Correlation between chromosome 1p/19q status and *VEGF* mRNA expression in gliomas

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ABSTRACT. The chromosome 1p/19q deletion has been reported as a good prognosis marker for gliomas. However, the detection of 1p/19q status alone in glioma patients is not sufficient. The identification of a combination of molecular factors could effectively enhance the prediction accuracy. Thus far, the potential correlation between the 1p/19q status and vascular endothelial growth factor (*VEGF*) expression has not been elucidated. The level of *VEGF* mRNA expression in the tumor and the adjacent normal tissues was determined by real-time polymerase chain reaction. The 1p/19q status of glioma patients was determined by fluorescence *in situ* hybridization. The association between the 1p/19q status and *VEGF* mRNA expression, as well as the glioma grade, was evaluated. A higher *VEGF* mRNA expression level was observed in gliomas, compared to matched normal tissues ($P < 0.01$). The 1p/19q status was significantly correlated with glioma grade ($P = 0.018$) and *VEGF* mRNA expression in the tissues ($P = 0.005$). A higher percentage of patients with high-grade gliomas displayed an intact 1p/19q

and higher *VEGF* mRNA expression than those with low-grade gliomas. A survival analysis revealed that patients (with high- and low-grade gliomas) with intact 1p/19q and higher *VEGF* mRNA expression showed a shorter overall survival time. Moreover, tissue *VEGF* mRNA expression and WHO grade were found to be independent risk factors for gliomas. In conclusion, the 1p/19q status and *VEGF* mRNA expression in tissues could be used in combination to predict the prognosis of gliomas.

Key words: 1p/19q status; VEGF; Gliomas; Prognosis marker

INTRODUCTION

Gliomas, arising from the glial cells, are the most common solid brain malignancies affecting both adults and children (Ricard et al., 2012). Despite the considerable advancement in surgery, radiotherapy, chemotherapy, and targeted therapy, the prognosis of patients with gliomas, especially malignant gliomas, remains extremely poor (Gao et al., 2015). A high percentage of recurrence post-treatment is the major contributing factor for the unsatisfactory clinical outcome. The identification of molecular characteristics of gliomas might assist in improving the pathological diagnosis, and predict the prognosis of this malignant disease.

Genetic events have been closely correlated with the initiation and progression of gliomas. p53 mutations, isocitrate dehydrogenase mutation, epidermal growth factor receptor (EGFR) amplification, loss of heterozygosity (LOH) in chromosomes 17 and 10, as well as the 1p/19q co-deletion/translocation are frequently observed in gliomas (Cohen and Colman, 2015; Sabha et al., 2014). The 1p/19q co-deletion occurs as a result of the combined loss of genetic material in the short arm of chromosome 1 and the long arm of chromosome 19, due to an unbalanced translocation. Previous studies have reported that the 1p/19q co-deletion is associated with good outcome, and might be a valuable tool for clinical treatment of gliomas (Alentorn et al., 2014; Zhang et al., 2014). However, the magnitude of correlation varies among different studies; some investigations even failed to detect this association (Kuo et al., 2009; Weller et al., 2009). Therefore, the 1p/19q status identification alone might not be sufficient for the clinical therapy of gliomas; combination of 1p/19q status with other molecular biomarkers is a good strategy to solve this problem.

Vascular endothelial growth factor (VEGF) is the main mediator of angiogenesis, and plays an important role in the development of gliomas. Abdulrauf et al. (1998) reported that astrocytoma patients expressing higher concentrations of VEGF displayed shorter overall survival rates and a poor clinical outcome. Additionally, VEGF is an independent prognostic factor for astrocytoma. VEGF overexpression has been frequently overexpressed in glioblastoma and tumor cells, suggesting that VEGF might promote the growth of gliomas through angiogenesis (Clara et al., 2014). Moreover, the currently employed anti-angiogenic approaches that focus on the down-regulation of VEGF are also believed to be effective against gliomas.

