadherin (1:1000), anti-N-cadherin (1:1000), anti-HES1 (1:1000), anti-MMP2 (1:1000), anti-MMP9 (1:1000) and anti-β-actin (1:1000) antibodies overnight at 4°C. Then, the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit antibody (1:5,000 dilution in TBS; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The brands were visualized via chemiluminescence using an electrochemiluminescence (ECL) detection kit (Amersham Inc., Arlington Heights, IL, USA). All antibodies were purchased from Cell Signaling Technology (Inc., Danvers, MA, USA).

Generation of SERPINE1 knockdown cell lines

The effective shRNA targeting SERPINE1 was synthesized by GenePharma (Shanghai, China). 2×10^5 cells were seeded in a 60 mm culture dish before transfection. 4 μg nmol shRNA were adiministered using the LipofectamineTM RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA). siRNA targeting HES1 was synthesized by Ruibo (Guangzhou, China). 48 h later, the experiment was conducted followed by subsequent assays.

Establishing stable SERPINE1 overexpressing glioma cell lines

The full length of SERPINE1 was cloned into a pGL3.0-basic vector. LipofectamineTM reagent (Invitrogen, Carlsbad, CA, USA) was used for transfection. Cells were transferred to G418 medium for selection after 24-h incubation.

Cell proliferation assay

The cell proliferation rate was determined by CCK-8 analysis. Cells lines were added with culture medium containing 10% (v/v) CCK-8 for 1 h and repeated after 24, 48 and 72 h of seeding in the range of 3.5×10^4 cells/well in 6-well plates with growth culture medium and detected the absorbance value at 450 nm.

In vitro assay of invasive activity

The invasive activity of glioma cell lines was assessed using a rapid Transwell technique assay and Wound healing assay as described previously [16]. In brief, for transwell assay, 200 μL of cell suspension (3×10^4/mL) was added to each well coated with Matrigel (Becton- Dickinson, Franklin Lakes, NJ, USA). The culture with 10% FBS was placed in the lower chamber. After 20-h incubation at 37°C, cells invading to the lower chamber were fixed with 100% methanol for 2 mins, followed by staining with 0.5% crystal violet for 5 min. Cells on the undersides of the filters were observed.

Wound healing assay

Cells were plated on 6-well culture plates (Corning, NY, USA), followed by scratching with a P-200 pipette tip. After 24 h the width of the scratch was detected and the migration rate was quantified.

Statistics

Statistical analyses were performed using the SPSS v.22 (IBM Corporation, Armonk, NY, USA) software. The significance of mean values between two groups were analyzed using two-tailed unpaired Student's t-test. The

