Kaohsiung Journal of Medical Sciences (2012) 28, 316–321

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.kjms-online.com

ORIGINAL ARTICLE

The alteration of plasma TGF-β1 levels in patients with brain tumors after tumor removal

Joon-Khim Loh [a,b,c,d], Ann-Shung Lieu [a,b], Yu-Feng Su [a,b,c], Chi-Yun Cheng [a], Tai-Hsin Tsai [a,b], Chih-Lung Lin [a,b], Kung-Shing Lee [a,b], Shiuh-Lin Hwang [a,b], Aij-Lie Kwan [a,b], Chih-Jen Wang [a,b], Yi-Ren Hong [c], Shen-Long Howng [a,b], Chung-Ching Chio [e,f,*]

[a] *Division of Neurosurgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[b] *Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[d] *Faculty of Medical Information Management, College of Health Science, Kaohsiung Medical University, Kaohsiung, Taiwan*
[e] *Center for General Education, Southern Taiwan University of Technology, Tainan 710, Taiwan*
[f] *Department of Neurosurgery, Chi Mei Medical Center, Tainan 710, Taiwan*

Received 14 January 2011; accepted 24 May 2011
Available online 4 April 2012

KEYWORDS
Brain tumor;
Transforming growth factor-beta 1;
Tumor removal

Abstract Transforming growth factor (TGF) β1 may be a candidate for a serologic tumor marker. In this study, the plasma levels of TGF-β1 in patients with brain tumors were measured using enzyme-linked immunosorbent assay before and after tumor removal. Patients were divided into four groups, the control group and the benign, malignant, and metastatic brain tumor groups. All brain tumor groups showed significant increases in the levels of TGF-β1 before tumor removal (6.36 ± 3.94, 17.0 ± 9.7, and 12.2 ± 10.3 ng/ml for the benign, malignant, and metastatic groups, respectively). When compared with the results obtained in the control group (1.12 ± 0.74 ng/ml), significant decreases in TGF-β1 concentrations after total tumor removal were found in both the benign and malignant brain tumor groups (2.55 ± 2.00 and 8.93 ± 5.73 ng/ml, respectively; $p = 0.0001$ and $p = 0.003$, respectively). On the other hand, plasma TGF-β1 levels in the metastatic brain tumor group showed a slight but significant increase (14.7 ± 9.3 ng/ml, $p = 0.035$) after tumor removal. In a case of low-grade astrocytoma, plasma levels of TGF-β1 were found to be 3.6 and 1.1 ng/ml before and after tumor removal, respectively. However, recurrent tumor was noted in this patient 7 months later, and the levels of TGF-β1 were 26.2 and 8.4 ng/ml before and after the second

* Corresponding author. 901, Chung Hwa Road, Yung Kang City, Tainan, Taiwan.
E-mail address: chiocc@ms28.hinet.net (C.-C. Chio).

doi:10.1016/j.kjms.2011.11.012

operation, respectively. The data show that plasma TGF-β1 was elevated in the circulation of patients with brain tumors and that significant decreases in TGF-β1 levels were observed after the removal of benign and malignant tumors. The results also suggest that TGF-β1 may be a useful serologic marker for brain tumors.

Introduction

Transforming growth factor beta (TGF-β) is a family of multifunctional cytokines that regulate cell proliferation, differentiation, and extracellular matrix production [1,2]. Deregulation of the expression or signaling of TGF-β has been implicated in the pathogenesis of a variety of diseases, including cancer [3] and fibrosis [4]. Under physiologic conditions, TGF-β inhibits the proliferation of normal astrocytes but it loses its growth-inhibitory potential toward gliomas due to alterations in the expression of cell cycle inhibitors. Lines of evidence suggest that TGF-β is actively secreted by tumor cells in the late stages of cancer development, and it contributes to cell growth, invasion, metastasis as well as to the decrease in host-tumor immune responses [1]. There are three distinct TGF-β isoforms (namely, TGF-β1, TGF-β2, and TGF-β3) and their effects depend on the type, differentiation state, and physiologic conditions of the target cells [3]. Among the TGF-β isoforms, TGF-β1 is the most abundant and most universally expressed compared with the other two [4,5]. Because of these properties, TGF-β1 has been the focus of most of the preclinical and clinical studies.

