J Cancer Res Clin Oncol (2008) 134:193–201
DOI 10.1007/s00432-007-0270-5

ORIGINAL PAPER

Relationship between VEGF and p53 expression and tumor cell proliferation in human gastrointestinal carcinomas

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Received: 28 May 2007 / Accepted: 19 June 2007 / Published online: 17 July 2007

Abstract
Purpose The vascular endothelial growth factor (VEGF) and p53 play important roles in the growth of tumor. However, the relationship between the expression of VEGF and p53 and tumor cell proliferation in human gastrointestinal cancer remains unknown. In the present study, therefore, we have examined the relationship between VEGF and p53 expression and tumor cell proliferation in gastrointestinal carcinoma (GITC), as well as the association between these biomarkers and clinicopathological factors.
Methods Surgical specimens from 30 patients with GITC were examined for VEGF, p53, and proliferating cell nuclear antigen (PCNA) expression by immunohistochemical staining.
Results We found a predominant VEGF expression of moderate intensity in 16(54.84%) of 30 GITC cases, while p53 expression was mainly high in 13(45.16%) of 30 GITC cases. PCNA expression was high in 20(64.52%) of 30 GITC cases. Tumor size, infiltration, vascular invasion, and gastritis were significantly correlated with VEGF, p53, and PCNA expression. There was a significant correlation between VEGF and p53 expression ($P = 0.0001$), VEGF and PCNA expression ($P = 0.00004$), and between p53 expression and PCNA expression ($P = 0.0016$). When the VEGF and p53 expression, and PCNA expression were considered together, both VEGF and p53 expression were not significantly associated with PCNA. A significant correlation between the PCNA expression and the mitotic index ($P = 0.0016$) was also found.
Conclusion These results demonstrate that VEGF and p53 expression are significantly correlated as independent prognostic factors with tumor cell proliferation, and might be associated with relevant events involved in gastrointestinal tumor biology.

Keywords VEGF · p53 · PCNA · Gastrointestinal carcinomas · Clinicopathological factors · Immunohistochemistry

Abbreviations

VEGF	Vascular endothelial growth factor
PCNA	Proliferating cell nuclear antigen
GITC	Gastrointestinal carcinomas

Introduction

Angiogenesis plays a critical role in the growth and metastasis of solid tumors (Folkman 1971; Folkman et al. 1971; Doussis-Anagnostopoulou et al. 2002). It is known that angiogenesis is regulated by a balance between stimulator and inhibitor factors (Kondo et al. 2000; Takahashi et al. 1998, 2003). Vascular endothelial growth factor (VEGF) is most commonly associated with tumor angiogenesis (Ellis et al. 2000; Fontanini et al. 2002; La Rosa et al. 2003; Takahashi et al. 2003; Uehara et al. 2004; Cristi et al. 2005; Mattioli et al. 2007), and has been involved in the proliferation, migration and survival of endothelial cells (Ferrara 1999, 2000; Ellis et al. 2000; Scoazec 2000; Lappi-Blanco et al. 2002; Cristi et al. 2005), the induction of fenestrations and increase of the cell permeability (Ferrara 1999, 2000; Scoazec 2000).

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In other studies, p53 tumor suppressor gene have been found to play a crucial regulatory role in the control of angiogenesis through the VEGF expression (Maeda et al. 1998a; Takahashi et al. 1998; Saito et al. 1999; Kondo et al. 2000; Maehara et al. 2000, 2001; Lanz et al. 2002; Lim et al. 2003; Cristi et al. 2005). A significant correlation between VEGF and p53 expression have been shown in gastric carcinoma (Maeda et al. 1998a), colon cancer (Takahashi et al. 1998; Cristi et al. 2005), non-small cell lung carcinoma (Fontanini et al. 1998), and oral squamous cell carcinoma (Maeda et al. 1998b).

p53 is the most commonly mutated gene in human tumors (Takahashi et al. 1998; Maehara et al. 1999; Saito et al. 1999; Okuyama et al. 2002; Sangrajrang et al. 2003; Capello et al. 2006; Guan et al. 2006; Mattioli et al. 2007; Qiu et al. 2007). The p53 family plays an important role in gastrointestinal malignancies (Guan et al. 2006). The abnormal overexpression of p53 protein has been correlated with a poor prognosis in human cancers (Maehara et al. 1999; Okuyama et al. 2002; Guan et al. 2006), including gastric (Saito et al. 1999), colon (Takahashi et al. 1998; Okuyama et al. 2002) and hepatocellular carcinomas (Guan et al. 2006).

