Int J Clin Exp Med 2017;10(2):3606-3611
www.ijcem.com /ISSN:1940-5901/IJCEM0042927

Original Article

Correlation analysis of the clinicopathological features of glioma and expression of p53 and VEGF

Jianguo Yang*, Zhaohui Zhao*, Xingming Zhong

*Department of Neurosurgery, The First People's Hospital of Huzhou, The First Affiliated Hospital of Huzhou University, Huzhou 313000, Zhejiang, China. *Equal contributors.*

Received July 5 2016; Accepted December 19, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: Glioma is a common type of brain and neurological cancer with high degree of malignancy. Expression of p53 has been found in a variety of tumor cells and VEGF has been associated with the angiogenesis in cancers. In this study, the expressions of p53 and VEGF in glioma were examined. The relationship between clinicopathological features of glioma and the expression profiles of p53 and VEGF was analyzed. Levels of p53 and VEGF in the serum of glioma patients were detected by ELISA. Immunohistochemical staining was used to measure the protein expressions of p53 and VEGF. The correlation between the changes of p53 and VEGF and clinical pathological features of glioma was analyzed. The expression levels of P53 and VEGF in glioma group were significantly higher than that in the control group before surgery. The expression levels of P53 and VEGF in glioma group were significantly reduced after surgery, compared with those before surgery ($P < 0.05$). In glioma group, p53 positive rate was 73.3% and VEGF positive rate was 76.7%, which were significantly higher than that in adjacent normal tissues or control group ($P < 0.05$). Expression of p53 was positively correlated with the expression of VEGF ($P < 0.05$). The expression levels of P53 and VEGF were correlated with pathological type of glioma, WHO classification, distant metastasis, and survival time ($P < 0.05$), but not age, sex, or PS score ($P > 0.05$). The expression levels of P53 and VEGF were up-regulated in both the serum and tumor tissues of glioma patients. The expression levels of P53 and VEGF were correlated with the pathological type of glioma, WHO classification, distant metastasis, and survival time, suggesting they might play a pivotal role in the development of glioma.

Keywords: p53, VEGF, glioma

Introduction

Originated from neural ectoderm, glioma accounts for about 40%-60% of brain cancers. Glioma, characterized by strong invasion capability, high recurrence rate, poor prognosis, and high mortality, seriously affects patients' lives [1]. Study showed that the pathogenesis and progression of glioma are affected by multiple factors, multiple genes, and multiple stages [2]. It has been reported that angiogenesis regulates the malignant tumor growth, invasion, and migration. VEGF not only can increase the permeability of the blood vessel, but also effectively promote the proliferation of endothelial cells [3]. p53 is a very important tumor suppressor gene and regulates the growth of tumor cells as well as induces DNA damage in tumor cells. It also can prevent the mutation of genes which have malignant tendency, which to some extent plays a suppression role in the development of malignant tumors [4, 5]. In this study, levels of p53 and VEGF in the serum and tumor tissue of glioma patients were detected by ELISA and IHC. The correlation between the changes of p53 and VEGF and clinical pathological features of glioma was analyzed.

Materials and methods

General information

A total of 60 glioma patients from January 2015 to January 2016 in the First People's Hospital of Huzhou including 12 cases with grade I, 18 cases grade II, 19 grade III, and 11 grade IV were included in this study. According to pathological changes, there were 20 cases of astrocytoma, 18 cases of oligodendroglioma, 13 cases of ependymoma, and 11 cases of glio-

Table 1. ELISA analysis of the serum levels of p53 and VEGF

Groups	Cases	p53 (ng/ml)	VEGF (ng/ml)
Glioma group	60		
Before surgery		1.57 ± 0.12*	1.25 ± 0.14*
After surgery		0.08 ± 0.02#	0.07 ± 0.02#
Prognosis		0.07 ± 0.01	0.06 ± 0.02
Normal control group	30		
Before surgery		0.06 ± 0.03	0.05 ± 0.04
After surgery		0.05 ± 0.07	0.05 ± 0.03
Prognosis		0.05 ± 0.02	0.05 ± 0.01

*Compared with normal control group, $P < 0.05$; #Compared with before surgery, $P < 0.05$.

