CHAPTER NINE

Expression of Twist associated to microcirculation patterns of human glioma correlated with progression and survival of the patient

Cong Li[a,†,‡], Yinsheng Chen[a,†], Qingping Zhang[b,†], Chengcheng Guo[a], Furong Chen[a], Shaoyan Xi[a], Jing Zeng[a], Chao Ke[a], Hari Shanker Sharma[c,*], Zhongping Chen[a,*]

[a]Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China
[b]Department of Neurosurgery, Shenzhen Nanshan People's Hospital (Shenzhen University Sixth Affiliated Hospital), Shenzhen, China
[c]International Experimental Central Nervous System Injury & Repair (IECNSIR), Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University Hospital, Uppsala University, S-75185 Uppsala, Sweden
*Corresponding authors: e-mail address: Sharma@surgsci.uu.se; chenzp57@mail.sysu.edu.cn

Contents

† Contributed equally to this article.
‡ Cong Li is now working in Chinese Medicine Hospital of Guangdong Province; The Second Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou, China

International Review of Neurobiology, Volume 151
ISSN 0074-7742
https://doi.org/10.1016/bs.irn.2020.03.026

Abstract

Twist is a transcription factor involved in the process of epithelial to mesenchymal transition (EMT) of carcinoma cells, and the promotion of invasion of gliomas through the mesenchymal adjusting process. However, its clinical significance in human glioma has not yet to be understood. To delineate the clinical-pathological significance and prognostic value of Twist, the expression of Twist was evaluated by Immunohistochemistry for 187 glioma samples. We found that Twist demonstrated frequent nuclear expression in the glioma samples and its expression levels were associated with tumor grade ($P<0.001$). Furthermore, high Twist expression was correlated with a poor outcome in patients with glioma ($P=0.001$), particularly with high grade glioma ($P=0.026$). Interestingly, Twist expression showed positive correlation with microvascular density (MVD) ($r=0.145$, $P=0.048$) as well as vasculogenic mimicry (VM) ($r=0.273$, $P<0.001$) in the tumors. These results suggest that Twist could be a predictor for poor prognosis in glioma patients. Additionally, Twist expression was associated with two major microcirculation patterns: endothelial-dependent vessels and VM in glioma, indicating that Twist could be a potential molecular target for anti-glioma therapy.

1. Introduction

Glioma is the most common primary brain tumor and also one of the most lethal human cancers despite current treatment with surgery, irradiation, and chemotherapy. The median survival time for patients with glioblastoma multiforme (GBM), the most malignant glioma, is at best still only 12–15 months (Wen & Kesari, 2008). Invasion and microvascular proliferation are the most important features of human glioma, especially in the high grade gliomas (WHO grade III and IV). Thus, development of effective anti-invasion and optimization of anti-vascular therapy should be one of the important strategies.

Twist is a basic helix-loop-helix transcription factor which plays a fundamental role in regulating epithelial to mesenchymal transition (EMT), which is a key step in tumor cell invasion and metastasis. Mesenchymal change, similar to EMT, is increasingly recognized in human glioma (Carro et al., 2010; Phillips et al., 2006). Recent studies have shown that Twist is widely expressed in human glioma cell lines and clinical samples (Elias et al., 2005). Notably, Twist also can promoted invasion in glioma through mesenchymal change (Mikheeva et al., 2010). Furthermore, recent

studies have demonstrated that Twist is not only involved in tumor angiogenesis (Hu, Roth, Brooks, Ibrahim, & Karpatkin, 2008; Mironchik et al., 2005) but also plays an important regulatory role in vasculogenic mimicry (VM) (Ma et al., 2011; Sun et al., 2010), defined as tumor cells mimicking endothelial cells to form extracellular matrix–rich channels. However, whether Twist is linked to angiogenesis or VM in glioma is still unknown.

A number of studies have indicated that Twist is over expressed in breast cancer (Soini et al., 2011), prostate cancer (Kwok et al., 2005), and gastric cancer (Yan-Qi et al., 2007) etc. These findings suggest that Twist is associated with tumor genesis or tumor progression as a novel oncogene. Twist expression also has an impact on survival of patients with bladder cancer (Fondrevelle et al., 2009), esophageal squamous cell carcinoma (Xie, Li, & Ouyang, 2009), colorectal carcinomas (Gomez et al., 2011) or nasopharyngeal carcinoma (Song et al., 2006). However, there are limited studies regarding the clinical significance of increased Twist expression in human gliomas. The present study was designed to evaluate the clinical significance of Twist expression and the relationship between Twist expression, angiogenesis, and VM in human gliomas.

2. Materials and methods

2.1 Ethics statement

All patients signed the informed consent that allowing researchers to use their glioma specimens for study (two patient consent form samples were attached). The current study was approved by the Institutional Review Board and the ethics committee of Sun Yat-sen University Cancer Center.

2.2 Tissue samples of glioma patients

Glioma specimens were obtained from 187 patients who underwent craniotomy for tumor resection between 2000 and 2008 at Sun Yat-sen University Cancer Center. All samples were examined by two pathologists and histopathologically classified according to the World Health Organization (WHO) classification (2007) criteria of central nervous system tumors. There were 19 cases of grade I glioma, 64 cases of grade II, 52 cases of grade III and 52 cases of grade IV. Grades I and II gliomas were considered as a low grade group, and grades III and IV as a high grade group. Detailed clinical follow-up data of all patients were collected.