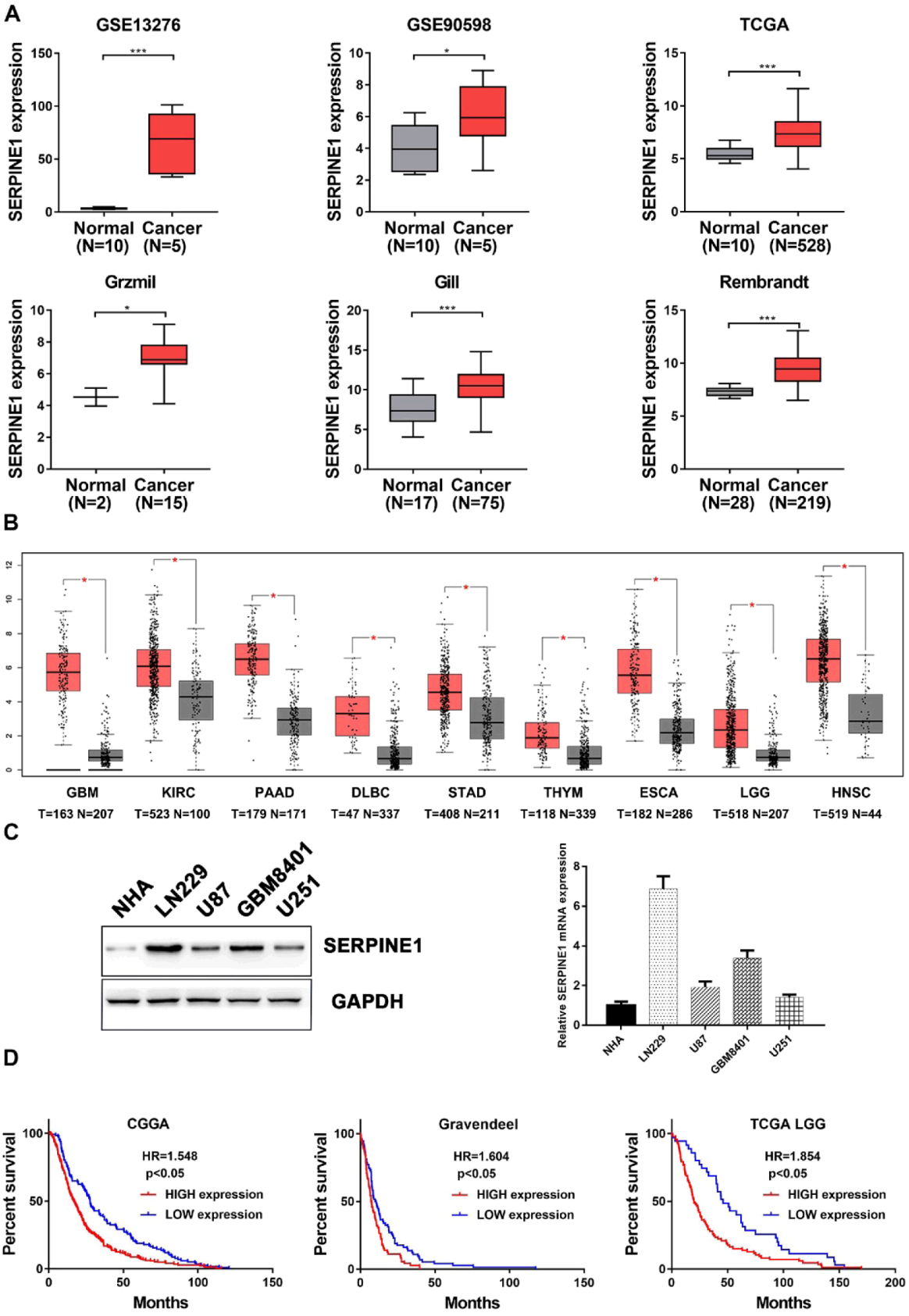

Figure 1. SERPINE1 is significantly upregulated in glioblastoma. **A:** Analysis of the expression pattern of SERPINE1 in normal brain tissue and glioblastoma based on the microarray datasets from GEO, TCGA and Gliovis. **B:** Analysis of the expression pattern of SERPINE1 in other types of cancers from TGCA database. **C:** Protein and mRNA level of SERPINE1 in NHA cell line (normal human astrocyte) and four GBM cell lines detected by western blot and RT-PCR. **D:** Kaplan-Meier analysis of overall survival in GBM patients based on SERPINE1 expression. Data are presented as mean ± SEM (*p<0.05, ***p<0.001).