Previous studies have reported a correlation between the 1p/19q co-deletion and various clinical features of gliomas, such as frontal lobe location, intratumoral heterogeneity, and tumor border (Zlatescu et al., 2001; Jenkinson et al., 2006). The VEGF and 1p/19q statuses are important clinical parameters of gliomas. However, the exact correlation between these factors remains unknown. The aim of this study was to evaluate the association between VEGF and 1p/19q and to identify the potential clinical value of this association.

MATERIAL AND METHODS

Study population

The study was approved by the Research Ethics Committee of the Affiliate Hospital of Qingdao University; written informed consent was obtained from all participants. One hundred and twenty-eight patients pathologically diagnosed with gliomas were recruited to the study; the gliomas were graded according to the WHO grading system. The researchers were blinded to all clinical samples. The tissues were stored at -80°C until further use. The age of the included participants (71 males and 57 females) ranged from 21 to 83 years.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from frozen tissues using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer protocols. First-strand cDNA was synthesized using the standard PrimeScript RT reagent kit (TaKaRa Bio Inc., Shiga, Japan). Real-time PCR was performed on an ABI 7500 Fast Real-time PCR platform (Applied Biosystems, Foster City, CA, USA). The PCR conditions were set as follows: denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 15 s. The *VEGF* mRNA levels were measured relative to that of glyceraldehyde-3-phosphate dehydrogenase mRNA; all reactions were performed in triplicate.

Fluorescence *in situ* hybridization (FISH)

Formalin-fixed paraffin-embedded blocks were analyzed using a 1p/19q fluorescent probe kit (Vysis Inc., Des Plaines, IL, USA) as described in a previous study (Ren et al., 2012). FISH signals were assessed under an epifluorescence microscope (ECLIPSE 80i; Nikon, Tokyo, Japan). A minimum of 100 non-overlapping cells were analyzed in the targeted region per sample for each hybridization, and the 1p/19q deletion was characterized by DNA loss in >30% of the observed nuclei.

Statistical analysis

An independent paired *t*-test was performed to reveal the *VEGF* mRNA expression level in glioma tissues, and matched adjacent normal tissues. The association between the 1p/19q status and glioma grade, as well as the *VEGF* mRNA expression in tissues were analyzed by a chi-squared test. Associations between a combination of the 1p/19q status and *VEGF* mRNA expression in the tissues, and overall survival was evaluated by Kaplan-Meier method and log-rank test. Multivariate cox regression model was conducted to determine the independent prognostic factors for gliomas. All calculations were performed using the SPSS 21.0 statistical package (SPSS Inc., Chicago, IL, USA); P values <0.05 were considered to be statistically significant.

RESULTS

Level of expression of *VEGF* mRNA in glioma tissues

Real-time PCR was performed to compare the *VEGF* mRNA expression in glioma tissues

and adjacent normal tissues. The results showed that gliomas expressed a significant higher amount of *VEGF* mRNA compared to the controls (8.36 ± 2.35 *vs* 1.41 ± 0.53; P < 0.001) (Figure 1).

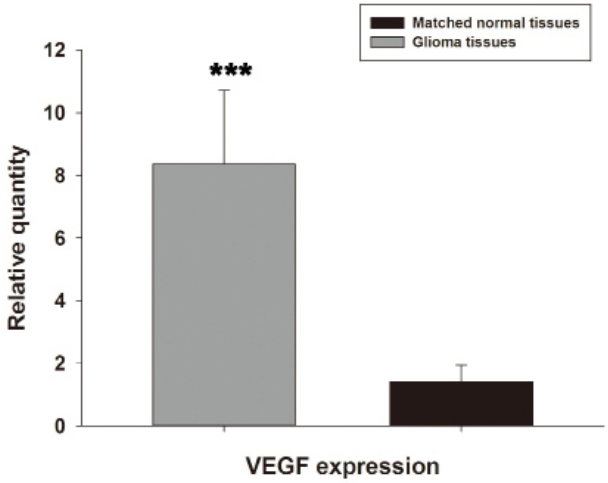

Figure 1. Level of expression of *VEGF* mRNA in glioma tissues. Glioma tissues expressed a significant higher amount of *VEGF* mRNA than the matched controls (8.36 ± 2.35 *vs* 1.41 ± 0.53; ***P < 0.001).