2.3 Immunohistochemistry studies

Immunohistochemistry assay was performed to evaluate the expression of Twist protein in the glioma tissues. Five-μm-thick tissue sections were

cut from paraffin-embedded glioma specimens. Sections were deparaffinized in xylene and dehydrated with a series of graded ethanol. The slides were immersed in a 3% hydrogen peroxide solution for 20 min at room temperature to block the endogenous peroxidase activity of specimens. Then the slides were heated in 0.01 M citrate buffer solution (pH = 6.0) for 15 min in a microwave for antigen retrieval. After washing three times with phosphate buffered saline (PBS) for 5 min each, nonspecific binding was blocked with normal goat serum for 20 min at room temperature. The blocked slides were incubated with primary anti-Twist antibody (mouse monoclonal antibody ab50887, dilutions 1:100; Abcam, Cambridge, UK) overnight at 4 °C. Then the slides were briefly washed in PBS and incubated at room temperature with the secondary anti-mouse IgG antibody (PV6002; Zhongshan Chemical Co., Beijing, China) for 50 min. After washing in PBS, the slides were color-developed using DAB solution (Dako Corporation, Carpinteria, CA, USA). The sections were then washed with water and counterstained with Mayer's hematoxylin. Breast cancer tissue with known positive Twist expression was used as a positive control and negative control was created by replacing the primary antibodies with PBS. The findings of the immunohistochemistry were evaluated by two pathologists independently. Only nuclear staining was considered as positive.

MGMT expression in glioma samples was determined by immunohistochemical analysis, which is routing examine. Briefly, 5-μm-thick tissue sections were cut from paraffin-embedded glioma specimens and stained with mouse anti-human-MGMT antibody (Invitrogen, USA) following protocol of the kit. Quantification was performed by counting the number of stained cells in 10 random fields at 400 × magnification for each tumor sample, and only those positive cell number $\geq$ 10% was considered as positive expression.

2.4 CD34 and PAS dual staining

As endothelial-dependent vessels are CD34-positive while vasculogenic mimicry is CD34-negative with PAS-positive patterns, CD34 and PAS dual staining were used to detect those two micro-circulation patterns in glioma specimens. Briefly, standard immunohistochemical staining was performed on paraffin-embedded tumor sections, at 5 μm, for CD34 (rabbit monoclonal anti-CD34 antibody 1:1000; Epitomics Inc., CA, USA) as described previously (Yue & Chen, 2005). The slides were rinsed with distilled water for

5 min, incubated with periodic acid-schiff (PAS) for 15 min, counterstained with Mayer's hematoxylin for 1 min, and viewed under a light microscope to detect CD34 and PAS signals.

2.5 Staining evaluation

The two-way scoring system was used for Twist immunostaining evaluation (Fondrevelle et al., 2009; Sasaki et al., 2009). The intensity of Twist staining was scored as: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The proportion of positive cells was also scored as: 0 (0%–5%), 1 (5%–25%), 2 (25%–50%), 3 (50%–75%), and 4 (75%–100%). Then overall staining scores were obtained by adding these two scores (0–7). An overall staining score of 0–5 and 6–7 were assigned as low and high expression group, respectively.

Endothelial-dependent vessels and vasculogenic mimicry (VM) were detected by CD34 and PAS dual staining. Microvascular density (MVD) was evaluated by counting CD34-positive channels in five randomly selected fields at 200 × magnification and the average counts were then calculated. According to the criteria of Folberg (Folberg, Hendrix, & Maniotis, 2000), VM was identified when the vessels were CD34-negative and PAS-positive, red blood cells could be found in the channel, and necrosis and inflammatory cells were absent around the channels.

2.6 Statistical analyses

All data involved in this study were analyzed by using SPSS version 13.0 software. Associations between Twist expression levels and clinical data were calculated by utilizing the Pearson's chi-square test. The prognostic value of Twist expression levels were evaluated using the Kaplan-Meier analysis and log-rank tests. The multivariate analysis was applied for survival analysis according to the Cox proportional hazards regression model. Spearman's rank correlation test and Pearson's chi-square independence test were used to analyze the correlation between Twist immunoreactivity scores and MVD, and the association between Twist expression levels and vasculogenic mimicry, respectively. In all statistical tests, a value of $P \leq 0.05$ was considered as significant.

3. Results

3.1 Clinical data of the patients

The general clinic-pathological characteristics of 187 investigated patients are summarized in Table 1. One hundred and sixteen (62%) patients were

Table 1 Associations between Twist expression and clinic- pathological characteristics of glioma patient.

