Biochemical and Biophysical Research Communications 446 (2014) 272–279

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Overexpressed homeobox B9 regulates oncogenic activities by transforming growth factor-β1 in gliomas

Liping Fang [a], Yinghui Xu [b], Lijuan Zou [a,*]

[a] Department of Oncology, 2nd Affiliated Hospital of Dalian Medical University, 467 Zhong Shan Road, Dalian 116023, Liaoning, China
[b] Department of Neurosurgery, The First Affiliated Hospital of Dalian Medical University, Dalian 116023, Liaoning, China

ARTICLE INFO

Article history:
Received 17 February 2014
Available online 28 February 2014

Keywords:
HOXB9
Glioma
Transforming growth factor-β1
Proliferation

ABSTRACT

Glioma is the leading cause of deaths related to tumors in the central nervous system. The mechanisms of gliomagenesis remain elusive to date. Homeobox B9 (HOXB9) has a crucial function in the regulation of gene expression and cell survival, but its functions in glioma formation and development have yet to be elucidated. This study showed that HOXB9 expression in glioma tissues was significantly higher than that in nontumor tissues. Higher HOXB9 expression was also significantly associated with advanced clinical stage in glioma patients. HOXB9 overexpression stimulated the proliferation, migration, and sphere formation of glioma cells, whereas HOXB9 knockdown elicited an opposite effect. HOXB9 overexpression also increased the tumorigenicity of glioma cells in vivo. Moreover, the activation of transforming growth factor-β1 contributed to HOXB9-induced oncogenic activities. HOXB9 could be used as a predictable biomarker to be detected in different pathological and histological subtypes in glioma for diagnosis or prognosis.

1. Introduction

Glioma is the most common primary brain tumor and the leading cause of tumor-related death in the central nervous system [1]. The prognosis for patients with malignant glioma is poor, and the mechanisms of gliomagenesis remain elusive to date. Multiple molecular dysfunctions involving TGF-β1, VEGF, p53, pRb, EGFR, p21, p27, $p16^{INK4a}$, $p19^{ARF}$, and telomerase have been associated with formation and growth of glioma and medulloblastoma [2–6]. Considerable effort has been carried out to determine the mechanisms underlying gliomagenesis, but an effective therapy has not been established. In addition, patients diagnosed with glioma have a median survival of only 12–15 months [7,8].

Homeobox (HOXB9) is a member of the HOX family gene cluster [9,10] and is involved in cell proliferation and embryonic segmentation [11]. Recent studies have demonstrated the implication of HOXB9 in tumorigenesis [12,13]. Several studies have suggested that the HOXB9 protein is upregulated in breast tumors. This protein induces several tumorigenic phenotypes and promotes disease progression by altering the microenvironment [14–16]. HOXB9 expression has also been reported in lung cancer cell lines [10]. Changes in HOXB9 potentially exert a wide pleiotropic effect that alters cell functions including proliferation, differentiation, and apoptosis [17,18]. Thus, HOXB9 may function as an oncogene, but whether HOXB9 is involved in glioma development and metastasis remains unknown.

HOXB9 expression has been studied in some types of cancer, but little is known on its expression in gliomas and its function in tumorigenesis. This study investigated the function of HOXB9 in gliomagenesis. The results showed that HOXB9 expression was elevated in most of the human glioma tissues. HOXB9 overexpression in human glioma cells significantly enhanced cell proliferation, migration, and sphere formation in vitro. HOXB9 overexpression also increased the tumorigenicity of T98G cells in nude mouse xenograft. Furthermore, HOXB9 increased the protein levels of TGF-β1 and its downstream target protein p-Smad2, as well as the expression level of CD133, Oct4, nestin, and Bmi-1. By contrast, HOXB9 knockdown by short hairpin RNA inhibited the proliferation, migration, and sphere formation of glioma cells. These results indicate the pivotal function of HOXB9 in the proliferation and invasion of glioma cells.

2. Materials and methods

The details of methods in Supplemental Materials and Methods.

* Corresponding author.
E-mail address: zoulijuantg@126.com (L. Zou).

http://dx.doi.org/10.1016/j.bbrc.2014.02.095
0006-291X/

2.1. Samples, cells, and antibodies

Human normal brain tissues and glioma tissue samples were obtained from patients who underwent surgery therapeutic procedures at the department of Neurosurgery, the first affiliated hospital of Dalian medical University. The adult normal brain tissues as normal controls were obtained from surgical resections of six trauma patients. Clinicopathological features and treatment strategies of all the glioma patients are shown in Supplemental Table 1. All experiments were approved by the ethics committee of Dalian medical University and informed consent was obtained from all patients prior to specimen collection. Human glioma cell lines, U87, T98G, SW1088, and SW1783, were obtained from American Type Culture Collection (ATCC, Manassas, VA). The cells were maintained in Minimum Essential Medium (MEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen). Mouse monoclonal HOXB9, p-Smad2, Smad2, TGF-β1, CD133, Oct4, nestin and Bmi-1 antibodies were purchased from Abcam. Mouse monoclonal β-actin antibody was product of Santa Cruz Biotech (Santa Cruz, CA).