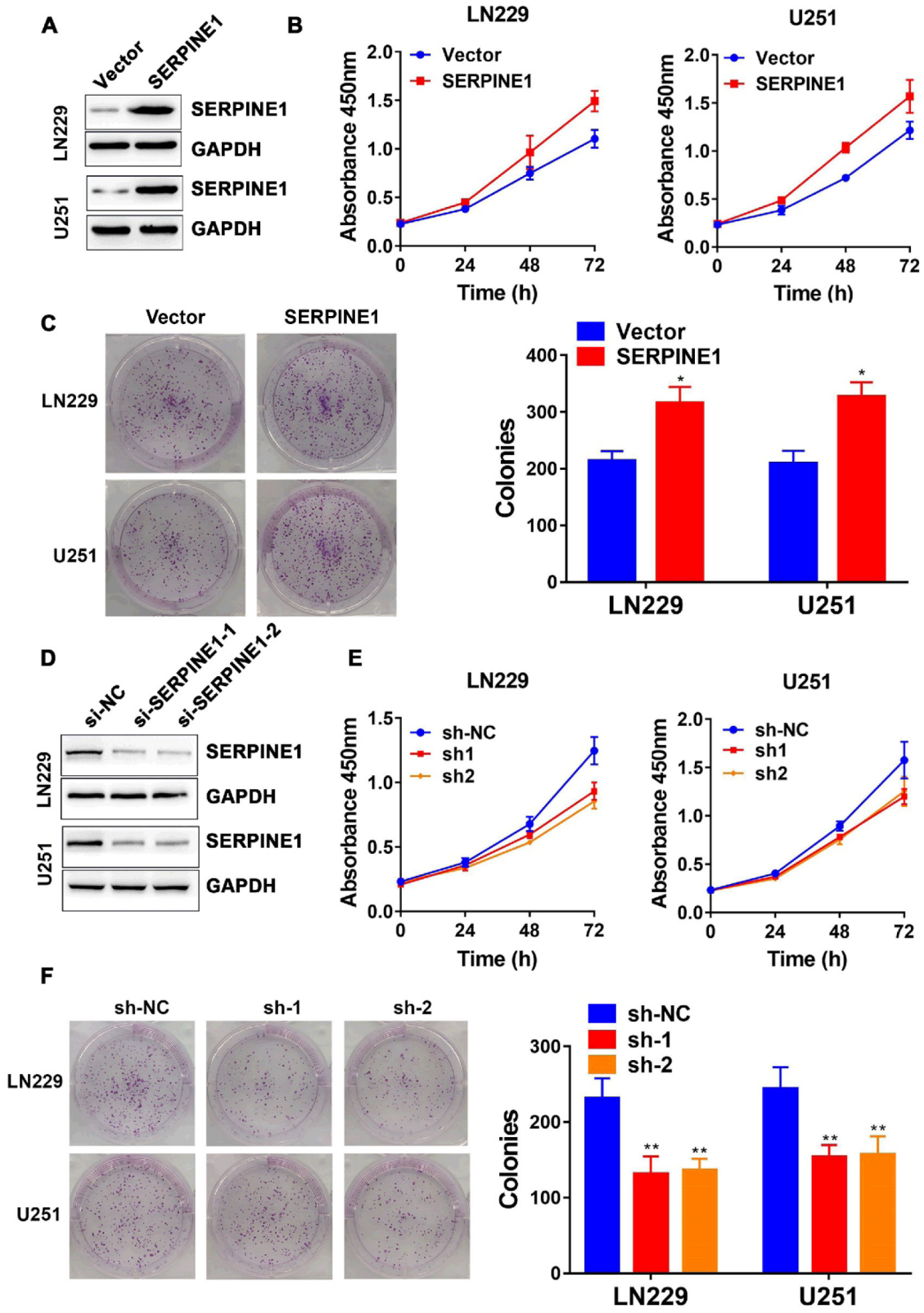

Figure 2. The expression of SERPINE1 is essential for proliferation of glioblastoma. **A:** Western blot analysis of SERPINE1 in constructed cell lines LN229 and U251 with higher expression of SERPINE1. Vector represents transfection of the pcDNA3 vector served as control groups. SERPINE1 represents establishing stable SERPINE1 overexpression groups. **B and C:** The relative growth rates were measured using CCK8 analysis and colony formation assay in SERPINE1 overexpressed cell lines and control groups. Data are presented as mean ± SEM. *p<0.05; **p<0.01. **D:** Western blot and RT-PCR analysis of SERPINE1 in constructed cell lines U251 with lower expression of SERPINE1. Sh-NC represents Negative Control shRNA. Sh-1 and Sh-2 represents two shRNAs targeting SERPINE1. **E and F:** The relative growth rates were measured using CCK8 analysis and colony formation assay in SERPINE1 silenced cell lines and control groups. Data are presented as mean ± SEM (*p<0.05, **p<0.01).

significance of mean values between multiple groups was analyzed using one-way analysis of variance (ANOVA). The correlation of the two variables was analyzed by Spearman rank correlation coefficient. Differences were considered significant at p values<0.05 in all the applied tests. Experiments were conducted in triplicate and three independent experiments were carried out. Data are presented as the mean ± SEM.

Results

SERPINE1 expression is upregulated in glioblastoma and predicts poor survival

In our previous study, we identified differential gene expression and pathway in GBM based on GSE13276 and GSE90598 by bioinformatics analysis. Among the different expressed genes, we found SERPINE1was higher expressed in GBM tissues than in normal tissues. To further explore the role of SERPINE1 in GBM, we analyzed the expression of SERPINE1 in other microarray datasets of Gliovis, CGGA and TCGA (Figure 1A and 1B) and found SERPINE1 was consistently highly expressed in GBM of GSE13276, GSE90598, TCGA, Grzmil, Gill and Rembrandt datasets. Furthermore, the expression of SERPINE1 was also upregulated in other types of cancers, including KIRC, PAAD, DLBC, STAD, THYM, ESCA, LGG, and HNSC. To validate the discovery above, we assessed the expression level of SERPINE1 in 4 glioma cell lines (LN229, LN229, GBM8401 and U251) and a normal human astrocyte cell line (NHA). As expected, the expression of SERPINE1 in malignant cell lines was higher than in normal human astrocyte cell line at both protein level and mRNA level (Figure 1D and 1E). Conducted survival analysis of SERPINE1 on GBM prognosis found that the upregulated of SERPINE1 was related to poor overall survival (Figure 1E). These results indicated that SERPINE1 is upregulated in GBM and might influence the glioma malignancy.

SERPINE1 promotes the proliferation of glioblastoma

Considering the unique properties of SERPINE1 in modulating collagen production and its location on the cell membrane in various types of cancers, the expression of SERPINE1 may have kinetic functions in tumor progression. We constructed SERPINE1 overexpressed glioma cell lines (Figure 2A) and CCK8 assays showed that the SERPINE1-overexpressing cells achieved faster proliferative ability (Figure 2B). Moreover, overexpressing SERPINE1 could remarkably enhance the colony formation ability (Figure 2C). On the contrary, SERPINE1 knockdown of glioma cell lines was established using shRNAs (Figure 2D). Inhibition of SERPINE1 markedly impaired the proliferation rate of glioma cell lines as detected by CCK-8 and colony formation assays (Figure 2E and 2F). These results indicated that SERPINE1 had a pro-tumor function in GBMs.

SERPINE1 promotes the migration and invasion of glioblastoma

We next sought to determine whether the ectopic over-expression or inhibition of SERPINE1 could affect cell migration and invasion by performing wound healing and transwell invasion assay to examine migration and invasion of SERPINE1-overexpression and silenced GBM cells. The numbers of invaded cells and the wound healing rate increased significantly in SERPINE1-overexpression groups (Figure 3A-3D), and decreased in SERPINE1-silenced LN229 and U251 cells compared with the control group (Figure 3E-3H).