A recent report demonstrated that elevated serum level of TGF-β1 was a more sensitive indicator of small hepatocellular carcinomas than alpha-fetoprotein, which suggests that measuring serum TGF-β1 levels may be clinically useful for the diagnosis of others tumors as well [6]. Furthermore, based on preclinical studies that showed the production of TGF-β1 by tumors, we hypothesized that the TGF-β1 level should decrease after the surgical removal of the tumors. Thus, we compared the plasma levels of TGF-β1 in patients with brain tumors before and after tumor removal and evaluated the potential of using this measurement as a new tool for the diagnosis and monitoring of malignancy in brain tumors.

Patients and methods

The study protocol was approved by the ethics review committee of the Medical Faculty of the Kaohsiung Medical University Hospital in Kaohsiung, Taiwan. All patients and controls gave their written informed consent. From September 2004 to December 2006, 68 patients were included in this study. Among them, 17 had benign brain tumors (13 with meningiomas, two with low-grade astrocytoma, and one each with pituitary adenoma and neurilemmoma), 14 had malignant brain tumors (six with glioblastoma multiforme, three each with anaplastic astrocytoma and malignant meningioma, and two with gliosarcoma), 24 had metastatic brain tumors (15 with lung carcinomas, three with melanoma, and one each with breast carcinoma, colon carcinoma, hepatoma, cervical carcinoma, neuroblastoma, and malignant thymoma) and 13 were controls (all received cranioplasty for skull bone defect). Blood samples (10 ml) were collected in tubes containing 10 mg of ethylenediaminetetraacetic acid and 0.15 ml of aprotinin from patients before and 7 days after surgery. The samples were centrifuged at 2000 × g for 10 minutes, and the supernatants were carefully aspirated and stored at −20°C until analysis. The plasma TGF-β1 level was measured with enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Carlsbad, California USA) of antibody sandwich format as described previously [7]. The first antibody used to coat the plates (capture antibody) is a monoclonal antibody 4A11, which was used as the detection antibody. Streptavidin-conjugated horseradish peroxidase (HRP) was used as the secondary antibody; H_2O_2 mixed with tetramethylbenzidine was the substrate. The 96-well microtiter plates were applied to an automatic plate reader to measure the absorbance at 450 nm using 540 nm as a wavelength correction. The TGF-β1 level was assayed by comparing the peroxidase activity in wells containing known amounts of purified TGF-β1 with the activity in the wells containing the plasma of the patient. The known amounts of purified TGF-β1 were used as a standard for ELISA. Samples were run in triplicate. Each assay was repeated to ensure the absorbance values

Table 1 The plasma levels of transforming growth factor β1 before and after tumor removal in benign brain tumor.

Case No	Sex	Age (yr)	Diagnosis	ng/ml	
				Preop	Postop
1	Woman	57	Meningioma	5.5	4.8
2	Woman	59	Meningioma	8.2	5.1
3	Man	66	Meningioma	4.9	2.8
4	Woman	44	Meningioma	6.2	2.1
5	Woman	79	Meningioma	6.1	3.6
6	Woman	48	Meningioma	3.4	0.5
7	Woman	63	Meningioma	6.1	1.6
8	Woman	10	Low-grade astrocytoma	3.6	1.1
9	Man	55	Low-grade astrocytoma	0.8	0.3
10	Man	52	Neurilemmoma	1.9	0.4
11	Woman	70	Meningioma	12.3	7.5
12	Man	24	Meningioma	9.6	3.3
13	Man	58	Meningioma	4.7	1.3
14	Woman	64	Meningioma	4.2	1.9
15	Woman	43	Pituitary adenoma	11.6	2.8
16	Woman	44	Meningioma	15.6	3.7
17	Woman	48	Meningioma	3.4	0.5

Table 2 The plasma levels of transforming growth factor β1 before and after tumor removal in malignant brain tumor.

Case No	Sex	Age (yr)	Diagnosis	ng/ml	
				Preop	Postop
1	Man	73	Gr III astrocytoma	7.5	4.0
2	Woman	57	Glioblastoma multiforme	24.9	9.1
3	Woman	70	Glioblastoma multiforme	6.5	5.3
4	Man	26	Gliosarcoma	27.0	8.6
5	Woman	52	Glioblastoma multiforme	10.8	6.5
6	Man	78	Glioblastoma multiforme	7.1	3.2
7	Man	51	Gliosarcoma	7.3	11.7
8	Woman	10	Glioblastoma multiforme	26.2	8.4
9	Woman	43	Malignant meningioma	11.0	2.8
10	Woman	65	Gr III astrocytoma	25.8	15.4
11	Woman	50	Malignant meningioma	29.2	15.2
12	Woman	42	Glioblastoma multiforme	7.0	1.0
13	Woman	75	Malignant meningioma	30.2	20.5
14	Man	42	Gr III astrocytoma	18.0	13.0

were within the range of the detectable standard cure. Since the procedure could not distinguish between the active and latent forms of TGFβ1, the measured TGFβ1 level was referred to as the total TGFβ1.