It is known that p53 acts as a key cell cycle regulator in response to stress signals, either by inducing cell growth arrest, senescence or apoptosis (Maeda et al. 1998a; Takahashi et al. 1998; McLeod and Murray 1999; Maehara et al. 2000, 2001; Lanz et al. 2002; Constantinou et al. 2003; Fenoglio-Preiser et al. 2003; Sangrajrang et al. 2003; Guan et al. 2006). Moreover, it has been suggested that p53 prevent the growth of potential tumor cells related to its function as a tumor suppressor gene (Haupt et al. 2002; Nayak and Das 2002).

The proliferating cell nuclear antigen (PCNA) is a cofactor protein of DNA polymerase delta that plays a major role in DNA synthesis (Linden et al. 1992; Maeda et al. 1999; McLeod and Murray 1999; Capello et al. 2006). The expression of PCNA is associated with cell proliferation (Maeda et al. 1999), particularly in the late G1 phase and S-phase (Linden et al. 1992; Maeda et al. 1999). Therefore, PCNA has been considered a useful marker for nuclear proliferative activity (Linden et al. 1992; Maeda et al. 1999; Capello et al. 2006).

Several studies have suggested that overexpression of VEGF and p53 in tumor cells is related to tumor growth (Maeda et al. 1998; Takahashi et al. 1998; Saito et al. 1999; Maehara et al. 2000; Lim et al. 2003). However, the relationship between the expression of VEGF and p53 and tumor cell proliferation in human gastrointestinal cancer remains unknown. In the present study, therefore, we have examined the relationship between VEGF and p53 expression and tumor cell proliferation in gastrointestinal carcinomas (GITC), as well as the association between these biomarkers and clinicopathological factors.

Patients and methods

Tumor specimens

A retrospective study was undertaken in surgical biopsies from patients ($n = 30$; 20 males and 10 females), mean age of 63.8 years and a histopathological diagnosis of GITC ($n = 4$ stomach, $n = 20$ colon, and $n = 6$ rectum). All tumors were surgically removed and collected from February 2000 to July 2003, and approved by the local ethics committee from the Hospital Clínico Universitario de Caracas, Universidad Central de Venezuela. No patient in this study was treated with chemotherapy or radiotherapy prior to surgery.

Histopathology

Resected specimens from the patients were fixed and embedded in paraffin, and stained with hematoxylin–eosin. One or two representative paraffin block from each case was processed for immunohistochemical analyses.

Immunohistochemical analysis

For immunohistochemical staining, we used the avidin–biotin–immunoperoxidase technique. Paraffin-embedded tissue were sectioned to 4 μm and de-paraffined. The endogenous peroxidase activity was blocked by 3% hydrogen peroxide in methanol for 5 min. The sections were then washed in phosphate-buffered saline (100 mM PBS, pH 7.2) and incubated with blocking reagent from labeled streptavidine biotine (LSAB) kit (DAKO, Carpinteria, CA, USA) for 30 min to reduce nonspecific antibody binding. The sections were incubated with anti-VEGF (A-20) (Santa Cruz Biotechnology, CA, USA) at a dilution of 1:300, anti-p53 (FL-393) (Santa Cruz Biotechnology, CA, USA) at a dilution of 1:100 and anti-PCNA (PC10) (DAKO, Carpinteria, CA, USA) at a dilution of 1:100 for 1 h at room temperature in a humid chamber. Following this, the sections were washed in PBS and incubated with biotinylated secondary antibody from LSAB kit for 30 min, and then incubated with streptavidin-peroxidase reagent from this kit for 30 min according to the manufacturer's instructions. Finally, immunoreactions were developed with 3-amino-9-ethyl carbazole solution for 10 min, counterstained with Mayer hematoxylin, and mounted in aqueous medium.