Table 2. IHC assay of levels of p53 and VEGF in tissues

Groups	Cases	p53 expression			Positive rate (%)	VEGF expression			Positive rate (%)
		-	+-++	+++		-	+-++	+++	
Glioma group	60	16	30	14	73.3*,#	14	33	13	76.7*,#
Adjacent tissues	60	40	16	4	33.3#	38	20	2	36.7#
Normal control	30	30	29	1	3.3	29	1	0	3.3

*Compared with adjacent tissues, $P < 0.05$; #Compared with normal controls, $P < 0.05$.

blastoma. Adjacent normal tissues were also collected. There were 30 males and 30 females with an average age of 52.5 ± 4.8 (range: 30-70 years). Healthy controls included 15 males and 15 females with an average age of 54.2 ± 4.5 (range: 31-70 years). All patients underwent microsurgical total resection of the tumor. No significant differences were found regarding gender or age between patients and controls ($P < 0.05$). This study was approved by the Medical Ethics Committee of the First People's Hospital of Huzhou and all patients signed informed consent forms.

Inclusion criteria: All patients should be confirmed by pathological diagnosis, without connective tissue disease or immune system disease; have not received radiotherapy, chemotherapy, or other anti-tumor immunotherapy; no heart, liver, lung, or other vital organ dysfunction.

Reagents and equipments

VEGF ELISA kit, p53 ELISA kit, VEGF blocking buffer, anti-VEGF antibody, p53 blocking buffer, anti-p53 antibody, and rabbit anti-mouse secondary antibody were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co.; Microscope was purchased from Olympus. Microplate reader was purchased from TECNA. Tissue slicer was purchased from Leica. Image analysis system was purchased from Hewlett-Packard.

Parameters detected and detection methods

ELISA assay of serum levels of p53 and VEGF: Fasting blood samples were collected before surgery, after surgery, and in the follow-up. Collected blood samples were centrifuged at 1500 r/min for 10 min, supernatants were collected for later use. Measurement of the serum levels of p53 and VEGF were performed according to the instructions of ELISA kits. Absorbance values were recorded and linear regression equation was used to calculate the levels of p53 and VEGF in the samples.

IHC assay of levels of p53 and VEGF in tissues: Tissues were formalin-fixed, dehydrated, transparented, then embedded and sliced. Heat antigen retrieval was performed and hydrogen peroxide was blocked. After blocking with normal goat serum in PBS, 50 μl of primary antibody (1:100 dilution) was added to each slice and incubated for 1 h followed by incubation with secondary antibody (1:100 dilution) for 10 min. Sections were then developed, counterstained, and mounted.

Section reading

Positive criteria: brown particles appeared at cytoplasmic membrane or cytoplasm and no nuclear staining. Positive rate ≤ 10% was designated as negative (-); positive rate between 11%-25% was designated as weakly positive (+); positive rate between 26%-50% was designated as positive (+); positive rate > 50% was designated as strong positive (+++) [6].

Statistical analysis

SPSS17.0 software was used for data statistical analysis. All data are expressed as mean ±