		Twist			
Characteristics	**N**	**Low expression**	**High expression**	χ^2	***P* values**
Gender					
Male	116	80	36	0.155	0.694[a]
Female	71	47	24		
Age					
<40	104	73	31	0.558	0.455[a]
≥40	83	54	29		
KPS					
<70	15	10	5	0.012	0.914[b]
≥70	172	117	55		
WHO grade					
I	19	17	2	19.167	<0.001[a,c]
II	64	53	11		
III	52	30	22		
IV	52	27	25		
Extent of tumor resection					
Complete resection	132	85	47	2.553	0.110[a]
Incomplete resection	55	42	13		
MGMT protein					
Negative	48	27	21		
Positive	45	21	24	0.137	0.754[a]

[a]Pearson Chi-Square test (asymptotic significance, two-sided).
[b]Fisher's exact test (two-sided).
[c]Significantly different.

male and 71 (38%) patients were female. The median age at surgery was 37 years old (range, 2–75 years old). The median preoperative Karnofsky performance score (KPS) of the patients was 80, with 172 of 187 (92%) patients presenting with a preoperative KPS of ≥70 and 15 (8%) with a preoperative KPS lower than 70. Complete tumor resection was achieved in

132 (70.6%) patients, while 55 (29.4%) patients received subtotal or partial tumor resection. The expression of MGMT protein was determined by immunohistochemistry in 93 patients, and revealed negative in 48 samples (51.6%) and positive in 45 samples (48.4%). There were 124 (66.3%) patients received radiotherapy and/or chemotherapy, while 63 (33.7%) patients without any other postoperative anti-tumor treatment. The mean follow-up time was 31.27 months.

3.2 Twist expression is correlated with tumor grade

Twist expression was found expressed in majority of glioma samples and its immunoreactivity was in the nucleus of tumor cell (Fig. 1). Of the 187 tumor samples examined, only 8 cases (4.3%) showed without Twist expression (overall staining scores = 0). To determine the clinical significance of Twist expression levels in gliomas, we compared the expression levels of Twist with clinic-pathological characteristics of the patients. Twist immunoreactivity scores were significantly higher in high-grade glioma (5.08 ± 1.51) compared to low-grade glioma (4.10 ± 1.59) ($Z = -4.650$, $P < 0.001$), and Twist expression levels were increased with ascending glioma grade (Table 1). However, Twist expression levels were not significantly associated with other clinic-pathological variants (Table 1).

3.3 Twist expression levels are related to overall survival of patients

The median survival time was 62.63 months (95% CI, 26.09–99.18 months) for those patients with low expression levels of Twist, compared with a median survival time of 14.23 months (95% CI, 11.43–17.04 months) for patients with high Twist expression levels. That is, patients with high expression levels of Twist had a shorter survival time than those with low expression levels (log-rank test, $P = 0.001$) (Fig. 2). By multivariate analysis using the Cox proportional hazards regression model, patients with high Twist expression showed a poor overall survival (RR = 1.748, 95% CI 1.120–2.727, $P = 0.014$) after adjusting for gender, age, WHO grade, KPS, extent of tumor resection, the expression of MGMT protein. Thus, according to these results, Twist expression in human glioma could be considered as a significant and independent prognostic factor.

Interestingly, the Kaplan-Meier analysis showed that high expression of Twist correlated with poor prognosis, but only in the high-grade glioma group ($P = 0.026$) (Fig. 3). We also analyzed the prognostic value of Twist expression levels according to other clinic-pathological parameters.