Negative controls for each tissue section were prepared replacing primary antibody with PBS. Control consisted of normal stomach, colon, and rectum tissues, obtained from a distant region of the tumors. The positive control consisted of tissue sections of known positive immunoreaction for each antibody. Intensity of immunostaining was scored as negative = 0, weak = 1, moderate = 2 and high = 3. Observations were performed at the microscope standard KF2

Zeiss with a digital camera (Casio QV-R40). The immunohistochemical evaluation of the specimens was performed on coded samples by two observers independently, without knowledge of the clinicopathological factors.

p53 expression and PCNA labeling index and mitotic index

The percentage of positive nuclear immunostaining of p53 (Fontanini et al. 2002; Lim et al. 2003) and positive nuclear immunostaining of PCNA (Maeda et al. 1995; McLeod and Murray 1999) was evaluated by counting 500 cells in ten fields of 40× of magnification and expressed as the labeling index.

Mitotic index was calculated as the percentage of mitotic cells in ten fields of 40× of magnification (van Diest et al. 1998; Takahashi et al. 2003).

Statistical analysis

The relationship between VEGF and p53 and PCNA expression were examined using ANOVA Kruskal–Wallis test, chi squared (χ^2) test or Student's t test as appropriate. The relationship between PCNA expression and mitotic index were examined using χ^2 test. The correlation between VEGF, p53 and PCNA and the clinicopathological factors was analyzed with the χ^2 test. Data were expressed as the mean ± SD. Statistical significance was defined as $P < 0.05$. All data were analyzed using Statistic 5.5 version software.

Results

The histology of GITC was represented by characteristics features including tumor cells with abundant stroma, and presence of cells in mitosis (Fig. 1a). The clinicopathological factors from GITC patients are shown in Table 1.

A total of 30 GITC were examined for the presence of immunoreactivity to VEGF, p53 and PCNA. The immunostaining pattern of distribution was diffuse in some tumors whereas focal in others. The VEGF expression was scored

