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Overexpressed KDM5B is associated with the progression of glioma and promotes glioma cell growth via downregulating p21

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ABSTRACT

Epigenetic alterations such as aberrant expression of histone-modifying enzymes have been implicated in tumorigenesis. Upregulation of lysine (K)-specific demethylase 5B (KDM5B) has been reported in a variety of malignant tumors. However, the impact of KDM5B in glioma remains unclear. The objective of this study was to investigate the expression and prognostic value of KDM5B in glioma. In clinical glioma samples, we found that KDM5B expression was significantly upregulated in cancer lesions compared with normal brain tissues. Kaplan–Meier analysis showed that patients with glioma and higher KDM5B expression tend to have shorter overall survival time. By silencing or overexpressing KDM5B in glioma cells, we found that KDM5B could promote cell growth both in vitro and in vivo. Moreover, we demonstrated that KDM5B promoted glioma proliferation partly via regulation of the expression of p21. Our study provided evidence that KDM5B functions as a novel tumor oncogene in glioma and may be a potential therapeutic target for glioma management.

1. Introduction

Gliomas are the most common brain tumors. Numerous studies indicate that the development and progression of glioma arises via miss-regulation of many related genes such as p53, p21, Akt, Gli1, and PTEN [1–6]. However, the regulatory mechanisms remain poorly understood. Therefore, discovery of critical carcinogenic pathways may be beneficial for the identification of new therapeutic targets for glioma.

Epigenetic regulation also plays a critical role in the pathogenesis of glioma [7,8]. DNA methylation is a component of the epigenetic gene-silencing complex, whereas histone (H3 and H4) post-translational modifications comprise a ubiquitous component of rapid epigenetic changes [9–11]. Epigenetic changes are associated with altered transcription. Histone methylation/demethylation generally deactivates and activates genes by controlling the access of transcription factors to DNA [10]. Histone dysregulation caused by genetic and epigenetic alterations is a hallmark of cancer [12]. KDM5B-mediated histone H3K4 demethylation contributes to the silencing of retinoblastoma target genes in senescent cells, presumably by compacting chromatin and silencing certain genes [13,14]. Previous studies have found that KDM5B depletion stimulated p16 and p21 transcription and suppressed tumor cell growth in vitro and in vivo [15,16], suggesting that it plays a role in cell growth regulation in human cancer.

In the present study, we aimed to explore the tumor oncogene function of KDM5B in glioma. We found that the expression of KDM5B was significantly up-regulated in human glioma lesions compared with normal brain tissues. We also demonstrated that KDM5B could promote the growth of glioma cell lines both in vitro and in vivo. Our present manuscript suggests that KDM5B acts as a potential oncogene in glioma.

2. Materials and methods

2.1. Patients and tissue samples

A total of 53 glioma tissue and 9 normal brain tissue samples were used in this study. All of the samples were obtained from the department of neurosurgery, Beijing Shijitan Hospital, Capital Medical University between 2010 and 2013. For all of the patients who participated in this study, written informed consent was obtained, which was approved by the Ethical Committee of Capital Medical University.

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2.2. Immunohistochemistry

Paraffin-embedded sections were deparaffinized, blocked, and incubated with antibody at 4 °C overnight. Horse-radish peroxidase-conjugated secondary antibody (1:500) was then added and further incubated for 1 h at room temperature. The sections were developed using a 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate kit (ThermoScien-tific, MA) at room temperature for 1–5 min and then counterstained with hematoxylin. The intensity of the immunostaining was categorized as follows: no brown particle staining (0), light brown particle (1), moderate brown particle (2), and dark brown particle (3). The extent of immunostaining was quantified by counting the percentage of positive cells and classified into four groups: 0, less than 25% positive cells; 1, 25–50% positive cells; 2, 51–75% positive cells; and 3, more than 75% positive cells. The sum of the extent and intensity scores was defined as staining index (SI). SI less than 3 was considered as a low expression, while SI of 4 or more was considered as a high expression. To avoid interindividual bias of IHC staining differentiations, all slides were determined by two experienced pathologists.

2.3. Cell culture and reagents

The glioma cell lines (SW1783, U-87, LN-18, Hs683, and T98G) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Antibodies that had been raised against KDM5B and p21 were obtained from Abcam (Cambridge, MA, USA). The signal silence p21 siRNA and its control siRNA were purchased from Sigma–Aldrich (St. Louis, MO, USA). All of the remaining reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise specified.

2.4. Plasmid construction and transfection

For overexpression, the cDNA representing the complete open reading frame of KDM5B was cloned into the pBabe vector to generate the KDM5B expression plasmid. The expression plasmid was verified by sequencing both strands and was used to transfect the SW1783 cells to establish the KDM5B overexpression cell line. For KDM5B RNA interference, the control (pSuper) and pSuper-shKDM5B plasmids were purchased from OligoEngine Biotechnology (Seattle, USA) and was used to transfect the LN-18 cells to establish the KDM5B knockdown cell line. The transfection efficiency of KDM5B was confirmed by Western blotting and quantitative reverse transcription PCR (qRT-PCR) analyses.

2.5. MTT Assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay was used to assess cell proliferation. The cells were seeded and 20 ml of the MTT solution (5 mg/ml) was then added to each well at the indicated time. The absorbance at 490 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.6. Colony formation assay

The cells were seeded in 6-cm dishes at a density of 300 cells per dish. After incubation for 14 days, the colonies were fixed with methanol for 10 min and stained with crystal violet for 15 min, after which point the number of colonies containing more than 50 cells was scored.