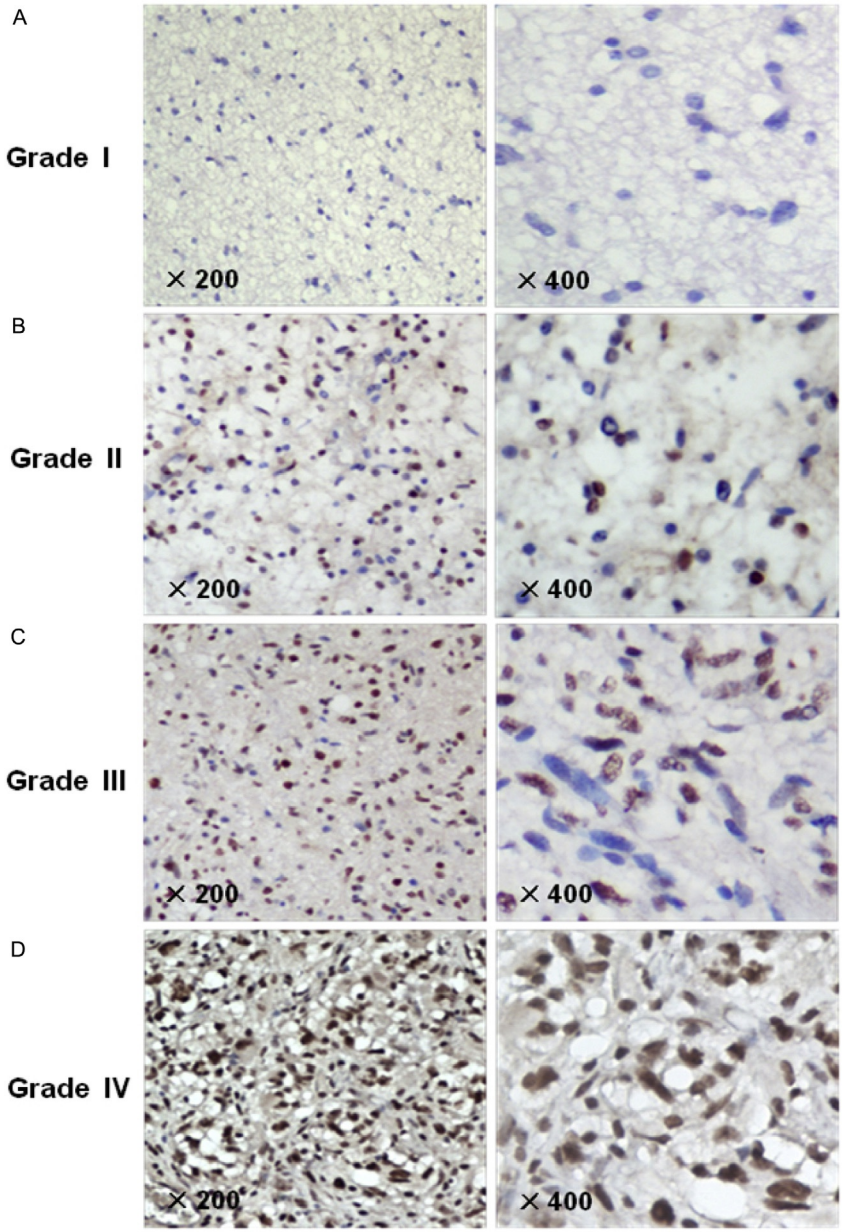

Fig. 1 Representative staining of Twist protein. Immunohistochemical staining of Twist protein in glioma samples and positive staining was seen in the nucleus of tumors cells. Twist expressions was up-regulated with ascending glioma WHO grades (a, WHO grade I; b, WHO grade II; c, WHO grade III; d, WHO grade IV) (original magnification $\times 200$ and $\times 400$).

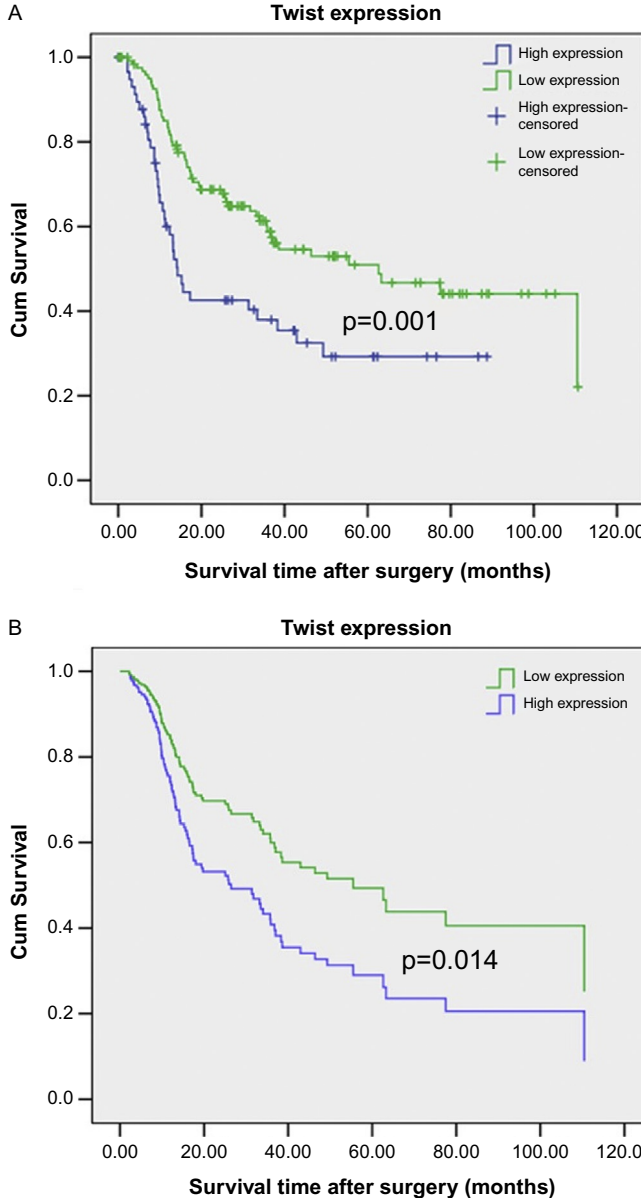

Fig. 2 The postoperative overall survival curves of patients with glioma according to Twist expression. (A) There was a significant difference in survival time between the glioma patients with low and high expression levels of Twist (Kaplan-Meier postoperative survival curve, $P = 0.001$). (B) Cox proportional hazards regression model after adjusting for gender, age, WHO grade, KPS, expression of MGMT protein, postoperative treatment and extent of tumor resection ($P = 0.014$, RR = 1.748, 95% CI 1.120–2.727).