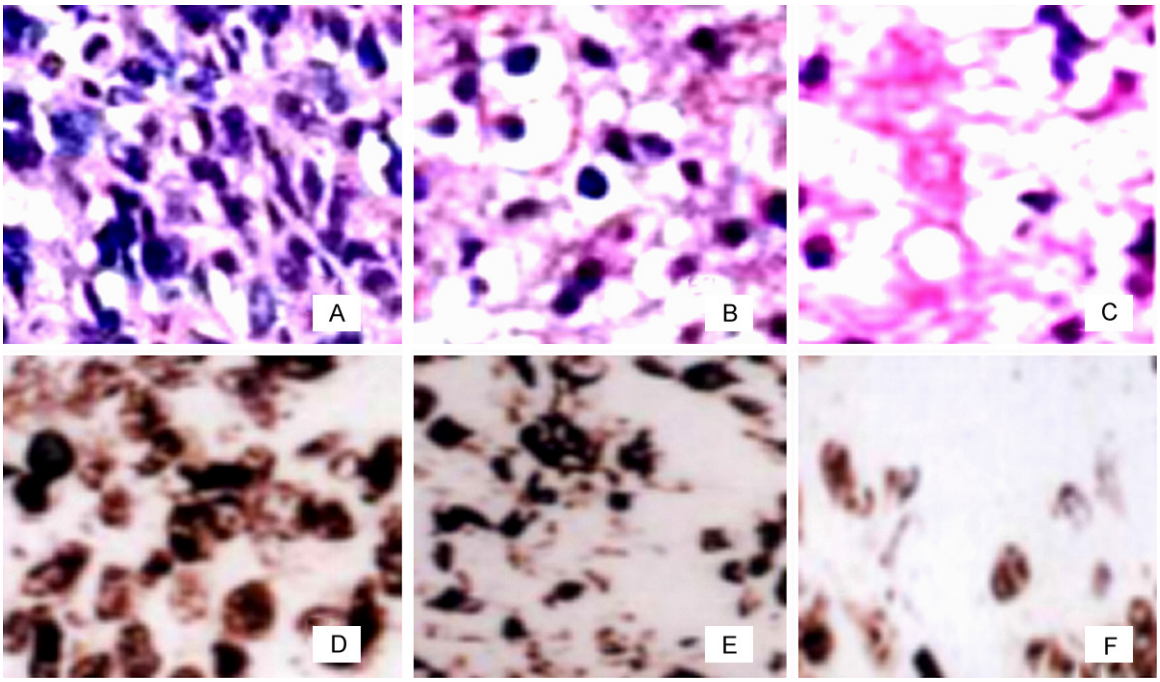

Figure 1. Expression of p53 and VEGF in brain tissues (× 200). A: Glioma tissue VEGF (+++); B: Adjacent tissue VEGF (+); C: Control tissue VEGF (-); D: Glioma tissue P53 (+++); E: Adjacent tissue P53 (+); F: Control tissue P53 (-).

Table 3. Relationship between p53 and VEGF in glioma

Groups	Cases	p53		χ^2	P
		-	+		
VEGF (-)	14	4	4		
VEGF (+)	46	12	40	8.179	0.002

standard deviation. χ^2 test was used to analyze counting data. T-test was used to do the comparison between groups. $P < 0.05$ was considered statistically significant.

Results

ELISA assay of serum levels of p53 and VEGF

ELISA assay results showed that the serum levels of p53 and VEGF in glioma patients were (1.57 ± 0.12) ng/ml and 1.25 ± 0.14 ng/ml before surgery, which were significantly higher than that in controls ($P < 0.05$). The levels of p53 and VEGF in glioma patients were significantly decreased after surgery compared with that before surgery ($P < 0.05$) (**Table 1**).

IHC assay of levels of p53 and VEGF in tissues

IHC was used to detect the expression of p53 and VEGF in brain tissue of patients. Results showed that the positive rate of p53 was 73.3% (30 cases were positive and 14 cases were strong positive) and the positive rate of VEGG was 76.7% with 33 positive cases and 13 strong positive cases, which were significantly higher than that in either adjacent normal tissues or normal controls. Moreover, the positive rates of p53 and VEGF in adjacent normal tissues were significantly higher than that in normal controls ($P < 0.05$) (**Table 2**; **Figure 1**).

Relationship between p53 and VEGF in glioma

The expression of p53 and VEGF was analyzed and results showed that 83.3% (50/60) of p53 positive patients showed VEGF positive. The positive rate of VEGF in p53 positive patients was significantly higher than that in p53 negative patients ($P < 0.05$). There was a significantly positive correlation between the expression of p53 and the expression of VEGF ($r = 0.419$, $P < 0.01$) (**Table 3**).