2.7. Western blot assay

Equal amounts of protein were separated using SDS polyacrylamide gels and were electrotransferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were immunoblotted overnight at 4 °C with primary antibodies, followed by their respective secondary antibodies. β-Actin was used as the loading control.

2.8. Quantitative reverse-transcription PCR

RNA was extracted using TRIzol reagent, according to the manufacturer's recommended protocol (Invitrogen). qRT-PCR was performed using Applied Biosystems (Foster City, CA, USA) StepOne and StepOne Plus Real-Time PCR Systems. GAPDH was used as a loading control. The experiments were repeated a minimum of 3 times to confirm the results.

2.9. Immunofluorescence staining

The cells were grown on the sterile coverslips, and the cells were fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton-X100. Cells were blocked with rabbit anti-Ki67 antibody followed by rhodamine-conjugated anti-rabbit secondary antibody. Finally, the cells were further stained with 4,6-diamidino-2-phenylindole (DAPI).

2.10. In vivo tumorigenesis assays

The in vivo tumorigenesis and metastasis assays were performed, as previously described [17]. Briefly, 1×10^6 cells were injected subcutaneously into the right flanks of severe combined immunodeficient (SCID) mice. Tumor length (L) and width (W) were measured every 3 days, and tumor volume was calculated using the equation: volume = $(W^2 \times L)/2$. After 6 weeks, the mice were killed and the tumor volume and weight were measured. All of the animal experiments were performed with the approval of the Capital Medical University Animal Care and Use Committee.

2.11. Statistical analysis

The results were analyzed using SPSS 18.0 software (Chicago, IL, USA). Each experiment was repeated a minimum of 3 times. A two-tailed t-test was used to determine statistical significance. The results were presented as the means ± S.D. P-values <0.05 were considered to be statistically significant.

3. Results

3.1. Expression of KDM5B was upregulated in glioma tissues

To examine the protein level of KDM5B in glioma tissues, IHC was performed (Fig. 1A and B). We observed that the protein expression level of KDM5B was markedly higher in glioma tissues than the level in the normal brain tissues ($P < 0.01$). To investigate the relationship between KDM5B expression and clinicopathological parameters in 53 cases with glioma, these cases were first divided into two subgroups: "low KDM5B expression" and "high KDM5B expression" as defined in the immunohistochemistry section of "Section 2". Significant correlations were found between KDM5B expression and pathological grade ($P = 0.006$) (Supplemental Table 1). There were no statistical connections between KDM5B expression and the rest clinicopathological parameters, such as patient age, gender, tumor size, and tumor locus (all $P > 0.05$). The association between KDM5B expression in glioma and the survival time of selected patients was analyzed with Kaplan–Meier survival analysis (Fig. 1C). The median overall survival time of high KDM5B expression group was significantly shorter than that of low KDM5B expression group ($P < 0.001$).