Due to SERPINE1 associations with epithelial-to-mesenchymal transition (EMT) and metastasis-related genes targeted by EMT [17], we detected the expression of EMT transition markers in constructed cell lines LN229 and U251 by Western blot, of which the results showed that overexpression of SERPINE1 upregulated the levels of N-Cadherin, MMP2 and MMP9, and downregulated the level of epithelial marker E-Cadherin (Figure 3I). Collectively, these results showed that the expression of SERPINE1 significantly motivated GBM migration and invasion.

SERPINE1 mediates malignant behaviors in glioblastoma via up-regulating HES1

To determine the downstream target genes of SERPINE1 we resorted Gliovis platform where the expression values were regained from the published Nutt Brain microarray dataset including SERPINE1, PLIN2, LDHA, HES1, etc. Performed on the plotted mRNA expression values, the expression of SERPINE1 and its co-expressed genes in Nutt Brain GBLs specimens were measured by Spearman correlation analysis, which revealed a moderate correlation (r=0.6798, p<0.0001) between them (Figure 4A). Observing deeper into the correlations between SERPINE1 and these related genes in this heatmap, HES1, one of downstream targets of Notch signaling pathways, which plays an important role in cell fates and many cancers [4,18,19], could be one of the potential downstream target genes of the SERPINE1. Further analysis of SERPINE1 and HES1 in Nutt Brain cancer cases disclosed a significant correlation between them presented in Figure 4B. (r= 0.6798, p<0.0001). Moreover, SERPINE1 expression was positively correlated with HES1 expression in other microar-