2.2. Chromatin immunoprecipitation assays

Cells were fixed in 1% formaldehyde for 10 min at 37 °C. Cross-linking was quenched by adding 125 mmol/L glycine. Cells were harvested and resuspended in SDS lysis buffer. Chromatin was sheared by sonication (average length 0.25–1 Kb) and incubated with 60 ml protein A/G agarose/salmon sperm DNA (50% slurry; Millipore) with gentle agitation for 30 min. The supernatant was then immunoprecipitated with anti-HOXB9 antibody 1:100 or its matched nonimmune crude serum 1:100 (IgG; Diagenode) at 4 °C overnight. Protein A/G agarose (60 mL of 50% slurry) was then added and incubated for 1 h. Pellets were washed and protein-DNA cross-links were reversed by overnight incubation at 65 °C with proteinase K. DNA was purified following a conventional phenol–chloroform protocol and eluted in 50 mL water. At least 2 independent Chromatin immunoprecipitation (ChIP) experiments were carried out. The primer sequences used in ChIP-PCR were: TGF-β1 region 1: sense 5′-GGAAAGGGTGGGAGTCCAA-3′ and antisense 5′-TTGCTCCAAACGCCAACC-3′; TGF-β1 region 2: sense 5′-CT TACTCGCCAAAGTCAGGGTT-3′ and antisense 5′-TGACCAGATGCAG-GATCAGA-3′.

2.3. Statistical analysis

Experimental data are shown as mean ± standard deviation (S.D.). The results from different treatment groups were compared using a two-tailed Student's *t*-test. The association between HOXB9 and p-Smad2 immunointensity on the same specimens was analyzed using Spearman rank correlation test. The Kaplan–Meier method was used to estimate the probability of patient survival, and differences in the survival of subgroups of patients were compared using Mantel's log-rank test. Differences were considered to be statistically significant at a value of P less than 0.05. Statistical analysis was done with SPSS/Win11.0 software (SPSS, Inc., Chicago, Illinois, USA).

3. Results

3.1. HOXB9 is overexpressed in human glioma samples

Semi-quantitative RT-PCR and Western blot were performed to measure the mRNA and protein levels of HOXB9, respectively, in six normal brain samples (Supplemental Fig. 1A), eight Grades I–III glioma samples (Supplemental Fig. 1B), and eight Grade IV glioma tissue samples (Supplemental Fig. 1C). The mRNA and protein levels of HOXB9 were upregulated in six of the eight (75%) Grades I–III samples and all of the eight (100%) Grade IV glioma samples compared with the normal brain samples. Densitometric evaluation of the relative expression showed that the expression level of HOXB9 was significantly higher in Grade III glioma than in Grades I–III gliomas (Supplemental Figs. 1D and E). Grade IV glioma expressed the highest HOXB9.

We analyzed HOXB9 expression in more samples by immunohistochemistry [normal brain = 10; low-grade glioma (Grades I and II) = 27; high-grade glioma (Grade III = 26 and Grade IV = 21) = 47]. No staining was detected in the normal human brain samples (Fig. 1A), and the HOXB9 protein was expressed in all human glioma samples. The protein was localized in the nuclei of the tumor cells (Fig. 1A). The nuclei of most high-grade glioma cells were more intensely stained compared with those of the low-grade tumors. The ratio of HOXB9-positive cells in the high-grade glioma samples was higher than that in the low-grade glioma samples and normal brain samples (Fig. 1B). These results indicate that HOXB9 expression in the nuclei of glioma cells was in a grade-dependent manner. A high level of HOXB9 expression was significantly more common in glioma tissues with high pathologic grade than those with low pathologic grade ($P = 0.002$, Supplemental Table 2). No significant association was found between HOXB9 expression and gender, KPS or age at diagnosis. Kaplan–Meier survival analysis showed significant survival extension of HOXB9 low-expression gliomas compared with HOXB9 high-expression gliomas (Fig. 1C). Survival analysis within a tumor grade in high grade gliomas (Grades III and IV) showed that the HOXB9 high-expressed patients had a shorter survival (Fig. 1D and E). The cut-off point was established using the X-tile software program (version 3.6.3; Yale University School of Medicine, CT, USA). These results suggest that HOXB9 expression in gliomas reveals the expression pattern of the tumor cells of origin and represents a consequence of therapeutic agents. More importantly, HOXB9 expression indicated an aberrant gene activation event associated with tumor progression.

3.2. HOXB9 promotes glioma cell proliferation

Semi-quantitative RT-PCR and Western blot were carried out to measure the mRNA and protein expression levels of HOXB9, respectively, in the four glioma cell lines (Fig. 2A and B). U87 expressed high level of HOXB9, whereas T98G, SW1088, and SW1783 expressed low levels of HOXB9.

The effect of HOXB9 overexpression was determined on cell proliferation to characterize the functions of HOXB9 in glioma. The HOXB9 expression plasmid pBabe-HOXB9 was first transfected into T98G cells. This study selected the T98G cell line because it exhibited the lowest expression level of HOXB9 among the four glioma cell lines. After selection with puromycin, HOXB9 expression was assayed by RT-PCR (Fig. 2C), Western blot (Fig. 2D), and immunofluorescence microscopy (Supplemental Fig. 2). The expression level of HOXB9 was high in the T98G-pBabe-HOXB9 cells but low in the control cells transfected with the empty pBabe plasmid (T98G-pBabe) and mock T98G cells. The MTT assay showed that the T98G-pBabe-HOXB9 cells displayed higher proliferation rates than the control cells (i.e., parent T98G cells and T98G-pBabe cells) (Fig. 2E).