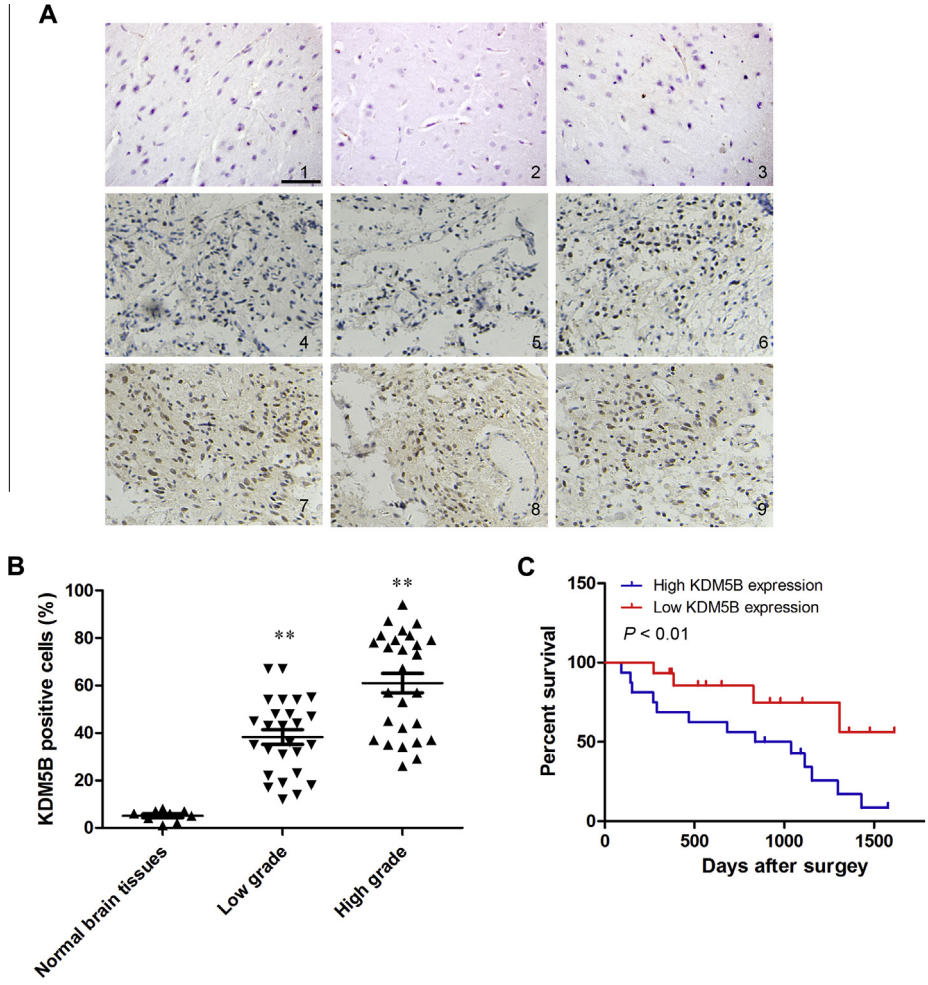

Fig. 1. Expression of KDM5B was upregulated in glioma tissues. (A) Immunohistochemical staining of KDM5B expression in human glioma and normal brain tissue. 1–3 Normal brain tissue. 4–6 Glioma with low grade (pathological grade I–II). 7–9 Glioma with high grade (pathological grade III–IV). (B) Ratio of KDM5B positive cells in normal brain, low-grade glioma, and high-grade glioma tissues. High-grade glioma expressed significantly higher ratio of KDM5B than normal brain and low-grade glioma tissues ($P < 0.01$). (C) Kaplan–Meier analyses of overall survival periods among 53 resected glioma patients are shown to be stratified according to KDM5B expression. $^{**}P < 0.01$ is based on Student's *t*-test. All results are from three or four independent experiments. Error bars indicate standard deviation.

3.2. Establishment of stable KDM5B transfectants in glioma cell lines

As showed in Fig. 2A and B SW1783 had the lowest expression level of KDM5B among the five glioma cell lines. So, we used SW1783 cells to establish a stable cell line that constitutively overexpressed the KDM5B protein with the aim of revealing the role that KDM5B expression has in the development or progression of glioma. We also used shRNA to generate a stable KDM5B knockdown in the LN-18 glioma cell line, which had a high KDM5B expression. The transfection efficiency was confirmed using Western blotting and qRT-PCR analyses. As shown in Fig. 2C and D, the SW1783 cells that had been transfected with the KDM5B expression plasmid displayed significantly increased KDM5B expression at both the mRNA and protein levels compared with the vector cell lines. In addition, the LN-18 cells that had been transfected with the KDM5B shRNA plasmid displayed significantly decreased KDM5B expression at both the protein (Fig. 2C) and mRNA (Fig. 2E) levels compared with the control cells.

3.3. KDM5B promoted glioma cell proliferation

We first explored the effects of KDM5B expression on cell growth using the MTT assay. As shown in Fig. 2F, KDM5B overexpression significantly enhanced the growth of SW1783 cells, whereas KDM5B knockdown significantly inhibited the growth of LN-18 cells. Next, we performed a clonogenic assay to confirm the effects of KDM5B on proliferation. We found that KDM5B overexpression dramatically increased the colony formation efficiency of SW1783 cells (Fig. 2G), whereas the colony formation efficiency was dramatically reduced in the LN-18 shRNA cell lines (Fig. 2H). As Ki67 is an important marker of cell proliferation, we next examined the Ki67 by immunofluorescence staining. As shown in Supplemental Fig. 1, we found that the overexpression of KDM5B in SW1783 cells significantly upregulated Ki67 staining (Supplemental Fig. 1A). Also knockdown of KDM5B in LN-18 cells dramatically downregulated the staining of Ki67 (Supplemental Fig. 1B). These results suggested that KDM5B could significantly promote the proliferation of glioma cells.

3.4. KDM5B promoted glioma cells proliferation via downregulating p21

The p21(now known as CDKN1A) has important roles in the proliferation of various cancer types, including glioma [18]. In addition, Wong et al. reported that KDM5B promotes cell cycle progression via direct p21 repression [16]. Thus, we determined