Relationship between expression levels of p53 and VEGF in gliomas and clinicopathological features

Relationships between expression levels of p53 and VEGF and patients' gender, age, PS score, pathological type, WHO classification,

Table 4. Relationship between the expression levels of p53 and VEGF in gliomas and clinicopathological features

Categories	Cases	P53	VEGF
Age			
< 45	29	21 (72.4)	23 (79.3)
≥ 45	31	23 (74.2)	23 (74.2)
P		> 0.05	> 0.05
Gender			
Male	30	22 (73.3)	23 (76.7)
Female	30	22 (73.3)	23 (76.7)
P		> 0.05	> 0.05
PS score			
≤ 1	28	20 (71.4)	22 (78.6)
> 2	32	24 (75)	24 (75)
P		> 0.05	> 0.05
Pathological type			
Astrocytoma	20	8 (40)	11 (55)
Oligodendroglioma	18	15 (83.3)	14 (77.8)
Ependymoma	13	11 (84.6)	11 (84.6)
Glioblastoma	11	10 (90.9)	10 (90.9)
P		< 0.05	< 0.05
WHO classification			
I	12	4 (33.3)	6 (50)
II	18	14 (77.8)	13 (72.2)
III	19	16 (84.2)	17 (89.5)
IV	11	10 (90.9)	10 (90.9)
P		< 0.05	< 0.05
Distant metastasis			
No	27	14 (51.8)	16 (59.3)
Yes	33	30 (90.9)	30 (90.9)
P		< 0.05	< 0.05
Survival time			
≤ 5 years	46	39 (84.7)	39 (84.8)
> 5 years	14	5 (35.7)	7 (50)
P		< 0.05	< 0.05

distant metastasis, survival time, or clinicopathological features were analyzed. Date showed that the expression levels of p53 and VEGF were correlated with glioma pathological type, WHO classification, distant metastasis, and survival time ($P < 0.05$), but not correlated with patients' gender, age, and PS score ($P > 0.05$). For glioblastoma, the expression levels of p53 and VEGF were significantly increased if the glioblastoma was classified as grade IV, which had distant metastasis, and the survival time ≤ 5 years (**Table 4**).

Discussion

The development and progression of malignant tumors is a multi-stage process in which proto-oncogenes are activated and tumor suppressor genes are inactivated under the effects of multiple cytokines. The over-expression of important cytokines or abnormal expression of multiple genes can induce uncontrolled proliferation and differentiation of malignant cells [7]. Pathological anatomy of brain with malignant glioma showed that glioma is rich in blood vessels. Glioma is characterized by tumor-associated angiogenesis and vascular endothelial cells proliferation and differentiation. Moreover, proliferation and differentiation of vascular endothelial cells increases along with the increase of the degree of malignancy of the tumors [8]. VEGF, produced by both normal cells and various types of malignant cells, is capable to stimulate proliferation and migration of vascular endothelial cells, and tumor angiogenesis via binding to its specific receptor [9]. p53 which aggregates in a variety of tumor cells regulates cycles of tumor cells [10, 11]. In the present study, we investigated the expression levels of p53 and VEGF in glioma patients to analyze the relationship between glioma clinical pathological features and the expressions of p53 and VEGF.

In the present study, peripheral venous blood samples were collect from both patients with glioma and patients who underwent brain surgery due to brain injury. ELISA results showed that the expression levels of p53 and VEGF in the serum of glioma patients were significantly higher than that of controls. IHC results showed that the positive rates of p53 and VEGF were 73.3% and 76.7% in glioma patients, which were significantly higher than that in either controls or adjacent tissues. This suggests that the expression levels of p53 and VEGF increased not only in serum but also in brain tissues of patients with glioma. It has been shown that VEGF is only expressed in invasive glioma tissues but not in normal brain tissues and the expression of VEGF was positively correlated with the pathological grade of glioma [12]. p53 gene is the most common tumor suppressor gene which is prone to mutations. Inactivation of p53 can significantly promote the uncontrolled growth of malignant tumor cells. Tumor cells can keep breeding asexually in the

absence of p53 which accelerates the transformation of glioma from low level malignancy to higher level of malignancy [13, 14]. Previous studies have showed that upon p53 gene inactivation, some glioma cells can be transformed from grade II to grade III or even grade IV [15], which is consistent with our study.

