PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2002;8(3):496-498
World Journal of Gastroenterology
 ISSN 1007-9327

• LARGE INTESTINAL CANCER •

TGF β_1 expression and angiogenesis in colorectal cancer tissue

Bin Xiong, Ling-Ling Gong, Feng Zhang, Ming-Bo Hu, Hong-Yin Yuan

Bin Xiong, Ling-Ling Gong, Feng Zhang, Ming-Bo Hu, Hong-Yin Yuan, Department of Oncology, Affiliated Zhongnan Hospital of Wuhan University,Wuhan 430071,Hubei Province,China
Supported by Hubei province Natural Science Foundation, No.2000J054
Correspondence to: Dr. Bin Xiong, Department of Oncology, Affiliated Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei, Provice, China. xbxh@public.wh.hb.cn
Telephone: +86-27-87325716
Received 2001-07-19 **Accepted** 2001-08-23

Abstract

AIM: **Transforming growth factor(TGF)β_1 is involved in a variety of important cellular functions, including cell growth and differentiation, angiogenesis, immune function and extracellular matrix formation. However, the role of TGF β_1 as an angiogenic factor in colorectal cancer is still unclear. We investigate the relationship between transforming growth factor β_1 and angiogenesis by analyzing the expression of transforming growth factor(TGF) β_1 in colorectal cancer, as well as its association with VEGF and MVD.**

METHODS: **The expression of TGF β_1, VEGF, as well as MVD were detected in 98 colorectal cancer by immunohistochemical staining. The relationship between the TGF β_1 expression and VEGF expression, MVD was evaluated. To evaluate the effect of TGF β_1 on the angiogenesis of colorectal cancers.**

RESULTS: **Among 98 cases of colorectal cancer,37 were positive for TGF β_1(37.8%), 36 for VEGF(36.7%), respectively. The microvessel counts ranged from 19 to 139.8, with a mean of 48.7(standard deviation,21. 8). The expression of TGF β_1 was correlated significantly with the depth of invasion, stage of disease, lymph node metastasis, VEGF expression and MVD. Patients in T3-T4, stage III-IV and with lymph node metastasis had much higher expression of TGF β_1 than patients in T1-T2, stageI-II and without lymph node metastasis (P<0.05). The positive expression rate of VEGF(58.3%) in the TGF-β_1 positive group is higher than that in the TGF-β_1 negative group(41.7%, P<0.05). Also, the microvessel count (54±18) in TGF-β_1 positive group is significantly higher than that in TGF-β_1 negative group (46±15, P<0.05). The microvessel count in tumors with both TGF-β_1 and VEGF positive were the highest (58±20,36-140, P<0.05). Whereas that in tumors with both TGF-β_1 and VEGF negative were the lowest (38±16, 19-60, P<0.05).**

CONCLUSION: **TGF β_1 might be associated with tumor progression by madulating the angiogenesis in colorectal cancer and TGF β_1 may be used as a possible biomarker.**

Xiong B, Gong LL, Zhang F, Hu MB, Yuan HY. TGF β_1 expression and angiogenesis in colorectal cancer tissue. *World J Gastroenterol* 2002;8(3):496-498

INTRODUCTION

Angiogenesis is essential for tumor growth and metastasis[1-6]. An association between poor prognosis and increase in microvascular density (MVD) of tumor has been reported in certain tumors[5-10]. This neoangiogenesis depends on the production of angiogenic factors by tumor cells and normal cells[7-15]. Vascular endothelial growth factor (VEGF) also plays a key role in angiogenesis of tumor[3-20], but the role of transforming growth factor-β1 is not clear yet. Now the expression of TGF-β_1 and VEGF, MVD were detected in 98 colorectal cancer by immunohistochemical staining, in order to investigate the correlation of TGF-β_1 and angiogenesis in colorectal cancer.

MATERIALS AND METHODS

Patients

All total of 98 colorectal adenocarcinoma patients who had undergone surgical resection in the Affiliated Zhongnan Hospital of Wuhan University (Wuhan) from July 1998 to December 2000 were included. There were 53 male and 45 female, with an age range from 23 to 74 years (mean, 56±11.2 years). Among the 98 adenocarcinoma patients, 17 were well differentiated, 47 moderately differentiated and 34 poorly differentiated. According to Dukes stage criteria, 34 cases were stageI, 29 stageII,30 stage III and 5 stage IV.