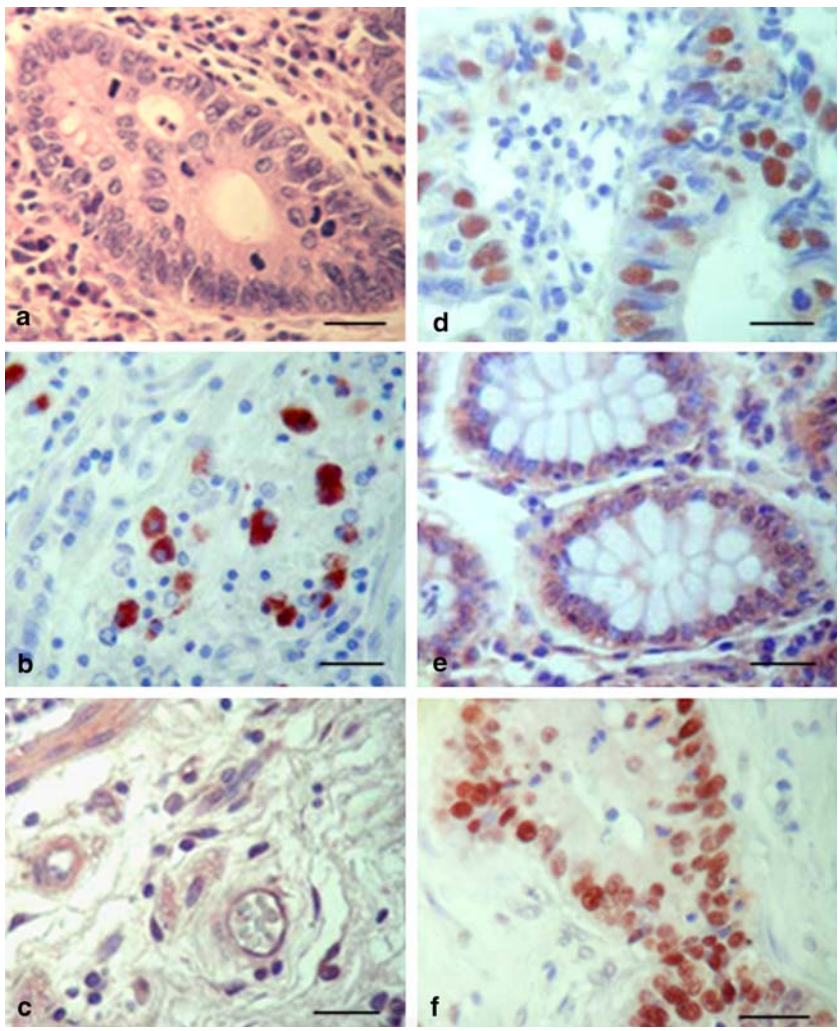

Fig. 1 Representative examples of GITC. Hematoxilin and eosin staining showing several mitosis (**a**). Immunohistochemical staining of VEGF in tumor cells (**b**) and blood vessels (**c**), p53 in the tumor cells nucleus (**d**) and cytoplasm (**e**), and PCNA in tumor cells (**f**). Bar = 25 μm (**a**–**f**)

Table 1 Correlation between VEGF, p53, PCNA expression, mitotic index and clinicopathological factors

	Total patients *n* (%)	VEGF			P53			PCNA			Mitotic index
		(−) *n* (%)	(+) *n* (%)	*P* value	(−) *n* (%)	(+) *n* (%)	*P* value	(−) *n* (%)	(+) *n* (%)	*P* value	*P* value
Age (years)											
>67	14(46.67)	0(0.00)	14(46.33)	0.31	1(3.33)	13(43.33)	0.80	3(10.00)	11(36.7)	0.09	<0.0001*
<67	16(53.33)	1(3.33)	15(50.33)		1(3.33)	15(46.63)		2(6.67)	14(46.7)		
Sex											
F	10(33.33)	1(3.33)	9(29.63)	0.06	1(3.33)	9(29.93)	0.21	3(10.00)	7(23.33)	0.36	<0.0001*
M	20(66.67)	0(0.00)	20(67.00)		1(3.33)	19(63.37)		2(6.67)	18(59.97)		
Tumor location											
Stomach	4(13.33)	0(0.00)	4(13.33)		0(0.00)	4(13.33)		0(0.00)	4(13.33)		
Colon	20(66.67)	0(0.00)	20(67.00)	0.29	2(6.67)	18(60.00)	0.68	1(3.33)	19(63.3)	0.68	<0.0001*
Rectum	6(20.00)	1(3.33)	5(16.60)		0(0.00)	6(19.97)		4(13.3)	2(6.67)		
Mean tumor size (cm)											
>6	8(26.70)	0(0.00)	8(26.33)	0.01*	1(3.33)	7(23.34)	0.015*	0(0.00)	8(26.70)	0.09	<0.0001*
<6	22(73.30)	1(3.33)	21(70.30)		1(3.33)	21(69.93)		5(16.70)	17(56.67)		
Histology grade											
Differentiated	28(93.40)	1(3.33)	27(79.63)	0.92	2(6.67)	26(86.60)	0.77	5(16.67)	23(76.70)	0.0003*	<0.0001*
Undifferentiated	2(6.60)	0(0.00)	2(6.70)		0(0.00)	2(6.67)		0(0.00)	2(6.67)		
Infiltration											
Positive	29(96.7)	1(3.33)	28(93.33)	<0.0001*	2(6.67)	27(90.00)	<0.0001*	4(13.3)	25(83.4)	<0.0001*	<0.0001*
Negative	1(3.33)	0(0.00)	1(3.33)		0(0.00)	1(3.33)		1(3.33)	0(0.00)		
Vascular invasion											
Positive	5(16.70)	0(0.00)	5(16.67)	0.002*	1(3.33)	4(13.33)	0.002*	1(3.33)	4(13.3)	0.03*	> 0.678
Negative	25(83.3)	1(3.33)	24(80.03)		1(3.33)	24(80.00)		4(13.30)	21(70.00)		
Metastasis											
Positive	16(53.30)	0(0.00)	16(53.63)	0.7	2(6.67)	4(13.33)	0.39	2(6.67)	14(46.63)	0.02*	<0.0001*
Negative	14(46.67)	1(3.33)	13(43.00)		0(0.00)	24(80.00)		3(10.00)	13(36.66)		
Gastritis											
Positive	3(10.00)	0(0.00)	3(10.00)	0.0002*	0(0.00)	3(10.00)	<0.0001*	0(0.00)	3(10.00)	0.004*	> 0.825
Negative	27(90.00)	1(3.33)	26(86.33)		2(6.67)	3(10.00)		5(16.70)	22(73.40)		

* Statistically significant

as negative in 1(3.23%), weak in 1(3.23%), moderate in 16(54.84%), and high in 12(38.71%) of 30 GITC cases. The VEGF expression was mainly localized in the cytoplasm and membrane of the tumor cells (Fig. 1b) and blood vessels (Fig. 1c).