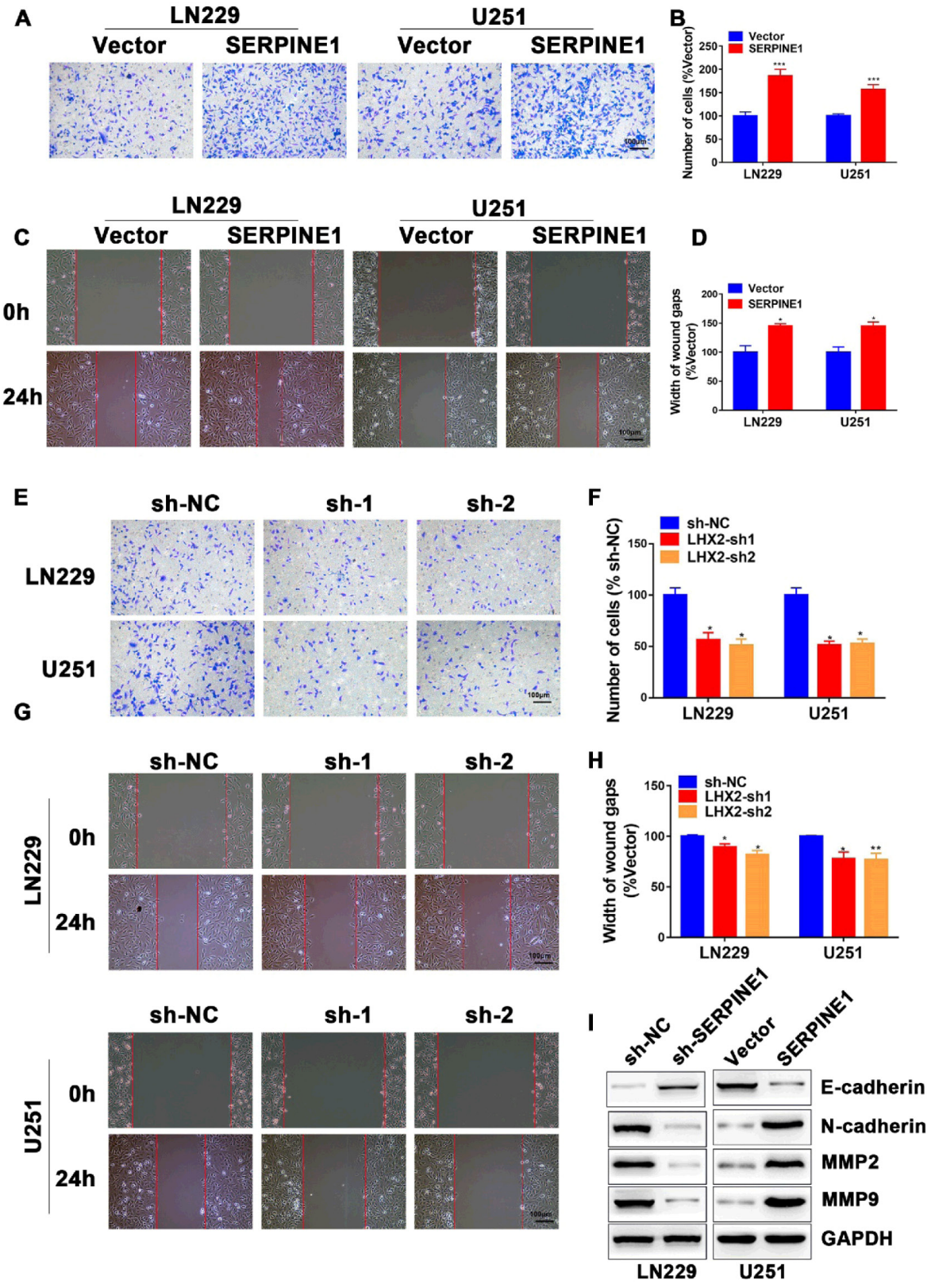

Figure 3. SERPINE1 promotes the migration and invasion of glioblastoma cells. **A and B:** The transwell invasion assay was evaluated of constructed LN229 and U251 compared between vector and SERPINE1 overexpression groups. **C and D:** wound healing assay of LN229 and U251 cells between vector and SERPINE1 overexpression groups. **E and F:** The transwell invasion assay was evaluated of constructed LN229 and U251 compared between sh-NC and SERPINE1 knockdown groups. **G and H:** wound healing assay of LN229 and U251 cells between sh-NC and SERPINE1 knockdown groups. **I:** Effects of constructed LN229 with silencing SERPINE1 and U251 with ectopic expressing SERPINE1 on E-cadherin, N- cadherin, MMP2 and MMP9 were determined by Western blot analysis (*p<0.05, **p<0.01, ***p<0.001).