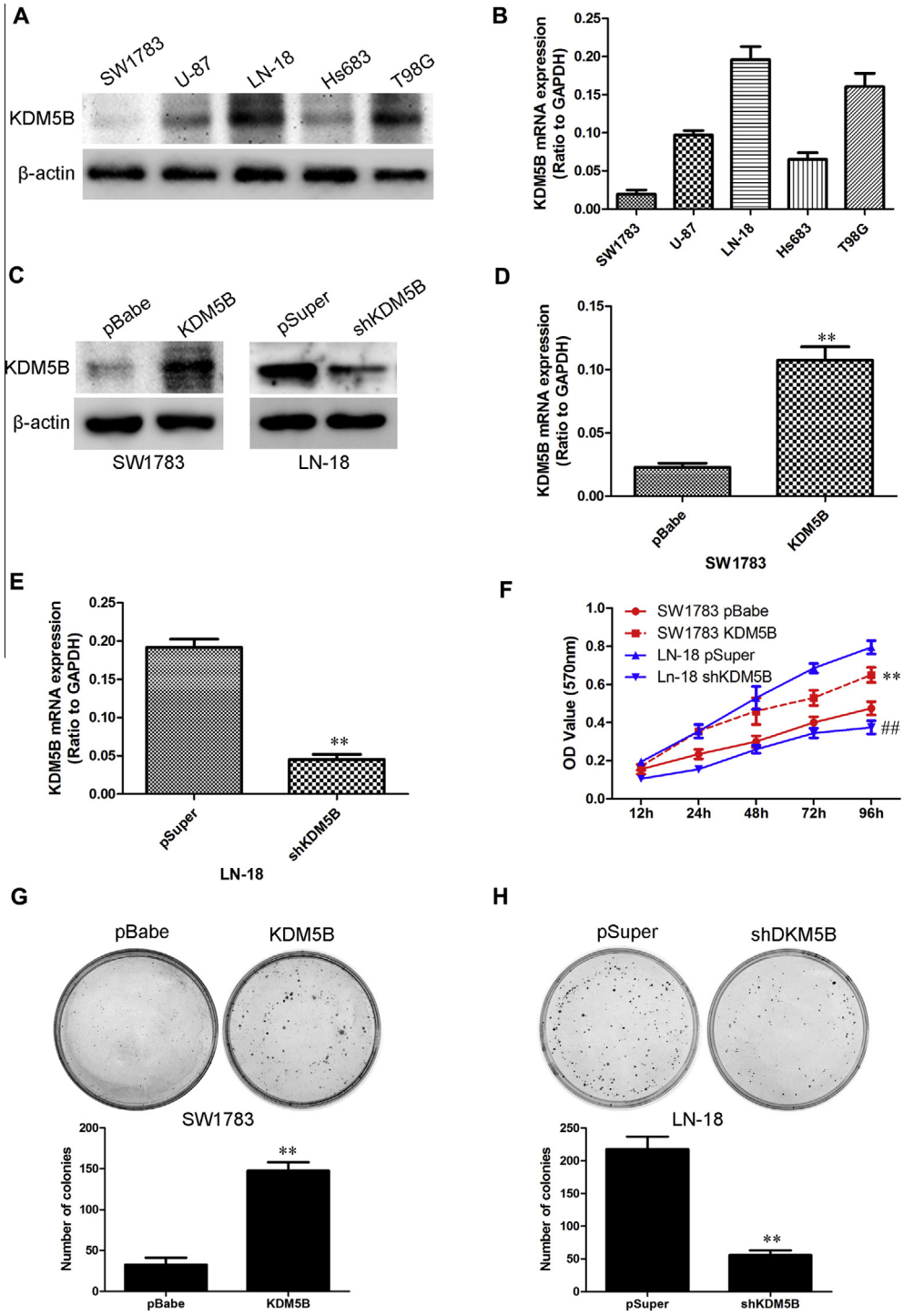

Fig. 2. KDM5B promoted glioma cell proliferation. KDM5B protein levels in five glioma cell lines (SW1783, U-87, LN-18, Hs683, and T98G) were revealed by Western blot. (B) KDM5B mRNA levels in five glioma cell lines (SW1783, U-87, LN-18, Hs683, and T98G) were revealed by qRT-PCR. (C) The transfection efficiency of KDM5B in SW1783 and LN-18 cells was analyzed by measuring protein levels by Western blotting. (D) The transfection efficiency of KDM5B in SW1783 cells was analyzed by measuring mRNA levels by qRT-PCR analyses. (E) The transfection efficiency of KDM5B in LN-18 cells was analyzed by measuring mRNA levels by qRT-PCR analyses. (F) Cell proliferation after KDM5B overexpression in SW1783 cells and KDM5B knockdown in LN-18 cells were measured using MTT assays. (G) The results of colony formation assays that were conducted after KDM5B overexpression in SW1783 cells. (H) The results of colony formation assays that were conducted after KDM5B knockdown in LN-18 cells. The data represent the means ± S.D. of three independent experiments $^{**}P < 0.01$.

whether the p21was involved in KDM5B-mediated tumor proliferation in glioma cells. We evaluated the effects of KDM5B on the p21 expression in SW1783 and LN-18 cells by Western blot. As shown in Fig. 3A, upregulation of KDM5B significantly downregulation p21 expression in SW1783 cells, while, knockdown of KDM5B dramatically upregulation p21 expression in LN-18 cells. To test whether p21 was involved in the KDM5B induced proliferation, we measured the role of p21 in shKDM5B-induced proliferation inhibition by knocking down p21 expression using siRNA in LN-18 cells. The p21 knockdown efficiency was detected using

Western blot at 24 h after transfection (Fig. 3B). As shown in Fig. 3C and D, the proliferation inhibition that was induced by shKDM5B was obviously reversed following p21 knockdown using siRNA. These results confirmed that p21 was involved in KDM5B-mediated proliferation ability in glioma cells.

3.5. KDM5B enhanced tumorigenesis in vivo

To explore the effects of KDM5B on tumorigenesis in vivo, different cell lines were injected subcutaneously into the flanks of nude mice. The diameters of the tumors were measured every 3 days. We found that the mice that had been injected with the KDM5B-overexpressing SW1783 cells formed tumors on the ninth day, while the mice that had been injected with control cells did not form tumors until the twelfth day (Fig. 4A). Similar results were observed in the LN-18 cells. We found that the control cells formed tumors earlier and that the tumor volumes were much larger in those that were formed from the control cells than in those that were formed from the KDM5B knockdown cells (Fig. 4C). As shown in Fig. 4B, the tumor volume of SW1783 control was much lower than KDM5B-overexpressing SW1783 cells. Similar with these, the control group was dramatically larger than the shKDM5B group in LN-18 cells (Fig. 4D). We next examined the p21 by immunohistochemistry staining in tumor tissues. As shown in Fig. 4E, we found that the overexpression of KDM5B in SW1783 cells significantly downregulated p21 staining. Also knockdown of KDM5B in LN-18 cells dramatically upregulated the staining of p21. These results suggested that KDM5B promotes glioma cell xenograft formation and growth in vivo.

4. Discussion

GBM is the most common primary brain tumor and the leading cause of tumor-related death in the central nervous system [19].