Comparison between various groups was conducted using Wilcoxon signed rank test, and a p value of less than 0.05 was considered to be statistically significant.

Table 4 Plasma levels of transforming growth factor β1 (TGF-β1) before and after tumor removal.

Group	TGF-β1 (ng/ml)		p value
	Preoperation (mean ± SEM)	Postoperation (mean ± SEM)	
Control ($n = 13$)	1.12 ± 0.74	1.43 ± 0.85	0.183
Benign ($n = 17$)	6.36 ± 3.94	2.55 ± 2.0	0.0001
Malignant ($n = 14$)	17.0 ± 9.7	8.93 ± 5.73	0.003
Metastatic ($n = 24$)	12.2 ± 10.3	14.7 ± 9.3	0.035

Statistical significance was conducted by Wilcoxon signed rank test ($p < 0.05$).

Results

The clinical data and plasma levels of TGF-β1 of benign brain tumor, malignant brain tumor, and metastatic brain tumor are shown in Tables 1 to 3 respectively.

The plasma levels of TGF-β1 in patients before and after tumor removal are shown in Table 4. Prior to surgery, plasma TGF-β1 levels in the benign (6.36 ± 3.94 ng/ml, mean ± SEM), malignant (17.0 ± 9.7 ng/ml), and metastatic (12.2 ± 10.3 ng/ml) brain tumor groups were significantly higher than the control (1.1 ± 0.7 ng/ml). Significant decreases in TGF-β1 levels were found in benign (2.55 ± 2.00 ng/ml; $p = 0.0001$) and malignant (8.93 ± 5.73 ng/ml; $p = 0.003$) brain tumor groups after tumor removal. However, a slight but significant increase in

Table 3 The plasma levels of transforming growth factor β1 before and after tumor removal in metastatic brain tumor.

Case No	Sex	Age (yr)	Diagnosis	ng/ml	
				Preop	Postop
1	Woman	65	Lung cancer with brain meta	4.1	4.1
2	Man	78	Lung cancer with brain meta	1.7	1.6
3	Woman	10	Neuroblastoma with brain meta	4.2	2.8
4	Woman	52	Melanoma with brain meta	1.5	3.3
5	Man	81	Melanoma with brain meta	2.1	3.7
6	Woman	77	Melanoma with brain meta	9.1	8.5
7	Woman	68	Lung cancer with brain meta	19.3	26.9
8	Woman	54	Breast cancer with brain meta	7.9	14.6
9	Man	54	Lung cancer with brain meta	17.9	26.8
10	Woman	74	Lung cancer with brain meta	18.7	30.6
11	Woman	65	Lung cancer with brain meta	13.9	20.2
12	Woman	65	Lung cancer with brain meta	15.0	21.9
13	Woman	71	Lung cancer with brain meta	26.3	25.0
14	Man	77	Lung cancer with brain meta	29.3	23.4
15	Man	72	Lung cancer with brain meta	29.7	21.8
16	Woman	55	Cervical cancer with brain meta	25.1	18.8
17	Man	70	Lung cancer with brain meta	29.0	23.3
18	Man	54	Lung cancer with brain meta	18.7	21.6
19	Man	65	Lung cancer with brain meta	10.4	15.2
20	Man	67	Thymoma with brain meta	1.7	5.7
21	Man	66	Lung cancer with brain meta	0.8	10.6
22	Man	65	Colon cancer with brain meta	4.6	12.8
23	Man	44	Hepatoma with brain meta	1.5	5.9
24	Man	75	Lung cancer with brain meta	0.5	4.0

plasma TGF-β1 levels was seen in the metastatic brain tumor group (14.7 ± 9.3 ng/ml; $p = 0.035$) after surgery (Table 4).