3.3. HOXB9 promotes tumorigenesis

The ability of the HOXB9-expressing T98G cells to form spheres was analyzed to determine the function of HOXB9 in cellular transformation. The number of spheres formed by the

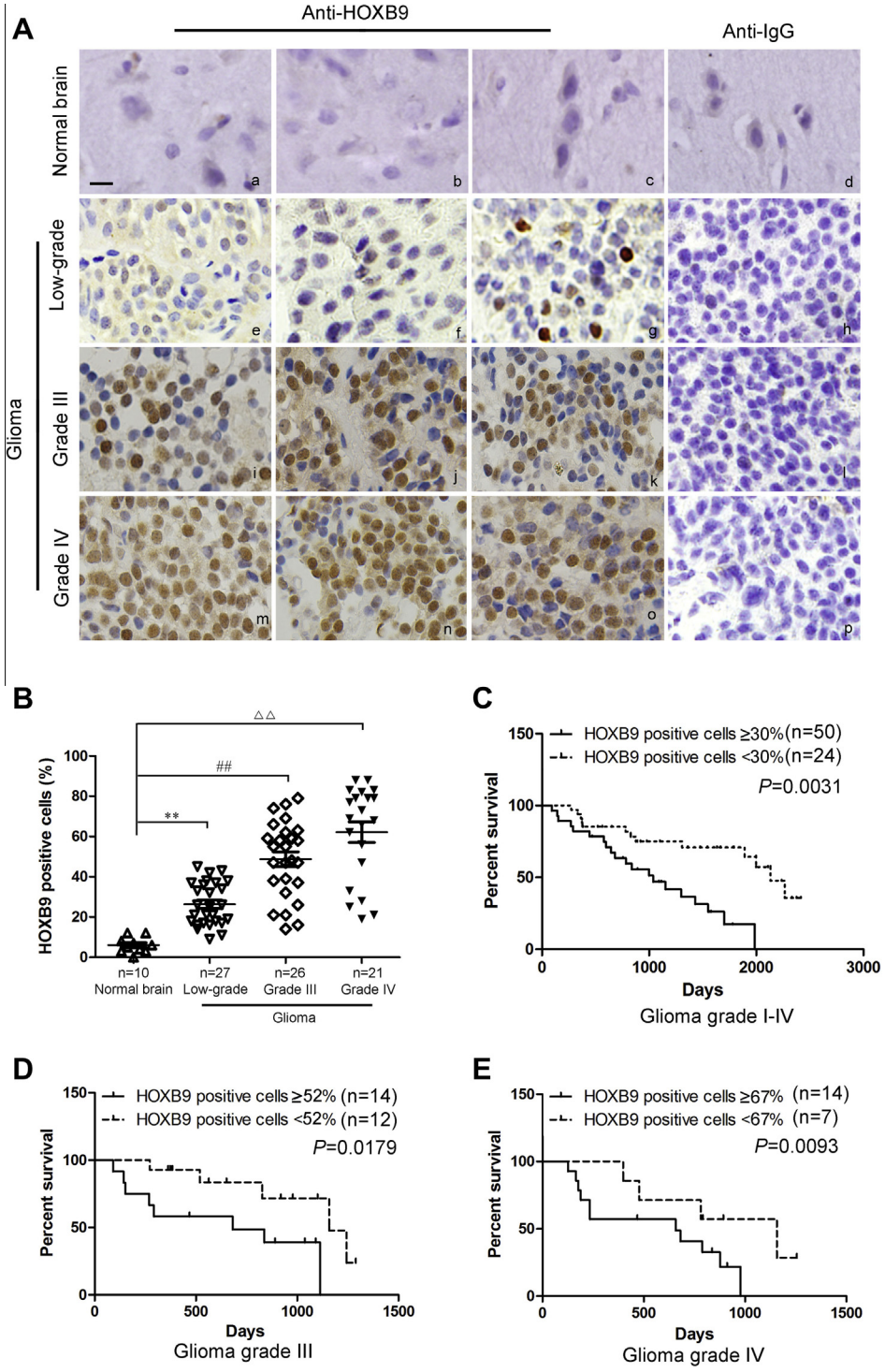

Fig. 1. HOXB9 overexpresses in gliomas and correlates with survival. Immunohistochemistry analysis showed that HOXB9 expression in paraffin-embedded sections formed normal brain (A: a–c), low-grade glioma (Grade I, II, A: e–g), and high-grade glioma (Grade III, A: i–k; Grade IV, m–o). HOXB9 expression was primarily localized in the nuclei of tumor cell (brown). Staining of HOXB9 was not observed in the normal brain tissues. Sections counterstained with hematoxylin. A: d, h, l, and p were negative control stained with IgG. (B) Ratio of HOXB9 positive cells in normal brain, low-grade glioma, and high-grade glioma tissues. High-grade glioma expressed significantly higher ratio of HOXB9 than normal brain and low-grade glioma tissues ($P < 0.01$). (C–E) Kaplan–Meier survival plot for samples with different HOXB9 expression. HOXB9 expression levels reverse correlated with patient survival. Scale bars = 20 μm.

T98G-pBabe-HOXB9 cells was significantly higher than that by the parent T98G cells and T98G-pBabe cells (Fig. 2I).

A glioma xenograft model was established by implanting T98G-pBabe and T98G-pBabe-HOXB9 cells subcutaneously into the right flanks of nude mice to examine the in vivo function of HOXB9 in glioma carcinogenesis. The tumor size was continuously monitored weekly. Mice tumors injected with T98G-pBabe-HOXB9 cells were significantly larger than those of the control mice injected with T98G-pBabe cells at 2 weeks after tumor cell injection (Fig. 3A–C). Immunohistochemical analysis confirmed the HOXB9 expression in the xenograft (Fig. 3D).