Correlation between glioma grade/*VEGF* mRNA expression and 1p/19q status

The 1p/19q status of glioma patients was determined by FISH. The results revealed that 67 patients (52.34%) had intact 1p/19q, while the remaining patients showed a deletion in the 1p/19q status (47.66%).

The included patients were categorized into the high grade (grade III-IV) or low grade (grade I-II) glioma group, according to the WHO grading system. A strong association was observed between the glioma grade and 1p/19q status (P = 0.018); patients with a lower grade glioma exhibited a higher percentage of 1p/19q deletion (57.14%) than those with a higher grade glioma (36.21%).

The average expression of *VEGF* mRNA was used as the cut-off point to divide the glioma patients into the high *VEGF* and low *VEGF* mRNA expression groups. We deduced a significant correlation between the *VEGF* mRNA expression in the tissues and the 1p/19q status (P = 0.005). A higher percentage of glioma patients included in the low *VEGF* mRNA expression group displayed the 1p/19q deletion (61.02%) than those included in the high *VEGF* mRNA expression group (36.23%) (Table 1).

Table 1. Correlation between glioma grade/tissue *VEGF* mRNA expression and 1p/19q status.

Variable	1p/19q status		χ^2	P value
	Intact	Deletion		
Gliomas				
Low grade	30	40		
High grade	37	21	5.573	0.018
Tissue *VEGF* mRNA				
Low	23	36		
High	44	25	7.832	0.005

Poor prognosis of glioma patients with intact 1p/19q status and high tissue *VEGF* mRNA expression

The patients were divided into three groups based on their 1p/19q status and *VEGF* mRNA expression level (group 1: patients with intact 1p/19q and high *VEGF* mRNA expression; group 2: patients with 1p/19q deletion and low *VEGF* mRNA expression; group 3: Others; patients with other 1p/19q statuses, and high/low tissue *VEGF* mRNA expression). We observed significant differences in the 1p/19q distribution pattern and *VEGF* mRNA expression between high-grade and low-grade glioma tissues (P = 0.010). A higher percentage of patients with high-grade gliomas (48.28%) expressed an intact 1p/19q and high tissue *VEGF* mRNA expression than those with low-grade gliomas (22.86%) (Table 2).

The mean survival time of patients with low-grade gliomas in group 1 was 20.56 ± 4.03 months, significantly shorter than that of similar patients in group 2 (35.75 ± 3.44 months; P = 0.005). No significant difference was found in the overall survival time between group 3 and group 2 (P = 0.187) or group 1 (P = 0.056) (Figure 2).

Table 2. Distribution of patients with gliomas categorized according to the 1p/19q status and *VEGF* expression.

Gliomas	1p/19q status and *VEGF* mRNA expression			χ^2	P
	Intact and High	Deletion and Low	Others		
Low grade	16 (22.86%)	24 (34.29%)	30 (42.86%)		
High grade	28 (48.28%)	12 (20.69%)	18 (31.03%)	9.229	0.010