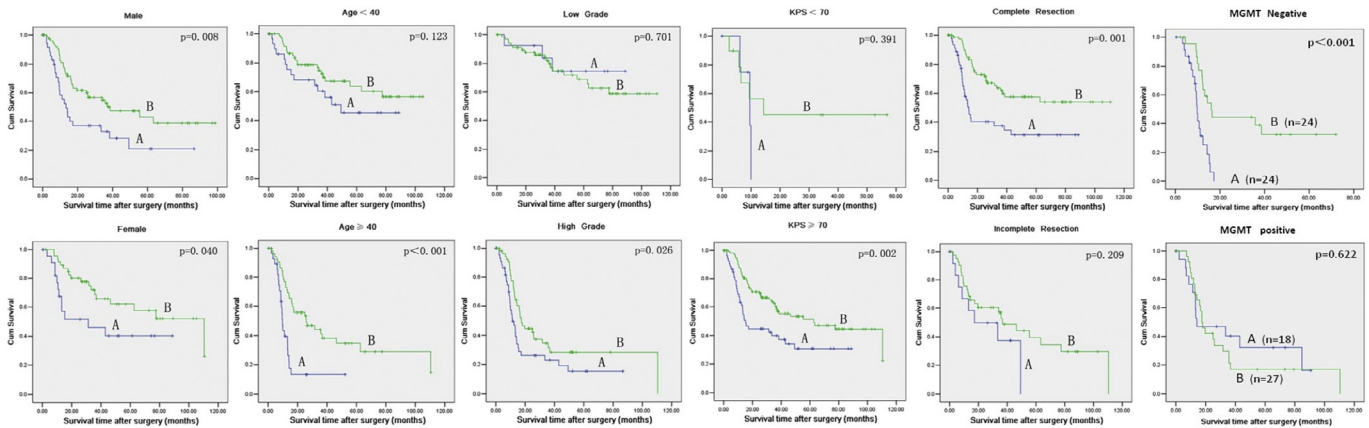

Fig. 3 The postoperative overall survival curves between the glioma patients with high or low expression of Twist according to subgroups of clinicopathological parameter. In the age $\geq$40, high grade, KPS $\geq$ 70, complete resection, MGMT negative and both gender subgroups, the patients with low Twist expression had a better outcome than those with high Twist expression levels. In the age $<$40, low grade, KPS $<$ 70, MGMT positive and incomplete resection subgroups, the survival curve was not significantly different according to the Twist expression levels.

High Twist expression levels correlated with significant shorter survival times compared to low expression levels of Twist at age ≥ 40, KPS ≥ 70, complete tumor resection and within both gender subgroups (Fig. 3).

3.4 Twist expression is associated with MVD and VM

The median MVD, which was evaluated by counting CD34-positive endothelial cell clusters (Fig. 4), was 28.20 (range, 7.20–83.20). The mean levels of MVD in low-grade and high-grade gliomas were 25.68 ± 11.71 and 35.54 ± 16.43, respectively. MVD was detected at a lower level in low Twist expression tumors (29.53 ± 15.47) than in high Twist expression tumors (34.62 ± 14.46) ($Z = -2.647$, $P = 0.008$) (Table 2). We then explored potential correlation between the Twist staining score and MVD in gliomas. Linear trend was found from the scatter plot ($r = 0.145$, $P = 0.048$) (Fig. 5), indicating that Twist expression positively correlated with MVD in the glioma samples.

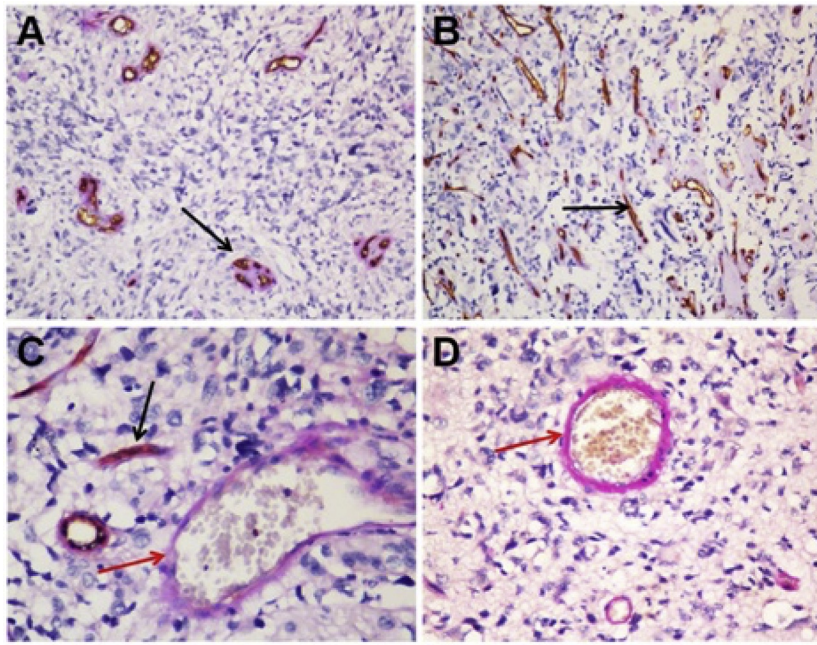

Fig. 4 Representative staining of microvessel density and vasculogenic mimicry. (A and B) Different microvessel density was showed as CD34-positive patterns (black arrows) (Original magnification $\times 200$). (C and D) Typical vasculogenic mimicry was defined as CD34-negative plus PAS-positive tunnel (red arrows) and red blood cells can find in this channel (Original magnification $\times 400$).

Table 2 Relationship between the expression levels of Twist and MVD, VM in human glioma.

	Twist low expression	Twist high expression	*P* values
MVD (mean ± SD)	29.53 ± 15.47	34.62 ± 14.46	0.008[a,b]
VM			
Negative (n)	119	44	<0.001[b,c]
Positive (n)	8	16	

[a]Wilcox on rank sum test of two independent samples (asymptotic significance, two-sided).
[b]Significantly different.
[c]Pearson Chi-Square test (asymptotic significance, two-sided).