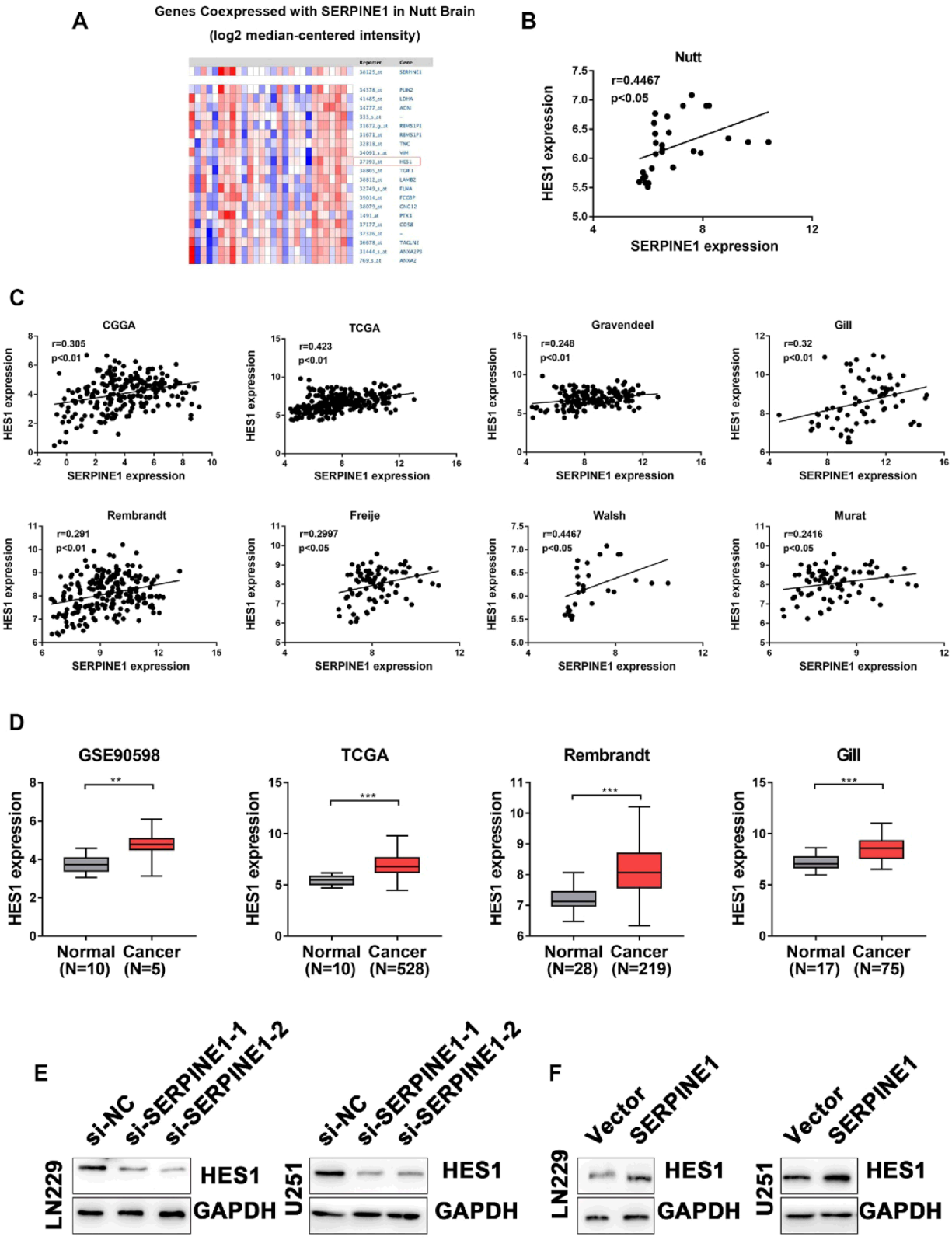

Figure 4. SERPINE1 up-regulating HES1 in GBM. **A:** Heat map from an oncomine (https://www.oncomine.org) microarray dataset of expression profiles for SERPINE1, PLIN2 LDHA HES1, etc. in Nutt brain dataset. **B:** SERPINE1 and HES1 relative expression were plotted on the basis of expression values. Spearman rank correlation coefficients between relative expression of HES (y-axis) and SERPINE1 (x-axis) in Nutt brain dataset were analyzed. **C:** SERPINE1 and HES1 relative expression were plotted on the basis of expression values. **D:** The expression level of HES1 in normal brain tissue and GBM, based on the microarray datasets from Gliovis, GEO and TCGA. **E:** HES1 expression of constructed LN229 and U251 cell lines infecting with SERPINE1 overexpressing plasmid and vector was determined by Western blot. **F:** HES1 expression of constructed LN229 and U251 cell lines infecting with two separated siRNA mediated SERPINE1 was determined by Western blot (*p<0.01, ***p<0.001).

ray dataset of GBM, including CGGA, TCGA, Gravendeel Rembrandt, Freije, Walsh, Murat datasets (Figure 4C). As expected, HES1 expression was much higher in GBM tissues than in normal tissues in GSE90598, TCGA, Rembrandt and Gill datasets (Figure 4D). In order to experimentally confirm this finding, Western blotting was performed to verify the regulatory role of SERPINE1 on HES1 expression levels. The results showed that overexpression of SERPINE1 increased the HES1 expression, while, in contract, SERPINE1 knockdown decreased HES1 expression (Figure 4E and 4F), which indicated that SERPINE1 might be the upstream regulator of HES1.

We next tested the HES1-mediated SERPINE1-induced proliferation, migration and invasion of GBM cells. LN229 cell was co-transfected with SERPINE1 overexpression plasmid and HES1 siRNA. CCK8 and colony formation assays were performed to determine the proliferation of LN229 in different transfected groups. As expected, overexpression of SERPINE1facilitated the proliferative ability, which could be partially abolished by the introduction of HES1 siRNA (Figure 5A and 5B). In addition, The SERPINE1-induced EMT of GBM cells could also be blocked by HES1 silencing (Figure 5C). What's more, the migration and invasion of LN229 cells was promoted by SERPINE1, which