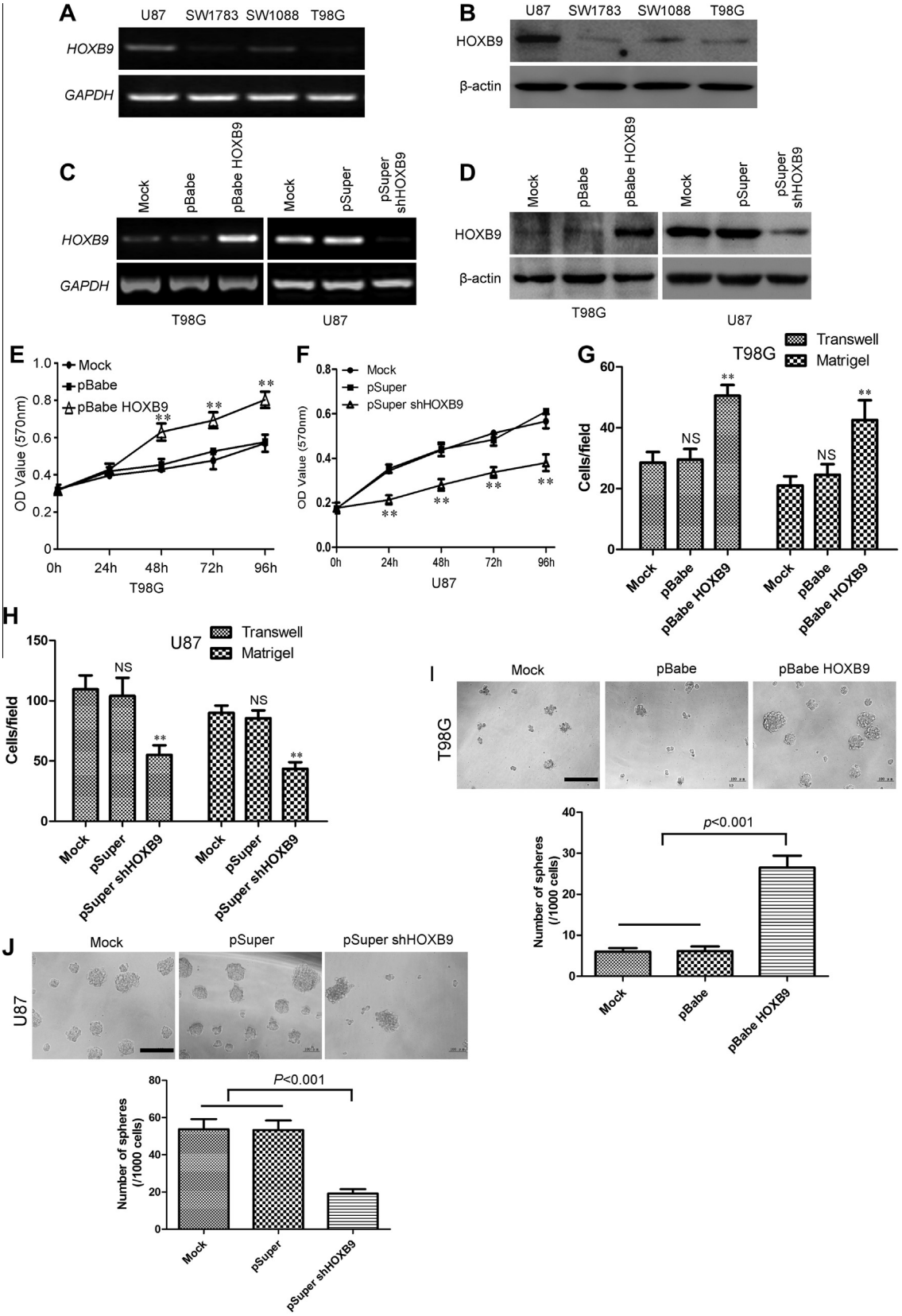

Fig. 2. HOXB9 promotes proliferation, migration, invasiveness, and sphere formation capacities of glioma cells. HOXB9 mRNA and protein levels in six glioma cell lines (SW1783, T98G, U87, and SW1088) were revealed by semi-quantitative RT-PCR (A) and Western blot (B), respectively. GAPDH and β-actin were used as internal controls for RT-PCR and Western blot, respectively. Overexpression and HOXB9 knockdown in stable transfectants of glioma cells were detected by RT-PCR (C) and Western blot (D). (E and F) The mean absorbance values of the cells at 570 nm were expressed as mean ± S.D. Quantification of migrated cells through the membrane and invaded cells through Matrigel of each cell line is shown as proportions of their vector controls (G and H). (I and J) Sphere formation of glioma cells was evaluated by sphere formation assay. Scale bars = 200 μm. The number of spheres in each culture well was scored, and the results are expressed as means ± S.D.; $^{**}P < 0.01$ is based on Student's *t*-test. All results are representative of three or four independent experiments.