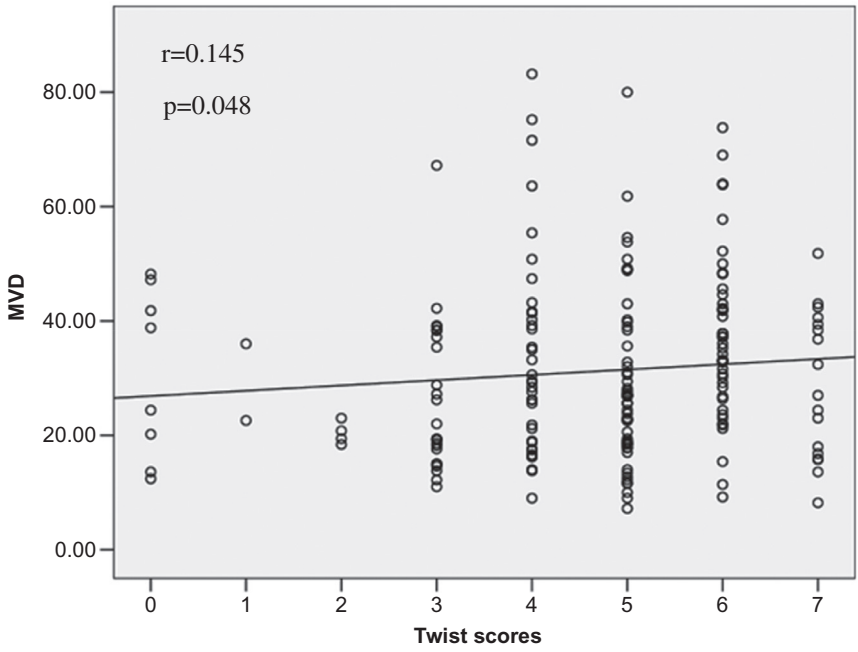

Fig. 5 Positive correlation between the immunoreactivity scores of Twist and MVD in human glioma (Spearman's rank correlation test, $r = 0.145$, $P = 0.048$).

Vasculogenic mimicry was identified as the presence of vessels lined by tumor cells (CD34-negative) instead of endothelial cells and surrounding extracellular matrix (PAS-positive) (Fig. 4). Vasculogenic mimicry was found in 24 out of the total 187 (12.83%) glioma samples. There were 2 cases of grade II, 7 cases of grade III and 15 cases of grade IV gliomas. High expression level of Twist was observed in 16 of the 24 (36.37%) VM-positive

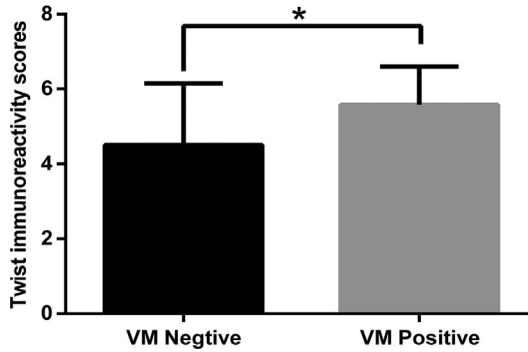

Fig. 6 Twist expression levels were significantly different between the VM-positive and VM-negative groups ($\chi^2 = 15.110$, $P < 0.001$).

tumor samples and in 44 of the 163 (27%) VM-negative samples (Table 2). Twist expression levels were significantly different between the VM-positive and VM-negative groups (Fig. 6, $\chi^2 = 15.110$, $P < 0.001$).

4. Discussion

The expression of Twist was examined in 187 human glioma samples. We found that Twist expression levels were associated with tumor grade, indicating that Twist might participate in the progression and promote the aggressiveness of glioma. Moreover, our data provided evidence that the expression of Twist was an independent prognostic factor for human glioma through the univariate survival analysis (Kaplan-Meier analysis) and the multivariate analysis (Cox proportional hazards regression model). We also demonstrated that there was a relationship between Twist expression and the two major microcirculation patterns, endothelium dependent vessels and vasculogenic mimicry in human gliomas.

Twist was originally described in *Drosophila* as a gene essential for the dorsoventral pattern (Thisse, el Messal, & Perrin-Schmitt, 1987). Recent studies have already highlighted the fundamental role of Twist in activating the EMT process in the context of embryogenesis (Yu, Kamara, & Svoboda, 2008) (Type 1 EMT), organ fibrosis (Bridges et al., 2009) (Type 2 EMT) and cancer metastasis (Yang et al., 2004) (Type 3 EMT). EMT mediated by Twist in carcinoma is a process where tumor cells lose their polarity and cell–cell adhesion, acquire a high degree of motility, and allowing invasion and metastasis through repression of the epithelial marker E-cadherin and acquisition of the mesenchymal marker N-cadherin, termed "cadherin switch."

Invasion is one of the most important features of human glioma and is responsible for the dismal prognosis in cancer patients. Diffuse tumor invasion into surrounding brain restricts radical resection and limits effective delivery of chemoradiotherapy. Mikheeva et al. (2010) demonstrated that Twist can also promote invasion in glioma through mesenchymal change, which is similar to the EMT process but without the "cadherin switch." Our study found that Twist expression was higher in high grade glioma than low grade glioma, which is less invasive than high grade glioma. This observation could support the idea that Twist expression is related to the invasive behavior of glioma.