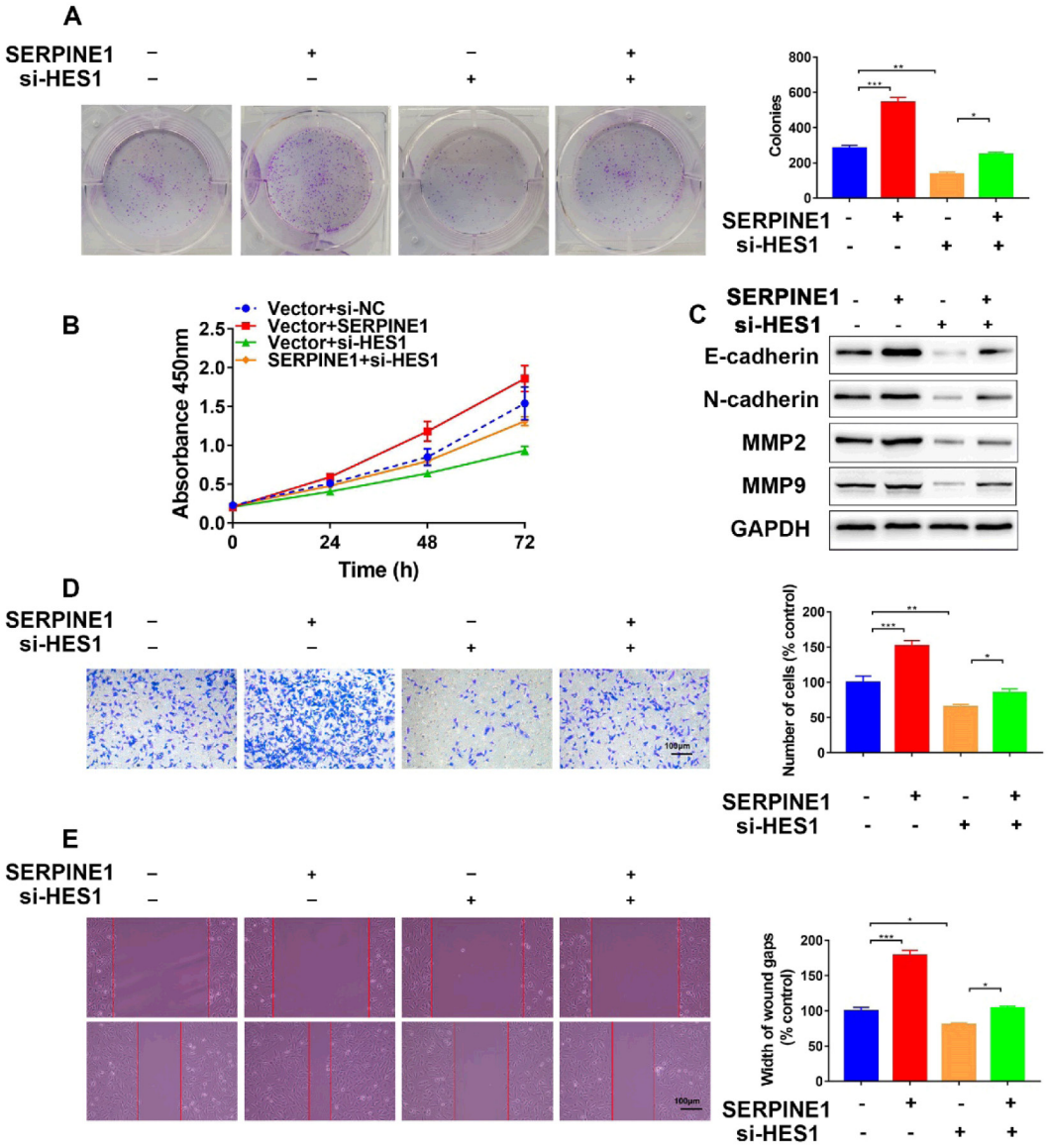

Figure 5. SERPINE1 mediates malignant behaviors in glioblastoma via up-regulating HES1. LN229 and U251 cell line was co-infected with SERPINE1 overexpression plasmid and HES1 siRNA. The relative growth rates were measured by CCK8 analysis **(A)** and colony formation assay **(B)**. The EMT-related protein levels were determined by Western blot **(C)**. The invasion and migration were measured by transwell invasion assay and wound healing assay. **(D and E)** Data are presented as mean ± SEM (*p<0.05, **p<0.01, ***p<0.001).

could be re-stained by HES1 silencing (Figure 5D and 5E). These results strongly suggested that the regulation of HES1 by SERPINE1 could markedly boost proliferation, migration and invasion in GBM cell lines.

Discussion

Glioblastoma multiforme (GBM) cells are highly resistant to chemotherapy and radiation, therefore a deeper understanding of the signaling pathways of GBM carcinogenesis is necessary for effectively eliminating the tumor [20-22]. In light of this, large-scale efforts should be taken in characterizing the genetic alterations in GBM which would reveal definite targets of its progression and invasion to treat tumors more precisely. In our study, we found that the expression of SERPINE1 was up-regulated in GBM than in normal brain tissue retrieved from the online database. However, further studies are still needed to explore the underlying mechanisms. Therefore, we performed a series of *in vivo* and *in vitro* studies, the results of which demonstrated that SERPINE1 manipulated malignant behaviors in GBM by enhancing the proliferative potential and invasion of GBM cell lines.

The mechanism underlying the regulation of SERPINE1 overexpression in GBM remains unclear at present. In consideration of the analysis of heat map of expression profiles (Figure 4A), we focused on HES1, targeted by Notch signaling pathways, which induced proliferation, increased cell growth, and enhanced colony-forming ability [23]. Previous studies have identified that *in vitro* Notch may directly suppress Hedgehog via HES1 mediated inhibition of Gli1 transcription, and targeting both pathways simultaneously may be more effective at eliminating GBM cells [24]. Meanwhile, up-regulation of Notch signaling is involved in the chemotherapy resistance and tumor recurrence contributing to GBM stem cells [25,26]. Moreover, Notch1 regulates the aggressive phenotypes of DTC, which could be mediated by SERPINE1 inhibition [27], revealing that Notch1/SERPINE1 axis may be served as a novel therapeutic target for carcinoma.

We hypothesized that HES1 may be a downstream molecule regulated by SERPINE1 in GBM. Our experiments showed that the migration and invasion of constructed SERPINE1- silencing GBM cell lines were all rescued and significantly increased when co-transfected with HES1–overexpressing plasmids. The results collectively supported an important role of SERPINE1 in mediating HES1 in GBM. However, we cannot exclude the possibility of crosstalk between SERPINE1 and any related protein targets of Notch signaling pathways, further studies will be needed. Whether other related molecules have the same or similar role in GBM remains to be investigated.

Conclusions

In summary, a significant overexpression of SERPINE1was observed in glioblastoma. Further studies revealed a critical role of SERPINE1 in the development and progression of GBM via up-regulating HES1. Our data may also introduce a potential crosstalk between SERPINE1 and activation of Notch signaling in tumors like GBM. Collectively, our findings provided evidence that SERPINE1 served as a useful independent prognostic factor and a novel therapeutic target to more effectively eliminate tumors for GBM patient.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81171147].