Brain magnetic resonance images of a case of low-grade astrocytoma are shown in Fig. 1 (top panel). Grossly, total removal of this low-grade astrocytoma was performed in this patient (Figs. 1A and 1B, top panel). Plasma TGF-β1 levels of this patient before and after tumor removal were 3.6 and 1.1 ng/ml, respectively (Fig. 1, bottom panel). The patient had a recurrent tumor 7 months later (Fig. 1C, top panel). A second operation was performed (Fig. 1D; top panel), and the pathologic report revealed glioblastoma multiforme. The TGF-β1 concentrations of this patient before and after the second surgery were 26.2 and 8.4 ng/ml, respectively (Fig. 1; bottom panel).

Discussion

Malignant tumors secrete into the circulation a number of proteins and peptides such as human chorionic gonadotropin, carcinoembryonic antigen, and alpha-fetoprotein,

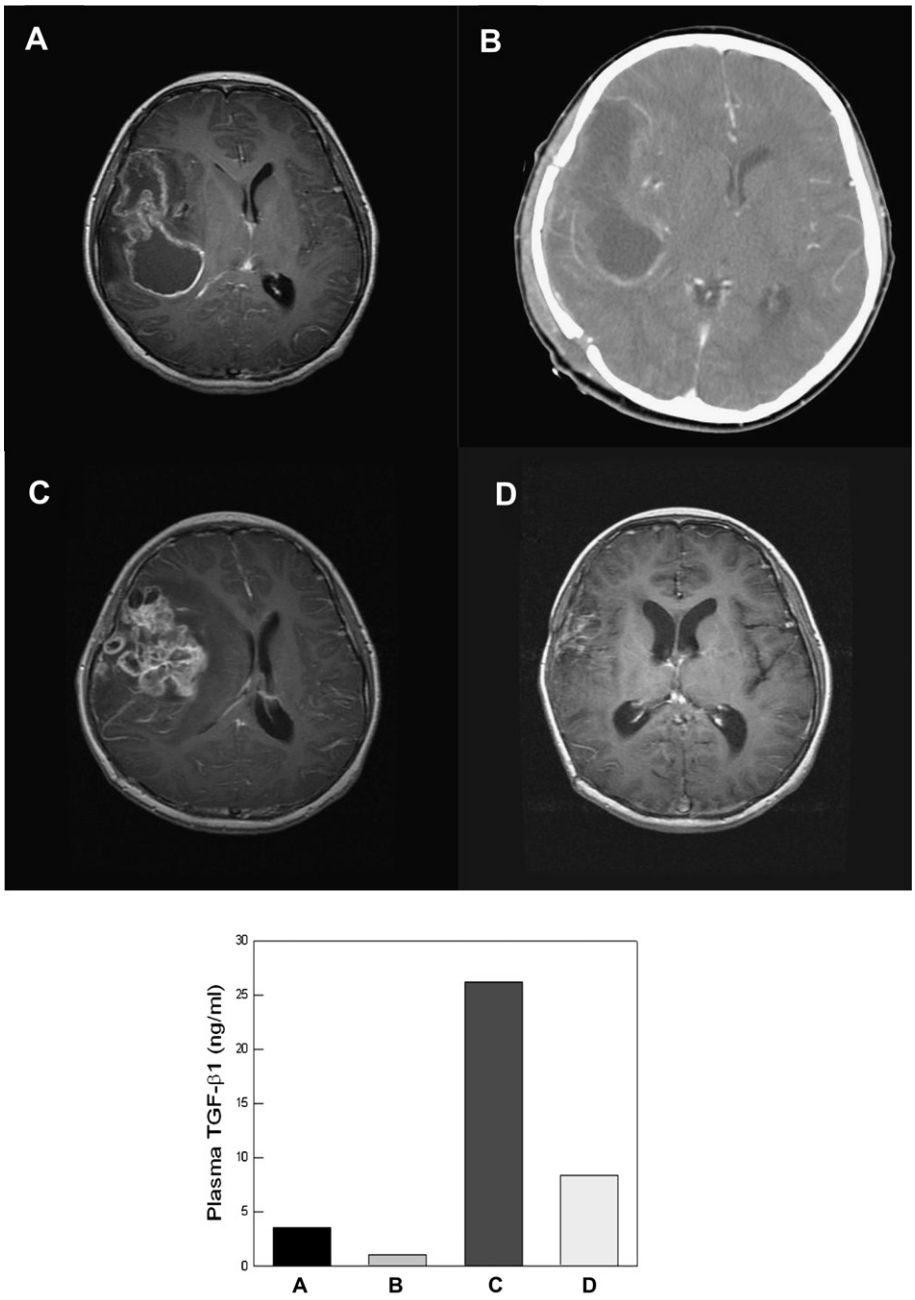

Figure 1. Brain magnetic resonance images (MRI) and plasma TGF-β1 levels of a patient with Grade II astrocytoma before and after tumor removal. Top panel: MRI of the right temporo-pariental lobe before (A) and after (B) a grossly total tumor removal was performed. Tumor recurred seven months later in this patient, and images were taken before (C) and after (D) a second operation. Bottom panel: corresponding plasma TGF-β1 levels for the same patient.

which can be used as helpful markers for the diagnosis, therapy, and prognosis of cancers. Likewise, TGF-β1 has recently emerged as such a marker for brain tumors [8—10].

In the present study, we measured plasma TGF-β1 levels in patients with brain tumors before and after tumor removal. Our data showed that the plasma TGF-β1 levels in patients with brain tumor before surgical management were significantly higher than those of healthy controls, suggesting that TGF-β1 was indeed produced by brain tumors. Furthermore, significant decreases in plasma TGF-β1 were found after tumor removal in both the benign and malignant brain tumor groups. These results are consistent with our hypothesis that surgical removal of the brain tumors can reduce the production of TGF-β1.

On the other hand, there was a slight but significant increase in plasma TGF-β1 levels in patients with metastatic brain tumor despite a significant tumor reduction by surgery. The results indicate that a quantity of unknown tumor cell clones may still reside outside the circumscribed tumor that has been removed surgically. These residual tumors together with another main tumor that were not removed were likely to be responsible for the production of TGF-β1 after surgery. Such an increase in TGF-β1 levels in the metastatic brain tumor group may also reflect the less favorable outcome in these patients.