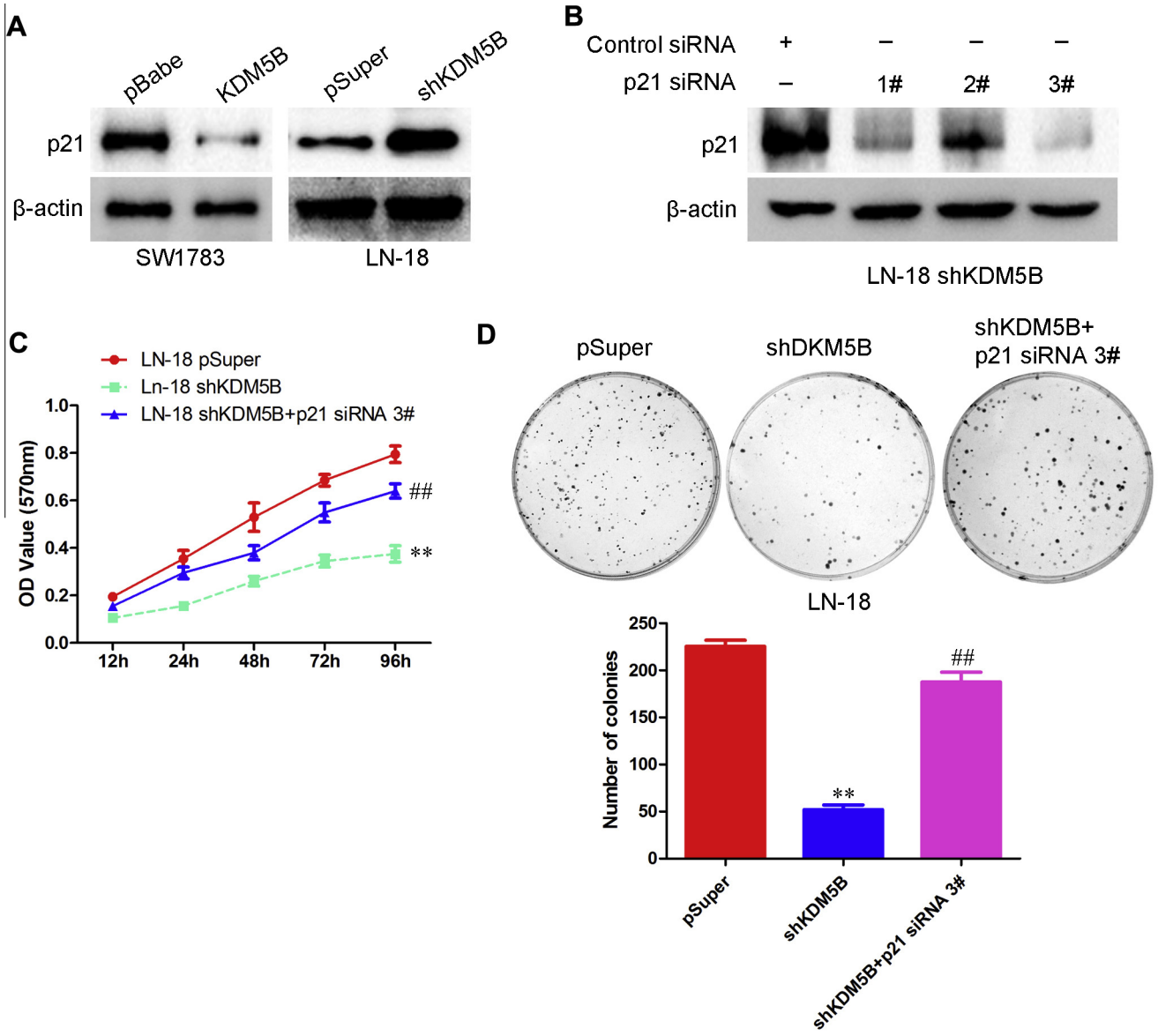

Fig. 3. KDM5B promoted glioma cells proliferation via downregulating p21. (A) The expression of p21 in KDM5B overexpression SW1783 cells and KDM5B knockdown LN-18 cells were examined using Western blotting. β-actin was used as a loading control. (B) The transfection efficiency of p21 siRNA 24 h after transfection was measured using Western blot analyses. β-actin was used as a loading control. (C) The graphs show the proliferation ability of KDM5B knockdown SN-18 cells after the cells had been pretreated with p21 siRNA 3#. (D) The results of colony formation assays that show the proliferation ability of KDM5B knockdown LN-18 cells after the cells had been pretreated with p21 siRNA 3#. The data represent the means ± S.D. of three independent experiments. $^{**}P < 0.01$ vs Control group; $^{\#\#}P < 0.01$ vs shKDM5B group.