In addition to invasion, abundant angiogenesis, characterized by microvascular proliferation and glomeruloid vascular structure, is another hallmark of glioma, especially in GBM. With current rapid development in molecular-targeted and anti-angiogenesis research, anti-vascular therapy shows promise in having a significant effect against gliomas. Recent studies reported that a new micro-circulation pattern termed vasculogenic mimicry, which describes the formation of fluid-conducting channels by highly invasive tumor cells instead of endothelial cells, co-exists with conventional endothelial-dependent vessels in a variety of cancer types, including melanoma (Maniotis et al., 1999), carcinomas (Liu, Xu et al., 2011; Sun, Fan, Zhang, & Ge, 2011), sarcomas (Sun, Zhang, Zhao, Zhang, & Hao, 2004), and even glioma (El Hallani et al., 2010; Yue & Chen, 2005). The discovery of VM reveals the heterogeneity of micro-circulation patterns in glioma, and presents a challenge in the development of anti-vascular therapy. Twist has also been found to not only induce angiogenesis in breast cancer (Mironchik et al., 2005) and hepatocellular carcinoma (Niu et al., 2007), but also plays an important role in VM formation in hepatomas (Ma et al., 2011; Sun et al., 2010). In addition, Twist has been implicated in the plasticity of cancer stem cells (Li & Zhou, 2011; Wu, 2011), now considered the initiative cell of VM (Ricci-Vitiani et al., 2010; Wang et al., 2010). These findings suggest that Twist might be a potential target both in anti-angiogenesis and anti-VM therapy. Our study, for the first time, found that Twist expression was associated with MVD and VM in human glioma, indicating that Twist might provide a chance to overcome the heterogeneity of micro-circulation patterns in human glioma, although more evidence is needed. As VM has been demonstrated to correlate with the survival of glioma patients (Liu, Zhang, et al., 2011), our results suggest the possibility that Twist influences patient survival by inducing VM formation.

In conclusion, our data shows that Twist expression levels correlated with glioma grade, and high expression levels of Twist may identify glioma

patients with poorer prognosis，especially in high grade gliomas. As such, Twist could be defined as a potential new predictor for poor outcome in patients with malignant glioma. Twist expression associated with MVD and VM in human glioma, indicating that Twist could be a target for anti-vascular therapy in human glioma,

Funding

National Natural Science Funds of China (No. 81372685), National Basic Research Program of China (No. 2015CB755505), Guangzhou Science Technology Project (No. 201508020125, 201803010056), and the Science and Technology Planning Project of Guangdong Province (No. 2016A020213004).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding received for this study.

Competing interests

The authors have declared that no competing interests exist.

References

Bridges, R. S., Kass, D., Loh, K., Glackin, C., Borczuk, A. C., et al. (2009). Gene expression profiling of pulmonary fibrosis identifies Twist1 as an antiapoptotic molecular "rectifier" of growth factor signaling. *The American Journal of Pathology*, *175*, 2351–2361.

Carro, M. S., Lim, W. K., Alvarez, M. J., Bollo, R. J., Zhao, X., et al. (2010). The transcriptional network for mesenchymal transformation of brain tumours. *Nature*, *463*, 318–325.

El Hallani, S., Boisselier, B., Peglion, F., Rousseau, A., Colin, C., et al. (2010). A new alternative mechanism in glioblastoma vascularization: Tubular vasculogenic mimicry. *Brain*, *133*, 973–982.

Elias, M. C., Tozer, K. R., Silber, J. R., Mikheeva, S., Deng, M., et al. (2005). TWIST is expressed in human gliomas and promotes invasion. *Neoplasia*, *7*, 824–837.

Folberg, R., Hendrix, M. J., & Maniotis, A. J. (2000). Vasculogenic mimicry and tumor angiogenesis. *The American Journal of Pathology*, *156*, 361–381.

Fondrevelle, M. E., Kantelip, B., Reiter, R. E., Chopin, D. K., Thiery, J. P., et al. (2009). The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status. *Urologic Oncology*, *27*, 268–276.

Gomez, I., Pena, C., Herrera, M., Munoz, C., Larriba, M. J., et al. (2011). TWIST1 is expressed in colorectal carcinomas and predicts patient survival. *PLoS One*, *6,* e18023.

Hu, L., Roth, J. M., Brooks, P., Ibrahim, S., & Karpatkin, S. (2008). Twist is required for thrombin-induced tumor angiogenesis and growth. *Cancer Research*, *68*, 4296–4302.

Kwok, W. K., Ling, M. T., Lee, T. W., Lau, T. C., Zhou, C., et al. (2005). Up-regulation of TWIST in prostate cancer and its implication as a therapeutic target. *Cancer Research*, *65*, 5153–5162.

Li, J., & Zhou, B. P. (2011). Activation of beta-catenin and Akt pathways by Twist are critical for the maintenance of EMT associated cancer stem cell-like characters. *BMC Cancer*, *11*, 49.

Liu, W. B., Xu, G. L., Jia, W. D., Li, J. S., Ma, J. L., et al. (2011). Prognostic significance and mechanisms of patterned matrix vasculogenic mimicry in hepatocellular carcinoma. *Medical Oncology*, *28*(Suppl. 1), S228–S238.