In this study, we found that the expression levels of p53 and VEGF was correlated with glioma histological type, WHO classification, distant metastasis, and survival time. The expression of p53 was increased and the expression of VEGF was decreased in patients with grade IV malignant glioblastomas with distant metastasis and survival time ≤ 5 years. Most patients showed p53 mutation and therefore its tumor suppression function is limited [16, 17]. Previous study showed that overexpression of p53 in malignant tumor indicated a lower degree of differentiation [18]. The positive rate of p53 gradually increased from patients with grades I glioma to patients with grade IV glioma, which is consistent with previous study. Study on VEGF showed that overexpression of VEGF significantly promoted the proliferation of glioma cells and made the cells more active [19]. Elevated VEGF expression stimulated the generation of new blood vessels which can provide more oxygen and blood, leading to accelerated growth of malignant cells [20].

Conclusion

The expression of p53 was decreased and the expression of VEGF was increased in the serum and tissues of patients with glioma. The expression of p53 and VEGF was correlated with pathological type, WHO classification, distant metastasis, and survival time. For grade IV glioblastoma patients with distant metastasis and survival time ≤ 5 years, the expression of p53 was decreased and the expression of VEGF was increased. Combined detection of p53 and VEGF has great significance in the prognosis and treatment of glioma.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xingming Zhong, Department of Neurosurgery, The First People's Hospital of Huzhou, The First Affiliated Hospital of Huzhou University, Huzhou 313000, Zhejiang, China. Tel: +86-057-22039316; Fax: +86-057-22039316; E-mail: feifeizhangtyu@sina.com

References

[1] Ki SY, Kim SK, Heo TW, Baek BH, Kim HS and Yoon W. Chordoid glioma with intraventricular dissemination: a case report with perfusion MR imaging features. Korean J Radiol 2016; 17: 142-146.

[2] Vandenberk L, Garg AD, Verschuere T, Koks C, Belmans J, Beullens M, Agostinis P, De Vleeschouwer S and Van Gool SW. Irradiation of necrotic cancer cells, employed for pulsing dendritic cells (DCs), potentiates DC vaccine-induced antitumor immunity against high-grade glioma. Oncoimmunology 2016; 5: e1083669.

[3] Vasconcelos RC, Costa Ade L, Freitas Rde A, Bezerra BA, Santos BR, Pinto LP and Gurgel BC. Immunoexpression of HIF-1alpha and VEGF in periodontal disease and healthy gingival tissues. Braz Dent J 2016; 27: 117-122.

[4] Cirkel GA, Kerklaan BM, Vanhoutte F, Van der Aa A, Lorenzon G, Namour F, Pujuguet P, Darquenne S, de Vos FY, Snijders TJ, Voest EE, Schellens JH and Lolkema MP. A dose escalating phase I study of GLPG0187, a broad spectrum integrin receptor antagonist, in adult patients with progressive high-grade glioma and other advanced solid malignancies. Invest New Drugs 2016; 34: 184-192.

[5] Lobo MR, Kukino A, Tran H, Schabel MC, Springer CS Jr, Gillespie GY, Grafe MR, Woltjer RL and Pike MM. Synergistic antivascular and antitumor efficacy with combined cediranib and SC6889 in intracranial mouse glioma. PLoS One 2015; 10: e0144488.

[6] Granados Lopez AJ and Lopez JA. Multistep model of cervical cancer: participation of miRNAs and coding genes. Int J Mol Sci 2014; 15: 15700-15733.

[7] Buckner JC, Shaw EG, Pugh SL, Chakravarti A, Gilbert MR, Barger GR, Coons S, Ricci P, Bullard D, Brown PD, Stelzer K, Brachman D, Suh JH, Schultz CJ, Bahary JP, Fisher BJ, Kim H, Murtha AD, Bell EH, Won M, Mehta MP and Curran WJ Jr. Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. N Engl J Med 2016; 374: 1344-1355.