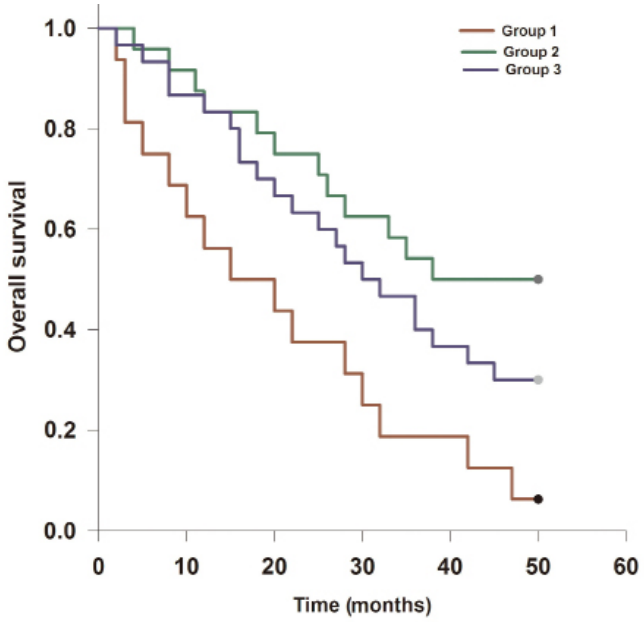

Figure 2. Poor prognosis of patients with low-grade glioma with intact 1p/19q status and high *VEGF* mRNA expression in the tissues. The mean survival rate of group 1 patients with low-grade gliomas was 20.56 ± 4.03 months, which was significant lower than that of group 2 (35.75 ± 3.44 months; P = 0.005). The overall survival rates of group 3 did not differ significantly from those of groups 2 (P = 0.187) and 1 (P = 0.056).

The mean survival time of high-grade glioma patients in group 1 (16.36 ± 1.85 months) differed significantly from those of patients in groups 2 (26.92 ± 4.18 months; P = 0.019) and 3 (23.00 ± 2.97 months; P = 0.032) (Figure 3).

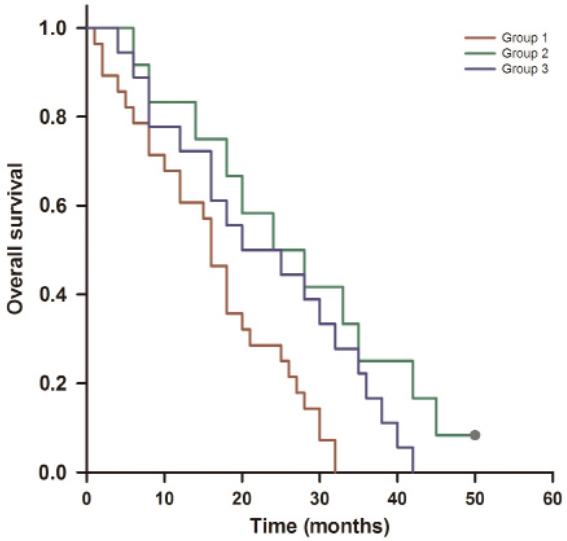

Figure 3. Patients with high-grade gliomas expressing an intact 1p/19q and high tissue *VEGF* mRNA showed poor prognosis. Patients with high-grade gliomas belonging to group 1 had a mean survival time of 16.36 ± 1.85 months, which differed significantly from the mean survival time of patients in groups 2 (26.92 ± 4.18 months; P = 0.019) and 3 (23.00 ± 2.97 months; P = 0.032).

Independent risk factors for gliomas

The Cox multivariate analysis showed that the WHO grade (HR = 5.561, P = 0.009) and *VEGF* mRNA expression levels (HR = 3.649, P = 0.031) were independent risk factors for gliomas. Although the 1p/19q status was not associated with overall survival in the multivariate Cox regression model, a borderline significance was achieved (HR = 2.158, P = 0.058) (Table 3).

Table 3. Cox multivariate analysis.

Parameter	HR	95%CI	P value
Age (>60 *vs* ≤60 years)	1.340	0.284-2.627	0.631
Gender (Male *vs* female)	1.083	0.523-1.895	0.826
Grade (High *vs* low)	5.561	2.351-8.398	0.009
1p/19q status (Intact *vs* deletion)	2.158	0.915-3.680	0.058
Tissue VEGF mRNA (High *vs* low)	3.649	1.457-4.817	0.031

HR = hazard ratio; CI = confidence interval.