Liu, X. M., Zhang, Q. P., Mu, Y. G., Zhang, X. H., Sai, K., et al. (2011). Clinical significance of vasculogenic mimicry in human gliomas. *Journal of Neuro-Oncology*, *105*, 173–179.

Ma, J. L., Han, S. X., Zhu, Q., Zhao, J., Zhang, D., et al. (2011). Role of Twist in vasculogenic mimicry formation in hypoxic hepatocellular carcinoma cells in vitro. *Biochemical and Biophysical Research Communications*, *408*, 686–691.

Maniotis, A. J., Folberg, R., Hess, A., Seftor, E. A., Gardner, L. M., et al. (1999). Vascular channel formation by human melanoma cells in vivo and in vitro: Vasculogenic mimicry. *The American Journal of Pathology*, *155*, 739–752.

Mikheeva, S. A., Mikheev, A. M., Petit, A., Beyer, R., Oxford, R. G., et al. (2010). TWIST1 promotes invasion through mesenchymal change in human glioblastoma. *Molecular Cancer*, *9*, 194.

Mironchik, Y., Winnard, P. T., Jr., Vesuna, F., Kato, Y., Wildes, F., et al. (2005). Twist overexpression induces in vivo angiogenesis and correlates with chromosomal instability in breast cancer. *Cancer Research*, *65*, 10801–10809.

Niu, R. F., Zhang, L., Xi, G. M., Wei, X. Y., Yang, Y., et al. (2007). Up-regulation of Twist induces angiogenesis and correlates with metastasis in hepatocellular carcinoma. *Journal of Experimental & Clinical Cancer Research*, *26*, 385–394.

Phillips, H. S., Kharbanda, S., Chen, R., Forrest, W. F., Soriano, R. H., et al. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*, *9*, 157–173.

Ricci-Vitiani, L., Pallini, R., Biffoni, M., Todaro, M., Invernici, G., et al. (2010). Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature*, *468*, 824–828.

Sasaki, K., Natsugoe, S., Ishigami, S., Matsumoto, M., Okumura, H., et al. (2009). Significance of Twist expression and its association with E-cadherin in esophageal squamous cell carcinoma. *Journal of Experimental & Clinical Cancer Research*, *28*, 158.

Soini, Y., Tuhkanen, H., Sironen, R., Virtanen, I., Kataja, V., et al. (2011). Transcription factors zeb1, twist and snai1 in breast carcinoma. *BMC Cancer*, *11*, 73.

Song, L. B., Liao, W. T., Mai, H. Q., Zhang, H. Z., Zhang, L., et al. (2006). The clinical significance of twist expression in nasopharyngeal carcinoma. *Cancer Letters*, *242*, 258–265.

Sun, W., Fan, Y. Z., Zhang, W. Z., & Ge, C. Y. (2011). A pilot histomorphology and hemodynamic of vasculogenic mimicry in gallbladder carcinomas in vivo and in vitro. *Journal of Experimental & Clinical Cancer Research*, *30*, 46.

Sun, B., Zhang, S., Zhao, X., Zhang, W., & Hao, X. (2004). Vasculogenic mimicry is associated with poor survival in patients with mesothelial sarcomas and alveolar rhabdomyosarcomas. *International Journal of Oncology*, *25*, 1609–1614.

Sun, T., Zhao, N., Zhao, X. L., Gu, Q., Zhang, S. W., et al. (2010). Expression and functional significance of Twist1 in hepatocellular carcinoma: Its role in vasculogenic mimicry. *Hepatology*, *51*, 545–556.

Thisse, B., el Messal, M., & Perrin-Schmitt, F. (1987). The twist gene: Isolation of a Drosophila zygotic gene necessary for the establishment of dorsoventral pattern. *Nucleic Acids Research*, *15*, 3439–3453.

Wang, R., Chadalavada, K., Wilshire, J., Kowalik, U., Hovinga, K. E., et al. (2010). Glioblastoma stem-like cells give rise to tumour endothelium. *Nature*, *468*, 829–833.

Wen, P. Y., & Kesari, S. (2008). Malignant gliomas in adults. *The New England Journal of Medicine*, *359*, 492–507.

Wu, K. J. (2011). Direct activation of Bmi1 by Twist1: Implications in cancer stemness, epithelial-mesenchymal transition, and clinical significance. *Chang Gung Medical Journal*, *34*, 229–238.

Xie, F., Li, K., & Ouyang, X. (2009). Twist, an independent prognostic marker for predicting distant metastasis and survival rates of esophageal squamous cell carcinoma patients. *Clinical & Experimental Metastasis*, *26*, 1025–1032.

Yang, J., Mani, S. A., Donaher, J. L., Ramaswamy, S., Itzykson, R. A., et al. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, *117*, 927–939.

Yan-Qi, Z., Xue-Yan, G., Shuang, H., Yu, C., Fu-Lin, G., et al. (2007). Expression and significance of TWIST basic helix-loop-helix protein over-expression in gastric cancer. *Pathology*, *39*, 470–475.

Yu, W., Kamara, H., & Svoboda, K. K. (2008). The role of twist during palate development. *Developmental Dynamics*, *237*, 2716–2725.

Yue, W. Y., & Chen, Z. P. (2005). Does vasculogenic mimicry exist in astrocytoma? *The Journal of Histochemistry and Cytochemistry*, *53*, 997–1002.