Methods

Immunohistochemistry All the tissue specimens were fixed in 100mL·L^{-1} neutral formalin and embedded in paraffin. Five-micrometer-thick sections were treated with xylene, dehydrated in ethanol. Tissue sections were washed three times in 0.05mol·L^{-1} PBS, incubated in endogenous peroxidase blocking solution. Non-specific antibody binding was blocked by pretreatment with PBS containing 5g·L^{-1} bovin serum albumin. Sections were then rinsed in PBS and incubated overnight at 4℃ with diluted anti-TGF β_1 protein polyclonal antibody, anti-VEGF protein polyclonal antibody and anti-CD34 protein monoclonal antibody. These steps were performed using immunostain kit according to the manufacturers instructions. PBS was used as substitutes of protein antibody for negative control groups. The sections were examined under light microscopy. Anti-TGF β_1 protein polyclonal antibody were purchased from Bosden Co (Wuhan). Anti-VEGF protein polyclonal antibody, anti-CD34 protein monoclonal antibody, and S-P detection kit were purchased from Fuzhou Maixin Co. Anti-TGF β_1 protein polyclonal antibody was diluted to 1:100. Anti-VEGF protein polyclonal antibody and anti-CD34 protein monoclonal antibody were impromptu type.

Results Positive signal was located in the cytoplasm or/and cell membrane. Immunoreactivity was graded as follows: +, ⩾10% stained tumor cells; -, <10% o stained tumor cells[21-23]. The microvessel counting procedures have been described in the published studies[21-24]. Briefly, the stained sections were screened at a magnification of ×100(×10 objective and ×10 ocular lens) under a light microscope to identify the 3 regions of the section with the highest microvessel density. Microvessels were counted in these areas at a magnification of ×200, and the average numbers of microvessels were recorded. The average number is known as MVD of the tumor.

Statistical analysis The difference between each group was analyzed by Chi-square test and correlativity. Significant difference was taken of P <0.05.

RESULTS

TGF β$_1$ expression in colorectal cancer and clinicopathologic findings

TGF β$_1$ was localized mainly in the cytoplasm and cell membrane of the tumor cells(Figure1). TGF β$_1$ expression was detected in 37 tumors (37.8%). The correlation between TGF β$_1$ expression and the clinicopathologic findings was shown in Table 1. The expression of TGF β$_1$ was correlated significantly with the depth of invasion, stage of disease and lymph node metastasis. Patients in T3-T4, stage III-IV and with lymph node metastasis had much higher TGF β$_1$ than patients in T1-T2, stageI-II and without lymph node metastasis (P<0.05). The expression of TGF β$_1$ was not correlated with age, gender and differentiation degree of the tumor.

Relationship between TGF β$_1$ expression, VEGF expression and MVD

VEGF was localized mainly in the cytoplasm and cell membrane of the tumor cells (Figure2). VEGF expression was detected in 36 tumors (36.7%), and TGF-β$_1$ expression was correlated closely with VEGF expression (Table 1). The positive expression rate of VEGF(58.3%) in the positive TGF-β$_1$ group was higher than that in the negative TGF-β$_1$ group(41.7%, P<0.05).

The number of the microvessel counts in all cases were 19-140 (±s, 49±22). Moreover, the microvessel counts were 54±18 in TGF-β$_1$ positive tumors and 46±15 in TGF-β$_1$ negative tumors (P<0.05, Table 1). TGF-β$_1$ expression, VEGF expression and MVD were significantly correlated one another (r=0.5816, 0.2619 and 0.5182, respectively. P<0.05). The microvessel counts in tumors with both positive TGF-β$_1$ and VEGF were the highest (58±20, 36-140; P<0.05). The microvessel counts in tumors with both negative TGF-β$_1$ and VEGF were the lowest (38±16, 19-60; P<0.05). The microvessel counts in tumors with positive TGF β$_1$ and negative VEGF were 25-128(49±18), and that in tumors with negative TGF β$_1$ and positive VEGF were 31-133 (50±20), lower than that in tumors with both positive TGF-β$_1$ and VEGF (P<0.05).