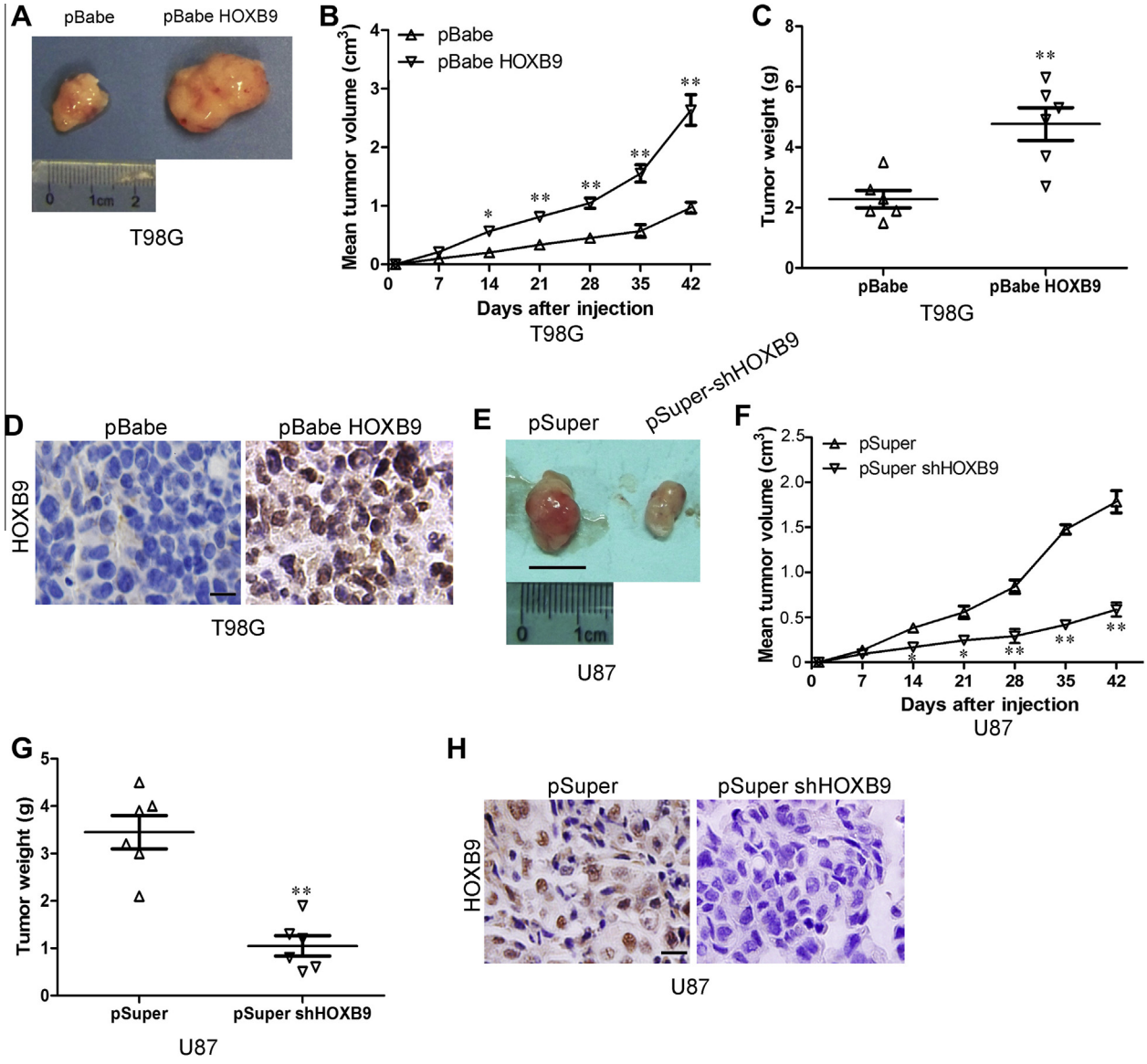

Fig. 3. HOXB9 promotes tumorigenesis in glioma cells in vivo. (A) Stable T98G transfectants were inoculated subcutaneously into the right axilla of four 5 to 6 week-old female BALB/*c-nu*/*nu* mice. (B) Tumor volumes were measured every week after tumor cell injection, and the results are expressed as means ± S.D. ($n = 5$; $^{*}P < 0.05$, $^{**}P < 0.01$). (C) Tumor weight was measured 42 days after tumor cell injection, and the results are expressed as means ± S.D. ($n = 5$; $^{**}P < 0.01$). (D) Immunohistochemical analysis confirmed the HOXB9 expression in xenograft. (E) Stable U87 transfectants were inoculated subcutaneously into the right axilla of four 5 to 6 week-old female BALB/*c-nu*/*nu* mice. (F) Tumor volumes were measured every week after tumor cell injection, and the results are expressed as means ± S.D. ($n = 5$; $^{*}P < 0.05$, $^{**}P < 0.01$). (G) Tumor weight was measured 42 days after tumor cell injection, and the results are expressed as means ± S.D. ($n = 5$; $^{**}P < 0.01$). (H) Immunohistochemical analysis confirmed HOXB9 expression in xenograft. Scale bars = 20 μm.

3.4. HOXB9 promotes the migratory and invasive capacities of glioma cells

Wound healing, Transwell, and Matrigel assay were performed using the established T98G stable transfectants and its parent cells to determine whether HOXB9 overexpression influences the migratory and invasive capacities of glioma cells. The migratory activity of T98G-pBabe-HOXB9 cells significantly increased compared with that of the control T98G-pBabe cells and parent cells. This result suggests that HOXB9 may also enhance the rate of glioma cell migration (Supplemental Fig. 3), which was confirmed by Transwell assay (Fig. 2G and Supplemental Fig. 4). Matrigel assay showed that the invasive capacity of T98G-pBabe-HOXB9 cells was higher than that of the control T98G-pBabe cells and parent cells (Fig. 2G and Supplemental Fig. 4).

3.5. Suppression of HOXB9 inhibits the proliferation, sphere formation, migration, and invasion of glioma cells

U87 is a highly tumorigenic human glioma cell line commonly used in glioma research. This cell line was infected with pSuper-shHOXB9 or the control (pSuper) to investigate the effect of HOXB9 knockdown on the proliferation, colony formation, migration, and invasion of glioma cells. RT-PCR (Fig. 2C) and Western blot (Fig. 2D) demonstrated that the mRNA and protein expression levels of HOXB9 were significantly suppressed in the cells infected with pSuper-shHOXB9. The results of the MTT assay also showed that HOXB9 suppression was associated with decreased cell proliferation (Fig. 2F). The sphere formation assay revealed that HOXB9 suppression significantly decreased the number of formed spheres (Fig. 2J). Transwell and Matrigel assays indicated that HOXB9