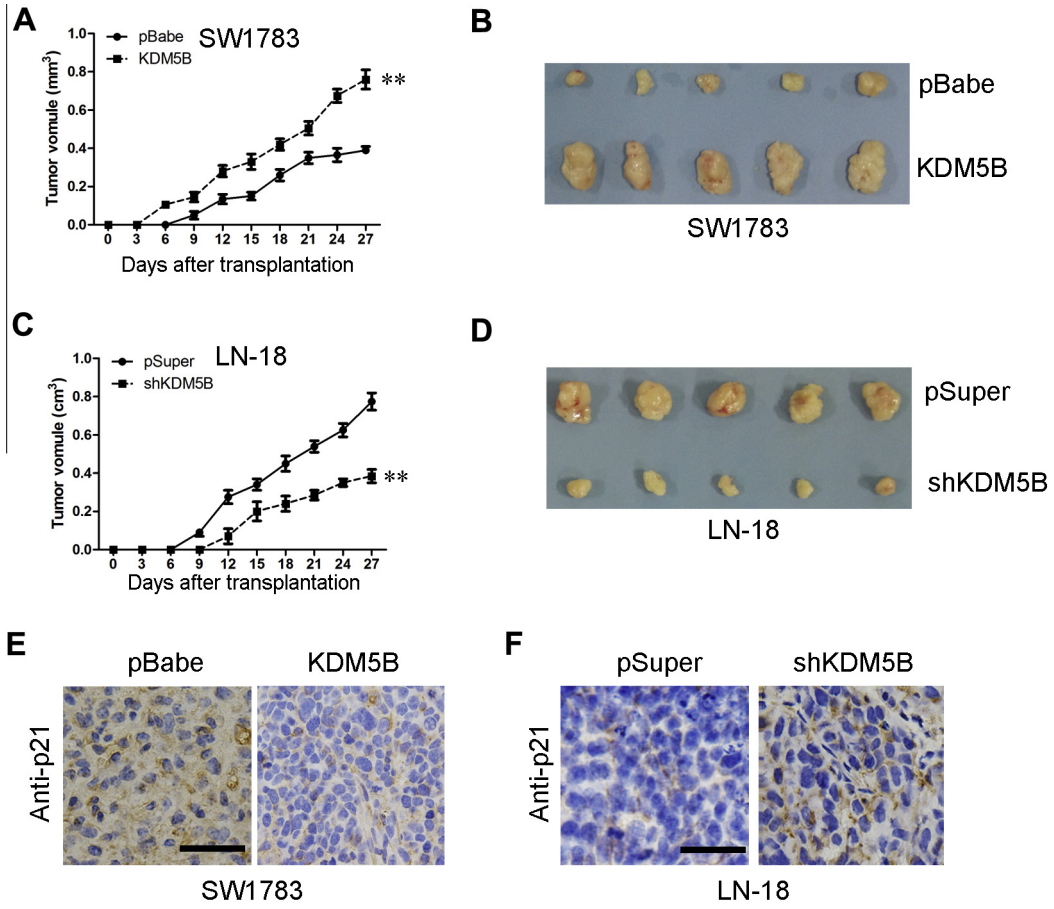

Fig. 4. KDM5B enhanced tumorigenesis in vivo. Growth curves of mammary tumors after the injection of KDM5B-overexpressing SW-1783 and control cells into SCID mice. The error bars represent the means ± S.D. ($n = 5$). (B) Representative tumors are presented from the experiments that were outlined in A. (C) Growth curves of mammary tumors after the injection of KDM5B-silenced LN-18 and control cells into SCID mice. The error bars represent the means ± S.D. ($n = 5$). (D) Representative tumors are presented from the experiments that were outlined in C. (E) Immunohistochemical staining of p21 in KDM5B overexpressed SW1783 and its control cells tumor tissues. (F) Immunohistochemical staining of p21 in KDM5B knockdown LN-18 and its control cells tumor tissues. $^{**}P < 0.01$ vs Control group.

The prognosis for patients with malignant glioma is poor, and the mechanisms of GBM genesis remain elusive. Multiple molecular dysfunctions are associated with GBM formation and growth, such as EGFR, TGF-β1, VEGF, p53, pRb, p21, p27, p16, p19, and telomerase [20–26]. Considerable effort has been made to determine the underlying mechanisms of gliomagenesis, but an effective therapy has not been established and patients diagnosed with GBM have a median survival of only 12–15 months [27,28]. In the present manuscript, we identified KDM5B as a candidate target gene for glioma growth.

A growing body of evidence indicates that overexpression or mutations of histone methyltransferases and demethylases have been linked to the development of many human cancers [29–31]. Histone H3K27 methyltransferase, EZH2, and H3K4 demethylases LSD1 and KDM5B have been thought to play important roles not only in tumor initiation, but also in tumor progression, since overexpression of these genes has been reported in many types of malignant tumors [30]. Previous papers revealed that upregulation of KDM5B enhanced cell migration, cell invasion and EMT to promote malignant progression of lung cancer cells [32,33]. However, the role of KDM5B in glioma carcinogenesis remains unclear. To confirm the oncogene function of KDM5B, we first examined the levels of KDM5B in glioma samples and normal brain tissue samples. We found that KDM5B was significantly overexpressed in gliomas. Kaplan–Meier analysis showed that patients with glioma and higher KDM5B expression tend to have shorter overall survival time. These results suggested that KDM5B was a candidate oncogene in glioma. To further explore the role of KDM5B in glioma, we transfected glioma cells either to ectopically express KDM5B or to inhibit its expression using RNA interference. Overexpression of KDM5B in vitro significantly enhanced the proliferation of glioma cells, while knockdown of KDM5B inhibited cell growth. Our in vivo experiments also demonstrated that KDM5B markedly promoted tumorigenesis. These data further supported the oncogene role of KDM5B in glioma.

Previous studies have shown that p21 was identified as a protein suppressing cyclin E/A-CDK2 activity and was originally considered as a negative regulator of the cell cycle and a tumor suppressor. It is now considered that p21 has alternative functions, and the view of its role in cellular processes has begun to change. At present, p21 is known to be involved in regulation of fundamental cellular programs: cell proliferation, differentiation, migration, senescence, and apoptosis. Wong et al. reported that KDM5B promotes cell cycle progression via direct p21 repression [16]. Thus, we determined whether the p21 was involved in KDM5B-mediated tumor proliferation in glioma cells. Our results indicated that the levels of p21 were significantly decreased in KDM5B-overexpressing cells and p21 were upregulated in KDM5B knockdown cells. When we pretreated the KDM5B knockdown cells with p21 siRNA, the decreased proliferation ability of KDM5B knockdown cells was reversed. All of these data revealed that KDM5B promotes the proliferation activities of glioma cells partly via inhibition p21.