In a murine malignant glioma model, high-grade human brain tumors have been shown to produce higher levels of TGF-β1 than those from low-grade brain tumors and from normal brain tissue [11—14]. When different histologic subgroups of gliomas were examined, TGF-β1 production was found to increase with anaplastic grade [15]. Furthermore, TGF-β1 production was significantly higher in glioblastomas than in the slow-growing glioma. In the present study we have also shown that plasma TGF-β1 levels in patients with malignant tumors are significantly higher than those with benign tumors before tumor removal (Table 4). The results imply that the malignant brain tumors synthesize higher amounts of TGF-β1 than benign tumors. Consequently, malignant brain tumors can induce more severe immunosuppression, and immunosuppression in a tumor-bearing host is a major obstacle in cancer treatment. TGF-β1 can down-regulate monocyte surface marker expression, cytokine secretion, cytotoxicity, and T-cell responsiveness, all of which paralyze the immune system of the host, thus contributing to the growth and progression of tumors [16—21].

In addition, immunosuppression may also lead to the development of tumor metastases in cancer patients. It has been widely accepted that the tumor-induced immunosuppression is associated with tumor burden. However, an important question remains as to whether immunosuppression could be reversed if the tumor burden is lessened. Previous studies have revealed that antitumor immune function could recover partially following reduction of tumor burden via primary tumor resection [22—25]. In other words, tumor-induced immunosuppression could be partially reversed by tumor resection in cancer patients. Our results reveal that the plasma levels of TGF-β1 decrease after brain tumor removal, which may lead to the possible lessening or relieving of host immune suppression.

In conclusion, the present study showed that plasma TGF-β1 levels in the benign and malignant brain tumor groups were elevated and that surgical removal of the tumors reduced the production of this cytokine. These results suggest that plasma TGF-β1 may be used as a marker for the diagnosis and monitoring of tumor malignancy. Further investigations are warranted to determine whether these findings contribute to morbidity and mortality or therapeutic outcome.