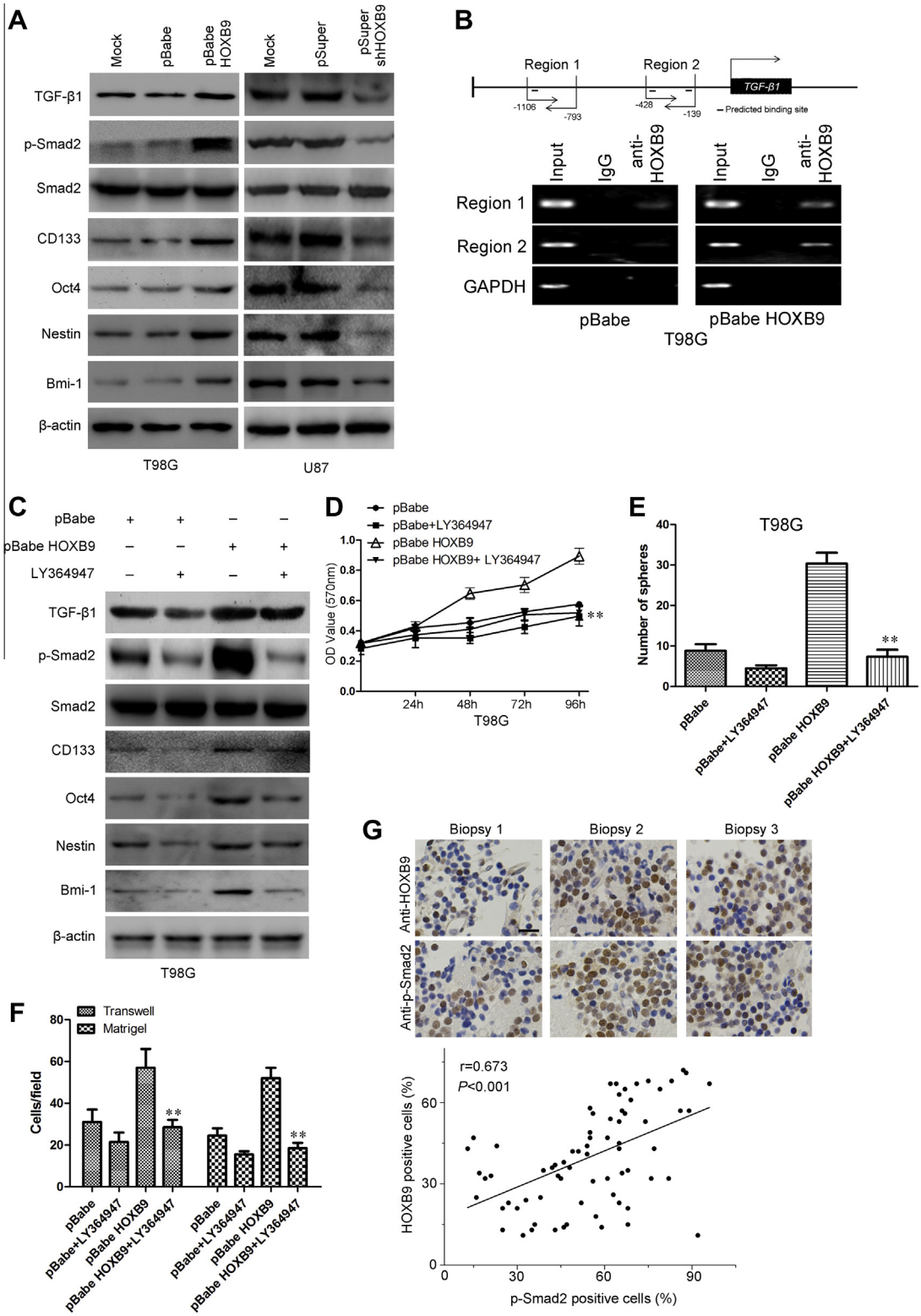

Fig. 4. Activation of TGF-β1 contributes to HOXB9-induced oncogenic activities. (A) Western blot analysis of TGF-β1, phosphor-Smad2, Smad2, CD133, Oct4, nestin, and Bmi-1 protein levels in glioma cells. (B) Schematic presentation of two regions associated with the TGF-β1 transcriptional start site used as primers to examine the abundance of HOXB9 (upper panel). ChIP-PCR was performed to assess HOXB9 abundance in T98G-pBabe and T98G-pBabe HOXB9 cells. IgG was used as negative control. (C) Western blot analysis of TGF-β1, phosphor-Smad2, Smad2, CD133, Oct4, nestin, and Bmi-1 protein levels in LY364947-treated HOXB9-T98G cells and the controls. β-Actin was used as the internal control for Western blot. (D) MTT measured the growth rates in LY364947-treated HOXB9-T98G cells and the controls. Data represent mean ± S.D. ($n = 6$; $^{**}P < 0.01$). (E) Sphere formation of the LY364947-treated HOXB9-T98G cells and the controls was evaluated by sphere formation assay. The number of spheres in each culture well was scored, and the results are expressed as means ± S.D. ($n = 6$; $P < 0.001$). (F) LY364947-treated HOXB9-T98G cells and the controls were subjected to transwell migration and Matrigel invasion assays. Quantification of migrated cells through the membrane and invaded cells through Matrigel of each cell line is shown as proportions of their vector controls. (G) p-Smad2 expression was positively correlated with HOXB9 expression in glioma tissues. $^{**}P < 0.01$ is based on Student's *t*-test. All results are representative of three or four independent experiments. Error bars indicate standard deviation. Scale bars = 20 μm.

knockdown significantly inhibited glioma cell migration and invasion (Fig. 2H and Supplemental Fig. 5). These results further confirm that HOXB9 was involved in the proliferation and migration of glioma cells.

3.6. Activation of TGF-β1 contributes to HOXB9-induced oncogenic activities

Previous studies have shown that TGF-β1 is induced in HOXB9-expressing breast cancer cells [13]. Thus, this study investigated whether TGF-β1 is induced by HOXB9 in glioma cells. HOXB9 overexpression in T98G cells significantly increased TGF-β1 expression and its downstream protein p-Smad2 (Fig. 4A). The expression of cancer stem cell-related markers, including CD133, OCT4, nestin, and Bmi-1, was significantly induced by HOXB9 in T98G-pBabe-HOXB9 cells compared with the parent and T98G-pBabe cells (Fig. 4A). By contrast, HOXB9 knockdown in U87 cells significantly decreased the expression levels of TGF-β1, p-Smad2, CD133, OCT4, nestin, and Bmi-1 (Fig. 4A).