However, how KDM5B might regulate the p21 is still need further study.

In conclusion, we found that KDM5B expression was generally higher in glioma lesions compared with normal brain tissues. Our data demonstrate that KDM5B has a vital function in promoting cell proliferation ability, which is at least partially controlled by the p21. Thus, we propose that the candidate tumor oncogene KDM5B may be an effective novel therapeutic target in the management of glioma.

Competing interests

The authors have no competing interests to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.078.

References

[1] Z. Zhou, Z. Ji, Y. Wang, J. Li, H. Cao, H.H. Zhu, W.Q. Gao, TRIM59 is upregulated in gastric tumors, promoting ubiquitination and degradation of P53, Gastroenterology (2014).
[2] X. Liu, H. Yu, H. Cai, Y. Wang, Expression of CD24, p21, p53, and c-myc in alpha-fetoprotein-producing gastric cancer: correlation with clinicopathologic characteristics and survival, J. Surg. Oncol. 109 (2014) 859–864.
[3] W. Zhou, X.Q. Fu, L.L. Zhang, J. Zhang, X. Huang, X.H. Lu, L. Shen, B.N. Liu, J. Liu, H.S. Luo, J.P. Yu, H.G. Yu, The AKT1/NF-kappaB/Notch1/PTEN axis has an important role in chemoresistance of gastric cancer cells, Cell Death Dis. 4 (2013) e847.
[4] M. El-Zaatari, J.Y. Kao, A. Tessier, L. Bai, M.M. Hayes, C. Fontaine, K.A. Eaton, J.L. Merchant, Gli1 deletion prevents Helicobacter-induced gastric metaplasia and expansion of myeloid cell subsets, PLoS ONE 8 (2013) e58935.
[5] S.S. Kim, N.J. Yoo, E.G. Jeong, M.S. Kim, S.H. Lee, Expression of NEDD4-1, a PTEN regulator, in gastric and colorectal carcinomas, APMIS 116 (2008) 779–784.
[6] Q. Wang, Y. Wang, Y. Zhang, Y. Zhang, W. Xiao, The role of uPAR in epithelial-mesenchymal transition in small airway epithelium of patients with chronic obstructive pulmonary disease, Respir. Res. 14 (2013) 67.
[7] P. Li, X. Chen, L. Su, C. Li, Q. Zhi, B. Yu, H. Sheng, J. Wang, R. Feng, Q. Cai, J. Li, Y. Yu, M. Yan, B. Liu, Z. Zhu, Epigenetic silencing of miR-338-3p contributes to tumorigenicity in gastric cancer by targeting SSX2IP, PLoS ONE 8 (2013) e66782.
[8] Z. Ali, Y. Deng, Y. Tang, S. Zheng, N. Ma, N. He, Epigenetic deregulations in gastric cancer, J. Nanosci. Nanotechnol. 13 (2013) 40–51.
[9] L.L. Thompson, B.J. Guppy, L. Sawchuk, J.R. Davie, K.J. McManus, Regulation of chromatin structure via histone post-translational modification and the link to carcinogenesis, Cancer Metastasis Rev. 32 (2013) 363–376.
[10] A.J. Bannister, T. Kouzarides, Regulation of chromatin by histone modifications, Cell Res. 21 (2011) 381–395.
[11] M. Litt, Y. Qiu, S. Huang, Histone arginine methylations: their roles in chromatin dynamics and transcriptional regulation, Biosci. Rep. 29 (2009) 131–141.
[12] H. Suzuki, R. Maruyama, E. Yamamoto, M. Kai, Epigenetic alteration and microRNA dysregulation in cancer, Front. Genet. 4 (2013) 258.
[13] X. Li, Y. Su, J. Pan, Z. Zhou, B. Song, E. Xiong, Z. Chen, Connexin 26 is down-regulated by KDM5B in the progression of bladder cancer, Int. J. Mol. Sci. 14 (2013) 7866–7879.
[14] Y. Sun, Y. Wang, C. Fan, P. Gao, X. Wang, G. Wei, J. Wei, Estrogen promotes stemness and invasiveness of ER-positive breast cancer cells through Gli1 activation, Mol. Cancer 13 (2014) 137.
[15] K. Ohta, N. Haraguchi, Y. Kano, Y. Kagawa, M. Konno, S. Nishikawa, A. Hamabe, S. Hasegawa, H. Ogawa, T. Fukusumi, M. Uemura, J. Nishimura, T. Hata, I. Takemasa, T. Mizushima, Y. Noguchi, M. Ozaki, T. Kudo, D. Sakai, T. Satoh, M. Fukami, M. Ishii, H. Yamamoto, Y. Doki, M. Mori, H. Ishii, Depletion of JARID1B induces cellular senescence in human colorectal cancer, Int. J. Oncol. 42 (2013) 1212–1218.
[16] P.P. Wong, F. Miranda, K.V. Chan, C. Berlato, H.C. Hurst, A.G. Scibetta, Histone demethylase KDM5B collaborates with TFAP2C and Myc to repress the cell cycle inhibitor p21(cip) (CDKN1A), Mol. Cell. Biol. 32 (2012) 1633–1644.