References

[1] Jennings MT, Pietenpol JA. The role of transforming growth factor beta in glioma progression. J Neurooncol 1998;36:123—40.
[2] Kaminska B, Wesolowska A, Danilkiewicz M. TGF beta signalling and its role in tumour pathogenesis. Acta Biochim Pol 2005;52:329—37.
[3] Elliott RL, Blobe GC. Role of transforming growth factor Beta in human cancer. J Clin Oncol 2005;23:2078—93.
[4] Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, et al. High levels of transforming growth factor beta 1 in patients with colorectal cancer: association with disease progression. Gastroenterology 1996;110:375—82.
[5] Huang F, Newman E, Theodorescu D, Kerbel RS, Friedman E. Transforming growth factor beta 1 (TGF beta 1) is an autocrine positive regulator of colon carcinoma U9 cells in vivo as shown by transfection of a TGF beta 1 antisense expression plasmid. Cell Growth Differ 1995;6:1635—42.
[6] Song BC, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, et al. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. Cancer 2002;94:175—80.
[7] Danielpour D. Improved sandwich enzyme-linked immunosorbent assays for transforming growth factor beta 1. J Immunol Methods 1993;158:17—25.
[8] Narai S, Watanabe M, Hasegawa H, Nishibori H, Endo T, Kubota T, et al. Significance of transforming growth factor beta1 as a new tumor marker for colorectal cancer. Int J Cancer 2002;97:508—11.
[9] Schneider T, Sailer M, Ansorge S, Firsching R, Reinhold D. Increased concentrations of transforming growth factor beta1 and beta2 in the plasma of patients with glioblastoma. J Neurooncol 2006;79:61—5.
[10] Shirai Y, Kawata S, Tamura S, Ito N, Tsushima H, Takaishi K, et al. Plasma transforming growth factor-beta 1 in patients with hepatocellular carcinoma. Comparison with chronic liver diseases. Cancer 1994;73:2275—9.
[11] Dong ZZ, Yao DF, Yao M, Qiu LW, Zong L, Wu W, et al. Clinical impact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2008;7:288—95.
[12] Kiefer R, Supler ML, Toyka KV, Streit WJ. In situ detection of transforming growth factor-beta mRNA in experimental rat glioma and reactive glial cells. Neurosci Lett 1994;166:161—4.
[13] Samuels V, Barrett JM, Bockman S, Pantazis CG, Allen Jr MB. Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. Am J Pathol 1989;134:894—902.
[14] Yamada N, Kato M, Yamashita H, Nister M, Miyazono K, Heldin CH, et al. Enhanced expression of transforming growth factor-beta and its type-I and type-II receptors in human glioblastoma. Int J Cancer 1995;62:386—92.
[15] Stiles JD, Ostrow PT, Balos LL, Greenberg SJ, Plunkett R, Grand W, et al. Correlation of endothelin-1 and transforming growth factor beta 1 with malignancy and vascularity in human gliomas. J Neuropathol Exp Neurol 1997;56:435—9.

[16] Chattopadhyay N, J TFH Godbole MM, Brown EM. Transforming growth factor beta receptor family ligands inhibit hepatocyte growth factor synthesis and secretion from astrocytoma cells. Brain Res Mol Brain Res 2004;121:146—50.
[17] Gomez GG, Kruse CA. Mechanisms of malignant glioma immune resistance and sources of immunosuppression. Gene Ther Mol Biol 2006;10:133—46.
[18] Kobie J. Akporiaye Egfb. Immunosuppressive role of transforming growth factor beta in breast cancer. Clin Applied Immunol Rev 2003;3:277—8.
[19] Weller M, Constam DB, Malipiero U, Fontana A. Transforming growth factor-beta 2 induces apoptosis of murine T cell clones without down-regulating bcl-2 mRNA expression. Eur J Immunol 1994;24:1293—300.
[20] Boucek J, Mrkvan T, Chovanec M, Kuchar M, Betka J, Boucek V, et al. Regulatory T cells and their prognostic value for patients with squamous cell carcinoma of the head and neck. J Cell Mol Med 2010;14:426—33.
[21] Moutsopoulos NM, Wen J, Wahl SM. TGF-beta and tumors—an ill-fated alliance. Curr Opin Immunol 2008;20:234—40.
[22] Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 2001;166:678—89.
[23] Barbieri C, Fujisawa MM, Yasuda CL, Metze IL, Oliveira EC, Santos LM, et al. Effect of surgical treatment on the cellular immune response of gastric cancer patients. Braz J Med Biol Res 2003;36:339—45.
[24] Danna EA, Sinha P, Gilbert M, Clements VK, Pulaski BA, Ostrand-Rosenberg S. Surgical removal of primary tumor reverses tumor-induced immunosuppression despite the presence of metastatic disease. Cancer Res 2004;64: 2205—11.
[25] Salvadori S, Martinelli G, Zier K. Resection of solid tumors reverses T cell defects and restores protective immunity. J Immunol 2000;164:2214—20.