DISCUSSION

Currently, gliomas are classified into four grades using the 2007 World Health Organization

(WHO) grading scale: grades I, II, III (anaplastic), and IV (glioblastoma) (Louis et al., 2007). This system separates gliomas according to their tissue histopathology, and remains the gold standard for glioma. The practicality and effectiveness of this system allows for its wide acceptance for clinical usage. However, the system, based solely on visual criteria, is not a subjective analysis (Vigneswaran et al., 2015); the latter might result in a different diagnosis because of inter-observer variation between multiple pathologists. One of the solutions for this conundrum is to combine the various molecular features of gliomas to better diagnose and classify this deadly disease.

Our results show that the level of expression *VEGF* mRNA was up-regulated significantly in tumor tissues compared to matched normal tissues. Moreover, *VEGF* mRNA in tissues was an independent risk factor for gliomas. Cao et al. (2014) revealed that an overexpression of the VEGF protein in glioma tissues was associated with disease progression. Stockhammer et al. (2000) detected high VEGF levels in the cyst of various types of gliomas, and associated this with local disease progression. Malignant gliomas are highly vascularized, and angiogenesis plays an indispensable role in the development of this disease; therefore, anti-angiogenesis therapy is a promising strategy for gliomas.

We observed a strong association between the 1p/19q status and tumor grade. Speirs et al. (2015) reported that both anaplastic oligodendroglioma and mixed anaplastic oligoastrocytoma patients with the 1p/19q co-deletion had superior overall survival, compared to those with intact 1p/19q, suggesting that the 1p/19q deletion might be a positive risk factor for gliomas, which was consistent with the results of a previous study. Recently, a meta-analysis conducted by Zhao et al. (2014) including 28 reports and 3408 cases indicated that co-deletion of 1p and 19q was associated with better survival rates in glioma. The 1p/19q deletion was also shown to be an independent predicator of better overall survival. Moreover, a combination of IDH mutations, 1p/19q co-deletion, and PTEN deletion was effective in discriminating the clinical outcome of hemispheric gliomas (Sabha et al., 2014). Although we discovered that the 1p/19q status was not an independent risk factor for gliomas, this result was almost of statistical significance; indicating that the loss of 1p/19q might signify a better prognosis.

To the best of our knowledge, this is first clinical study evaluating the potential association between 1p/19q co-deletion and *VEGF* expression. The 1p/19q status and *VEGF* expression were significantly correlated. A higher percentage of patients with intact 1p/19q and high tissue *VEGF* mRNA expression had high-grade gliomas. Moreover, glioma patients with a 1p/19q deletion and low tissue *VEGF* mRNA expression displayed a favorable prognosis. In keeping with our results, Kapoor et al. (2009) reported that glioma patients with LOH of 1p/19q, or 1p deletion, displayed an increase in *VEGF*, *CD31*, and *CD105* expression compared to those with an intact 1p/19q or 19q deletion; therefore, the 1p deletion (and not that of 19q) could possibly be correlated with VEGF expression. Additionally, the 1p/19q co-deletion was significantly associated with relative tumor blood volume, and EGFR and VEGF expression, using a multivariate linear regression model (Kapoor et al., 2009). Although the clinical data indicated a close correlation between the 1p/19q status and *VEGF* expression, it remains to be determined if the 1p/19q co-deletion could drive the angiogenesis process and promote *VEGF* expression. Further investigations must be conducted to reveal the potential molecular mechanisms affecting the association between the 1p/19q status and *VEGF* expression.

Taken together, our data indicated that the 1p/19q status was strongly associated with VEGF expression. The 1p/19q status and *VEGF* mRNA expression could be used in combination to predict the prognosis of patients with gliomas.

Conflicts of interest

The authors declare no conflict of interest.

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