Conflict of interests

The authors declare no conflict of interests.

References

1. Ostrom QT, de Blank PM, Kruchko C et al. Alex's Lemonade Stand Foundation Infant and Childhood Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2007-2011. Neuro Oncol 2015;16 Suppl 10:x1-36.
2. Kast RE. Adding perphenazine to increase effectiveness of standard glioblastoma chemoirradiation. JBUON 2020;25:1676-86.
3. Tarassishin L, Lim J, Weatherly DB, Angeletti RH, Lee SC. Interleukin-1-induced changes in the glioblastoma secretome suggest its role in tumor progression. J Proteomics 2014;99:152-68.
4. Narayanappa R, Rout P, Aithal MG, Chand AK. Aberrant expression of Notch1, HES1, and DTX1 genes in glioblastoma formalin-fixed paraffin-embedded tissues. Tumour Biol 2016;37:6935-42.

5. Alexiou GA, Kyritsis AP. Combination treatment for glioblastoma with temozolomide, DFMO and radiation. JBUON 2019;24:397-404.
6. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. Nat Rev Mol Cell Biol 2010;11:23-36.
7. Margheri F, Luciani C, Taddei ML et al. The receptor for urokinase-plasminogen activator (uPAR) controls plasticity of cancer cell movement in mesenchymal and amoeboid migration style. Oncotarget 2014;5:1538-53.
8. Mazar AP. Urokinase plasminogen activator receptor choreographs multiple ligand interactions: implications for tumor progression and therapy. Clin Cancer Res 2008;14:5649-55.
9. Ulisse S, Baldini E, Sorrenti S, D'Armiento M. The urokinase plasminogen activator system: a target for anticancer therapy. Curr Cancer Drug Targets 2009;9:32-71.
10. Sang Y, Chen MY, Luo D et al. TEL2 suppresses metastasis by down-regulating SERPINE1 in nasopharyngeal carcinoma. Oncotarget 2015;6:29240-53.
11. Duffy MJ, McGowan PM, Gallagher WM. Cancer invasion and metastasis: changing views. J Pathol 2008;214:283-93.
12. Speleman L, Kerrebijn JD, Look MP, Meeuwis CA, Foekens JA, Berns EM. Prognostic value of plasminogen activator inhibitor-1 in head and neck squamous cell carcinoma. Head Neck 2007;29:341-50.
13. Harris L, Fritsche H, Mennel R et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.
14. Harbeck N, Schmitt M, Meisner C et al. Ten-year analysis of the prospective multicentre Chemo-N0 trial validates American Society of Clinical Oncology (ASCO)-recommended biomarkers uPA and PAI-1 for therapy decision making in node-negative breast cancer patients. Eur J Cancer 2013;49:1825-35.
15. Colin C, Voutsinos-Porche B, Nanni I et al. High expression of cathepsin B and plasminogen activator inhibitor type-1 are strong predictors of survival in glioblastomas. Acta Neuropathol 2009;118:745-54.
16. Wang T, Zhang Z, Wang K et al. Inhibitory effects of BMP9 on breast cancer cells by regulating their interaction with pre-adipocytes/adipocytes. Oncotarget 2017;8:35890-901.
17. Ryu SH, Heo SH, Park EY et al. Selumetinib Inhibits Melanoma Metastasis to Mouse Liver via Suppression of EMT-targeted Genes. Anticancer Res 2017;37:607-14.
18. Strazzabosco M, Fabris L. Development of the bile ducts: essentials for the clinical hepatologist. J Hepatol 2012;56:1159-70.
19. Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol 2006;7:678-89.
20. Phillips HS, Kharbanda S, Chen R et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157-73.
21. Parsons DW, Jones S, Zhang X et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
22. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-8.
23. Jeon HM, Kim SH, Jin X et al. Crosstalk between glioma-initiating cells and endothelial cells drives tumor progression. Cancer Res 2014;74:4482-92.
24. Schreck KC, Taylor P, Marchionni L et al. The Notch target Hes1 directly modulates Gli1 expression and Hedgehog signaling: a potential mechanism of therapeutic resistance. Clin Cancer Res 2010;16:6060-70.
25. Yu JB, Jiang H, Zhan RY. Aberrant Notch signaling in glioblastoma stem cells contributes to tumor recurrence and invasion. Mol Med Rep 2016;14:1263-8.
26. Zhang JF, Chen Y, Qiu XX et al. The vascular delta-like ligand-4 (DLL4)-Notch4 signaling correlates with angiogenesis in primary glioblastoma: an immunohistochemical study. Tumour Biol 2016;37:3797-805.
27. Yu XM, Jaskula-Sztul R, Georgen MR et al. Notch1 Signaling Regulates the Aggressiveness of Differentiated Thyroid Cancer and Inhibits SERPINE1 Expression. Clin Cancer Res 2016;22:3582-92.