[8] Jung JH, Kim AA, Chang DY, Park YR, Suh-Kim H and Kim SS. Three-dimensional assessment of bystander effects of mesenchymal stem cells carrying a cytosine deaminase gene on glioma cells. Am J Cancer Res 2015; 5: 2686-2696.

[9] Renner DN, Malo CS, Jin F, Parney IF, Pavelko KD and Johnson AJ. Improved treatment efficacy of antiangiogenic therapy when combined

with picornavirus vaccination in the GL261 glioma model. Neurotherapeutics 2016; 13: 226-236.
[10] Tiwari RK, Saha K, Mukhopadhyay D, Datta C, Chatterjee U and Ghosh TK. Evaluation of preoperative serum levels of CA 125 and expression of p53 in ovarian neoplasms: a prospective clinicopathological study in a tertiary care hospital. J Obstet Gynaecol India 2016; 66: 107-114.
[11] Sawair F, Hassona Y, Irwin C, Stephenson M, Hamilton P, Maxwell P, Gordon D, Leonard A and Napier S. p53, Cyclin D1, p21 (WAF1) and Ki-67 (MIB1) expression at invasive tumour fronts of oral squamous cell carcinomas and development of local recurrence. Asian Pac J Cancer Prev 2016; 17: 1243-1249.
[12] Wang C, Ge Q, Zhang Q, Chen Z, Hu J, Li F and Ye Z. Targeted p53 activation by saRNA suppresses human bladder cancer cells growth and metastasis. J Exp Clin Cancer Res 2016; 35: 53.
[13] Ormenisan C, Kubik M, Legrand S, Kraemer D, Smotherman C and Masood S. The potential of ki67 and p53 assessment in development of individualized targeted therapy in breast cancer patients. Pathologica 2015; 107: 177-180.
[14] Telugu RB, Chowhan AK, Rukmangadha N, Patnayak R, Phaneendra BV, Prasad BC and Reddy MK. Histopathological and immunohistochemical evaluation of meningiomas with reference to proliferative markers p53 and Ki-67. J Clin Diagn Res 2016; 10: EC15-19.
[15] Bakry R, El-Sayed MI, Hamza HM and Hassan KH. Pretreatment levels of serum osteoprotegerin and p53 protein and urine telomerase as prognostic factors affecting survival in Egyptian bladder cancer patients. Oncol Lett 2016; 11: 823-830.
[16] Solga AC, Pong WW, Kim KY, Cimino PJ, Toonen JA, Walker J, Wylie T, Magrini V, Griffith M, Griffith OL, Ly A, Ellisman MH, Mardis ER and Gutmann DH. RNA sequencing of tumor-associated microglia reveals Ccl5 as a stromal chemokine critical for neurofibromatosis-1 glioma growth. Neoplasia 2015; 17: 776-788.
[17] Chen L, Jiang Z, Ma H, Ning L, Chen H, Li L and Qi H. Volatile oil of acori graminei rhizoma-induced apoptosis and autophagy are dependent on p53 status in human glioma cells. Sci Rep 2016; 6: 21148.
[18] Yu J, Guo M, Wang T, Li X, Wang D, Wang X, Zhang Q, Wang L, Zhang Y, Zhao C and Feng B. Inhibition of cell proliferation, migration and invasion by a glioma-targeted fusion protein combining the p53 C terminus and MDM2-binding domain. Cell Prolif 2016; 49: 79-89.
[19] Chu SH, Zhou ZM, Feng DF and Ma YB. Inhibition of human glioma U251 cells growth in vitro and in vivo by hydroxyapatite nanoparticle-assisted delivery of short hairpin RNAs against SATB1. Mol Biol Rep 2014; 41: 977-986.
[20] Ryu J, Yoon NA, Seong H, Jeong JY, Kang S, Park N, Choi J, Lee DH, Roh GS, Kim HJ, Cho GJ, Choi WS, Park JY, Park JW and Kang SS. Resveratrol induces glioma cell apoptosis through activation of tristetraprolin. Mol Cells 2015; 38: 991-997.