[17] Y. Wang, M. Wen, Y. Kwon, Y. Xu, Y. Liu, P. Zhang, X. He, Q. Wang, Y. Huang, K.Y. Jen, M.A. LaBarge, L. You, S.C. Kogan, J.W. Gray, J.H. Mao, G. Wei, CUL4A induces epithelial-mesenchymal transition and promotes cancer metastasis by regulating ZEB1 expression, Cancer Res. 74 (2014) 520–531.
[18] O. Tapia, I. Riquelme, P. Leal, A. Sandoval, S. Aedo, H. Weber, P. Letelier, E. Bellolio, M. Villaseca, P. Garcia, J.C. Roa, The PI3K/AKT/mTOR pathway is activated in gastric cancer with potential prognostic and predictive significance, Virchows Arch. 465 (2014) 25–33.
[19] Y. Wang, T. Jiang, Understanding high grade glioma: molecular mechanism, therapy and comprehensive management, Cancer Lett. 331 (2013) 139–146.
[20] M.L. Goodenberger, R.B. Jenkins, Genetics of adult glioma, Cancer Genet. 205 (2012) 613–621.
[21] J.V. Joseph, V. Balasubramaniyan, A. Walenkamp, F.A. Kruyt, TGF-beta as a therapeutic target in high grade gliomas – promises and challenges, Biochem. Pharmacol. 85 (2013) 478–485.
[22] M. Lindgren, M. Johansson, J. Sandstrom, Y. Jonsson, A.T. Bergenheim, R. Henriksson, VEGF and tPA co-expressed in malignant glioma, Acta Oncol. 36 (1997) 615–618.
[23] T. Abe, T. Tamiya, Y. Ono, A.H. Salker, K. Akiyama, T. Ohmoto, Accumulation of cell cycle regulatory proteins, p21 and p27, induced after hyperthermia in human glioma cells, Int. J. Hyperthermia 17 (2001) 499–507.
[24] B. Wagenknecht, M. Hermisson, K. Eitel, M. Weller, Proteasome inhibitors induce p53/p21-independent apoptosis in human glioma cells, Cell. Physiol. Biochem. 9 (1999) 117–125.
[25] M.J. Pykett, E. Azzam, W. Dahlberg, J.B. Little, Differential p53, p21, mdm2 and Rb regulation in glioma cell lines that overexpress wild-type p53, Int. J. Oncol. 13 (1998) 213–216.
[26] T. Komata, T. Kanzawa, H. Takeuchi, I.M. Germano, M. Schreiber, Y. Kondo, S. Kondo, Antitumour effect of cyclin-dependent kinase inhibitors (p16(INK4A), p18(INK4C), p19(INK4D), p21(WAF1/CIP1) and p27(KIP1)) on malignant glioma cells, Br. J. Cancer 88 (2003) 1277–1280.
[27] K. Anton, J.M. Baehring, T. Mayer, Glioblastoma multiforme: overview of current treatment and future perspectives, Hematol. Oncol. Clin. North Am. 26 (2012) 825–853.
[28] Y. Xu, Y. Wang, G. Ma, Q. Wang, G. Wei, CUL4A is overexpressed in human pituitary adenomas and regulates pituitary tumor cell proliferation, J. Neurooncol. 116 (2014) 625–632.
[29] S. Venneti, M.M. Felicella, T. Coyne, J.J. Phillips, D. Gorovets, J.T. Huse, J. Kofler, C. Lu, T. Tihan, L.M. Sullivan, M. Santi, A.R. Judkins, A. Perry, C.B. Thompson, Histone 3 lysine 9 trimethylation is differentially associated with isocitrate dehydrogenase mutations in oligodendrogliomas and high-grade astrocytomas, J. Neuropathol. Exp. Neurol. 72 (2013) 298–306.
[30] K. Xu, Z.J. Wu, A.C. Groner, H.H. He, C. Cai, R.T. Lis, X. Wu, E.C. Stack, M. Loda, T. Liu, H. Xu, L. Cato, J.E. Thornton, R.I. Gregory, C. Morrissey, R.L. Vessella, R. Montironi, C. Magi-Galluzzi, P.W. Kantoff, S.P. Balk, X.S. Liu, M. Brown, EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent, Science 338 (2012) 1465–1469.
[31] Z.S. Walters, B. Villarejo-Balcells, D. Olmos, T.W. Buist, E. Missiaglia, R. Allen, B. Al-Lazikani, M.D. Garrett, J. Blagg, J. Shipley, JARID2 is a direct target of the PAX3-FOXO1 fusion protein and inhibits myogenic differentiation of rhabdomyosarcoma cells, Oncogene 33 (2014) 1148–1157.
[32] S. Hayami, M. Yoshimatsu, A. Veerakumarasivam, M. Unoki, Y. Iwai, T. Tsunoda, H.I. Field, J.D. Kelly, D.E. Neal, H. Yamaue, B.A. Ponder, Y. Nakamura, R. Hamamoto, Overexpression of the JmjC histone demethylase KDM5B in human carcinogenesis: involvement in the proliferation of cancer cells through the E2F/RB pathway, Mol. Cancer 9 (2010) 59.
[33] Y. Wang, G. Ma, Q. Wang, M. Wen, Y. Xu, X. He, P. Zhang, Y. Wang, T. Yang, P. Zhan, G. Wei, Involvement of CUL4A in regulation of multidrug resistance to P-gp substrate drugs in breast cancer cells, Molecules 19 (2013) 159–176.