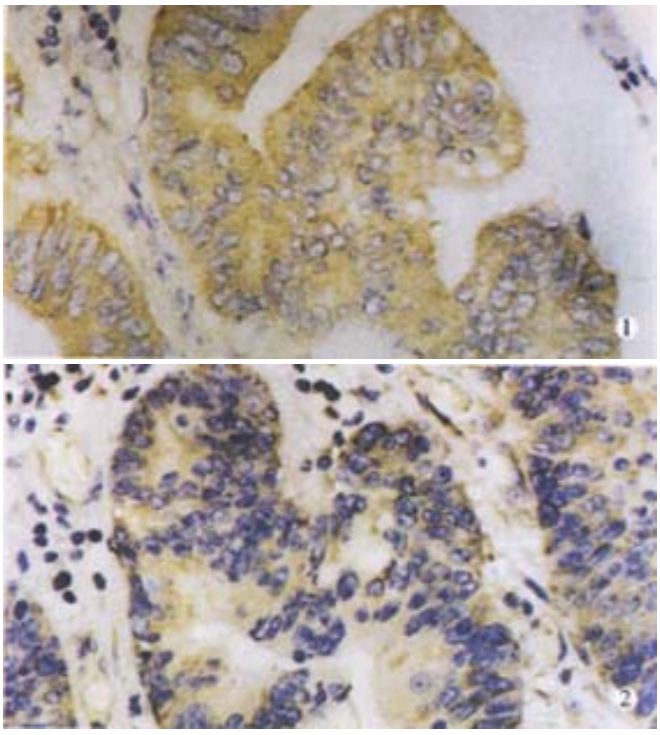

Figure 1 TGF β$_1$ mainly in cytoplasm and membrane of tumor cells, ×400
Figure 2 VEGF expression mainly in cytoplasm and membrane of tumor cell,×400

Table 1 Relationship between expression of TGF β$_1$ and clinicopathologic findings

Clinic-pathologic parameters	TGF β$_1$ expression(%)	
	Positive(n=37)	Negative(n=61)
Male	20 (37.8)	33 (62.3)
Female	17 (37.8)	28 (62.2)
Age (y)	55±13	57±12
Histology: differentiation		
Well	9(52.9)	8 (47.1)
Moderate	15 (31.9)	32 (68.1)
Poor	13 (38.2)	21 (61.8)
Depth of invasion		
T1-T2	17 (28.3)	43 (71.7)
T3-T4	20 (52.6)	18 (47.4)[a]
Lymph node metastasis		
Present	18 (51.4)	17 (48.6)
Absent	19 (30.2)	44 (69.8)[a]
Dukes Stage		
I	8 (23.5)	26 (76.5)
II	9 (31.1)	20 (68.9)
III+IV	20 (57.1)	15 (42.9)[a]
VEGF expression		
Positive	21 (58.3)	15 (41.7)
Negative	16 (25.8)	46 (74.2)[a]
MVD ($\bar{x}$±s)	54±18	46±15[a]

[a]P<0.05, *vs* positive

DISCUSSION

The process of angiogenesis is the outcome of an imbalance between positive and negative angiogenic factors produced by both tumor cells and normal cells. Numerous angiogenic factors have been described. Of these, VEGF play a key role in the angiogenesis in the colorectal cancer[3-25]. VEGF is a multi-functional cytokine, and has direct relationship with angiogenesis. The factors that regulate VEGF expression in tumor and non-tumor cells have now been elucidated[20-31]. The TGF βs represent a family of multifunctional cytokines that modulate the growth and function of many cells, including those with malignant transformation. The over-expression of TGF β$_1$ has been reported in tissue from patients with different carcinoma, and is believed to play a role in tumor transformation and progression, as well as in tumor regression[23-33]. Studied the correlation of TGF β$_1$ and angiogenesis of gastric cancer, and found TGF β$_1$ might regulate angiogenesis through an up-regulation of the expression of VEGF. A direct correlation between TGF β$_1$ expression and microvessel counts had not been identified in the current study[20-30]. TGF β$_1$ has no relationship with VEGF expression in breast cancer tissue, but is correlated with the expression of platelet-derived growth factor, and co-regulate angiogenesis[20-24]. The modulating mechanisms of TGF β$_1$ in angiogenesis are not entirely the same in different type of tumor.

The role of TGF β$_1$ in angiogenesis of colorectal cancer is not identified yet. This study found that the expression of VEGF and MVD in positive TGF β$_1$ group are significantly higher than that in TGF β$_1$ negative group. The expression of TGF β$_1$ is significantly positively correlated with the expression of VEGF. It demonstrated that TGF β$_1$ may be correlated indirectly with angiogenesis through an up-regulation of the expression of VEGF. The expression of TGF β$_1$ is also significantly positively correlated with MVD in colorectal cancer.

It demonstrates that TGF β_1 may modulate angiogenesis directly or indirectly through up-regulating the expression of other angiogenic factors. The microvessel counts in tumors that were both positive TGF-β_1 and VEGF were the highest of all. It demonstrates that TGF-β_1 and VEGF may co-modulate the angiogenesis.

TGFβ_1 expression was detected in 37 tumors (37.8%). The expression of TGF β_1 was correlated significantly with the depth of invasion, stage of disease and lymph node metastasis. Patients in T3-T4, stage III-IV and with lymph node metastasis had much higher expression of TGF β_1 than patients in T1-T2, stageI-II and without lymph node metastasis (P<0.05).

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Edited by Wu XN