ChIP assay was then performed using antibody against HOXB9 and primers specific to TGF-β1 promoter as indicated in Fig. 4B. The abundance of HOXB9 at the TGF-β1 promoter was significantly higher in T98G-pBabe HOXB9 cells compared with T98G cells with control vector (Fig. 4B). These data indicate that TGF-β1 was transcriptionally activated by HOXB9 directly. TGF-β1 is a well-known regulator of cancer stem cells; thus, we determined whether the activation of TGF-β1 contributes to HOXB9-induced oncogenic activities. Suppression of TGF-β1 receptor signaling using the inhibitor LY364947 suppressed Smad2 phosphorylation without affecting the TGF-β1 expression in the T98G-pBabe-HOXB9 cells (Fig. 4C). Treatment with LY364947, an inhibitor of TGF-β1 receptor signaling, suppressed the expression levels of CD133, Oct4, nestin, and Bmi-1 in the T98G-pBabe-HOXB9 cells (Fig. 4C). The suppression of TGF-β1 signaling also reduced the migration, invasion, proliferation, and sphere formation properties of T98G-pBabe-HOXB9 cells (Fig. 4D–F). We performed the co-staining for p-Smad2 and HOXB9, and analysis the relation between the expression of HOXB9 and p-Smad2. The p-Smad2 expression was positively correlated with HOXB9 expression in glioma tissues (Fig. 4G). The overall survival of glioma patients with $HOXB9^{High}$/$p\text{-}Smad2^{High}$ was significantly lower than that of glioma patients with $HOXB9^{High}$/$p\text{-}Smad2^{Low}$, $HOXB9^{Low}$/$p\text{-}Smad2^{High}$, and $HOXB9^{Low}$/$p\text{-}Smad2^{High}$ ($P < 0.001$) (Supplemental Fig. 6). These results indicate that the induction of TGF-β1 expression by HOXB9 activated critical signaling pathways implicated in glioma formation.

4. Discussion

This study characterized the functions of HOXB9 in glioma. Elevated mRNA and protein levels of HOXB9 were detected in the majority of the glioma tissues examined compared with the normal brain tissues. HOXB9 overexpression enhanced the proliferation, sphere formation, migration, and invasion of glioma cells and accelerated the tumor growth in nude mice in vivo. By contrast, HOXB9 knockdown significantly inhibited the proliferation, sphere formation, migration, and invasion of glioma cells; these HOXB9 properties were partly associated with TGF-β1. This study is the first to prove the contribution of HOXB9 in gliomagenesis.

HOXB9 is included in a cluster of Homeobox genes, and the encoded protein functions as a sequence-specific transcription factor [19]. Previous results have shown that HOXB9 is expressed differentially in normal and cancer tissues. HOXB9 overexpression has been associated with progression and metastasis in leukemia, pediatric acute myeloid leukemia, lung cancer, breast cancer, and gastric cancer [10,12,20,21]. However, the effect of altered HOXB9 expression on the progression of glioma cells remains elusive. Thus, the possible clinical significance of HOXB9 is still unknown in glioma patients. This study examined the protein expression of HOXB9 in glioma cancer and investigated the relationship between the HOXB9 expression and the clinicopathological characteristics of patients with glioma. The results showed that HOXB9 was not detectable in normal human brains and overexpressed in a majority of high-grade glioma tissues examined. To clarify the prognostic significance, the relationship of HOXB9 expression to the survival of 62 patients with glioma was analyzed, and a link between overexpression and poor survival was found. The survival rate of patients with high HOXB9 expression was significantly lower than that of patients with low expression.

HOXB9 overexpression increased glioblastoma cell proliferation and promoted tumorigenesis in vitro and in vivo, whereas HOXB9 knockdown by shRNA inhibited tumorigenesis. These data are consistent with a recent study that investigated the function of HOXB9 in gastric and breast carcinogenesis [14,22]. We found that HOXB9 overexpression enhanced cell migration and invasion, whereas HOXB9 knockdown produced an opposite effect. To our knowledge, this study is the first to demonstrate that HOXB9 could regulate the migration of glioma cells. All of these findings are supported by a recent study, which reported that the overexpression of HOXB9 protein in breast cancer alters tumor cell fate and promotes tumor progression and metastasis.

TGF-β signaling is involved in the regulation of proliferation, differentiation, and survival/apoptosis of many cells, including glioma cells [23]. TGF-β functions through specific receptors that activate multiple intracellular pathways, which resulted in the phosphorylation of receptor-regulated Smad2/3 proteins associated with the common mediator Smad4. This complex translocates to the nucleus, binds to the DNA, and then regulates the transcription of many genes [23]. The increased TGF-β expression correlates with a degree of malignancy of human gliomas [24]. TGF-β may contribute to tumor pathogenesis by directly supporting tumor growth, promoting self-renewal of glioma initiating stem cells, and inhibiting anti-tumor immunity. Previous studies have shown that HOXB9 expression leads to TGF-β1 production in breast cancer cells and normal breast cells [13]. Therefore, we speculate that HOXB9 may also modulate TGF-β1 in glioma cells. In this study, HOXB9 overexpression significantly increased TGF-β1 expression and its downstream protein p-Smad2, whereas HOXB9 knockdown produced an opposite effect. HOXB9 was also found to regulate CD133, nestin, Oct4, and Bmi-1, which are proteins relevant to glioma cancer stem cells. The inhibition of the TGF-β1 signaling pathway by LY364947 reversed the induction of HOXB9 on proteins associated with glioma cancer stem cells. Moreover, suppression of TGF-β1 signaling also reduced the migration, invasiveness, proliferation, and sphere formation properties of T98G-pBabe-HOXB9 cells. Thus, aberrant HOXB9 in gliomas may enhance the oncogenic effects of activated TGF-β1 signaling pathway. However, the mechanisms involved in the expression profile of the gene need further investigation.

In conclusion, this study demonstrated for the first time that HOXB9 was overexpressed in glioma tissues. HOXB9 overexpression increased the growth and migration of glioma cells in vitro and promoted glioma tumorigenesis in vitro and in vivo. Therefore, HOXB9 is potentially an important molecular target for the design of novel antiglioma therapy.

Conflicts of interest statement

No conflicts of interest exist.

Acknowledgment

This project was supported by National Natural Science Foundation of China (81171486).

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.2014.02.095.

References

[1] Y. Wang, T. Jiang, Understanding high grade glioma: molecular mechanism, therapy and comprehensive management, Cancer Lett. 331 (2013) 139–146.
[2] 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.
[3] 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.
[4] 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.
[5] 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.
[6] 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.
[7] 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.
[8] 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.
[9] J. Malicki, L.D. Bogarad, M.M. Martin, F.H. Ruddle, W. McGinnis, Functional analysis of the mouse homeobox gene HoxB9 in Drosophila development, Mech. Dev. 42 (1993) 139–150.
[10] D.X. Nguyen, A.C. Chiang, X.H. Zhang, J.Y. Kim, M.G. Kris, M. Ladanyi, W.L. Gerald, J. Massague, WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis, Cell 138 (2009) 51–62.
[11] F. Chen, M.R. Capecchi, Paralogous mouse Hox genes, Hoxa9, Hoxb9, and Hoxd9, function together to control development of the mammary gland in response to pregnancy, Proc. Natl. Acad. Sci. USA 96 (1999) 541–546.
[12] T. Hayashida, F. Takahashi, N. Chiba, E. Brachtel, M. Takahashi, N. Godin-Heymann, K.W. Gross, M. Vivanco, V. Wijendran, T. Shioda, D. Sgroi, P.K. Donahoe, S. Maheswaran, HOXB9, a gene overexpressed in breast cancer, promotes tumorigenicity and lung metastasis, Proc. Natl. Acad. Sci. USA 107 (2010) 1100–1105.
[13] N. Chiba, V. Comaills, B. Shiotani, F. Takahashi, T. Shimada, K. Tajima, D. Winokur, T. Hayashida, H. Willers, E. Brachtel, M.D. Vivanco, D.A. Haber, L. Zou, S. Maheswaran, Homeobox B9 induces epithelial-to-mesenchymal transition-associated radioresistance by accelerating DNA damage responses, Proc. Natl. Acad. Sci. USA 109 (2012) 2760–2765.
[14] B. Shrestha, K.I. Ansari, A. Bhan, S. Kasiri, I. Hussain, S.S. Mandal, Homeodomain-containing protein HOXB9 regulates expression of growth and angiogenic factors, facilitates tumor growth in vitro and is overexpressed in breast cancer tissue, FEBS J. 279 (2012) 3715–3726.
[15] H. Seki, T. Hayashida, H. Jinno, M. Takahashi, Y. Kitagawa, HOXB9 as a novel prognostic factor in breast cancer, Nihon Rinsho 70 (Suppl. 7) (2012) 166–169.
[16] H. Seki, T. Hayashida, H. Jinno, S. Hirose, M. Sakata, M. Takahashi, S. Maheswaran, M. Mukai, Y. Kitagawa, HOXB9 expression promoting tumor cell proliferation and angiogenesis is associated with clinical outcomes in breast cancer patients, Ann. Surg. Oncol. 19 (2012) 1831–1840.
[17] K. Rouault, V. Scotet, S. Autret, F. Gaucher, F. Dubrana, D. Tanguy, C. Yaacoub El Rassi, B. Fenoll, C. Ferec, Do HOXB9 and COL1A1 genes play a role in congenital dislocation of the hip? Study in a Caucasian population, Osteoarthritis Cartilage 17 (2009) 1099–1105.
[18] 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.
[19] D. Paul, L. Bridoux, R. Rezsohazy, I. Donnay, HOX genes are expressed in bovine and mouse oocytes and early embryos, Mol. Reprod. Dev. 78 (2011) 436–449.
[20] K. Ohnishi, T. Tobita, K. Sinjo, A. Takeshita, R. Ohno, Modulation of homeobox B6 and B9 genes expression in human leukemia cell lines during myelomonocytic differentiation, Leuk. Lymphoma 31 (1998) 599–608.
[21] S. Sha, Y. Gu, B. Xu, H. Hu, Y. Yang, X. Kong, K. Wu, Decreased expression of HOXB9 is related to poor overall survival in patients with gastric carcinoma, Dig. Liver Dis. 45 (2013) 422–429.
[22] N. Tomioka, K. Morita, N. Kobayashi, M. Tada, T. Itoh, S. Saitoh, M. Kondo, N. Takahashi, A. Kataoka, K. Nakanishi, M. Takahashi, T. Kamiyama, M. Ozaki, T. Hirano, S. Todo, Array comparative genomic hybridization analysis revealed four genomic prognostic biomarkers for primary gastric cancers, Cancer Genet. Cytogenet. 201 (2010) 6–14.
[23] B. Kaminska, M. Kocyk, M. Kijewska, TGF beta signaling and its role in glioma pathogenesis, Adv. Exp. Med. Biol. 986 (2013) 171–187.
[24] M.T. Jennings, J.A. Pietenpol, The role of transforming growth factor beta in glioma progression, J. Neurooncol. 36 (1998) 123–140.