The p53 expression was negative in 2(6.45%), weak in 13(9.68%), moderate in 12(38.71%), and high in 13(45.16%) of 30 GITC cases. The p53 immunoreaction was predominant in the nucleus of malignant cells (Fig. 1d), and in some cases a cytoplasmic diffuse immunostaining (Fig. 1e) was also seen. A strong to moderate positive immunostaining to p53 in endothelial cells, and a weak positive immunoreaction in smooth muscle cells, was present. In normal cells a cytoplasmatic diffuse expression of p53 was also found.

The PCNA expression was negative in 5(17.74%), moderate in 5(17.74%), and high in 20(64.52%) of 30 GITC cases. A moderate to strong nuclear PCNA immunostaining was present in tumor cells (Fig. 1f).

The association between VEGF, p53, and PCNA expression and several clinicopathological factors in patients with GITC is shown in Table 1. VEGF, p53 and PCNA immunostaining was significantly correlated with infiltration, vascular invasion, and gastritis. Taken together, these results indicate that overexpression of VEGF and p53 is associated with an increase of the tumor proliferation activity, and a poor prognosis in GITC, in correspondence with other malignant tumors (Maeda et al. 1998, 1999; Niu et al. 2000; Okuyama et al. 2002; Uehara et al. 2004). Both, VEGF and p53 expression were also related to the tumor size as previously reported (Takahashi et al. 2003). The PCNA expression was correlated with tumor location, histological grade and metastasis. These results suggest that the increase of the proliferative activity is related to the local tumor invasion and metastasis spread. VEGF, p53 and PCNA might be used as biomarkers of tumor activity, since they play a role in gastrointestinal tumor biology.

Kruskal–Wallis analysis, as shown in Table 2, showed that there was a significant correlation between VEGF and p53 expression ($P = 0.0001$), and between VEGF and PCNA expression ($P = 0.00004$). We used Student's t test to examine the correlation between p53 and PCNA expression ($P = 0.0016$, Table 3). When the VEGF and p53 expression, and PCNA expression were considered together, both VEGF and p53 expression were not significantly associated with PCNA (Table 4).

The mitotic index in GITC was 2.21 ± 1.54 (mean $\pm$ SD). The χ^2 test showed a significant correlation between the mitotic index and VEGF expression ($P = 0.0004$) and the mitotic index and p53 expression ($P < 0.0001$). Moreover, by the Student's t test there was a significant correlation between the PCNA expression and the mitotic index ($P = 0.0016$). The mitotic index showed significant correlation with age, sex, infiltration, tumor location, histological grade, and metastasis (Table 1).

Discussion

The present study, as far as we know, examines for the first time the relationship between VEGF and p53 expression, and tumor cell proliferation in GITC, and the association between these biomarkers and clinicopathological factors. It is known that VEGF have a role in the modulation of endothelial functions in angiogenesis and vascular remodelation (Lappi-Blanco et al. 2002; La Rosa et al. 2003; Rosa et al. 2003), through its binding to VEGF receptor 1 (Flt-1/VEGFR-1) and receptor 2 (Flk-1/VEGFR-2/KDR) (Ferrara 2004; Lappi-Blanco et al. 2002). Here, we demonstrated by immunohistochemistry that 29(96.77%) of 30 GITC patients clearly expressed VEGF. A diffuse cytoplasmatic pattern of distribution in endothelial and tumor cells was similar to gastric (Saito et al. 1999), and colorectal cancer (Takahashi et al. 1998; Gunsilius et al. 2002; Kondo et al. 2000). Smooth muscle cells have also been shown to express VEGF in GITC (Saito et al. 1999; Takahashi et al. 2003; La Rosa et al. 2003; Ellis et al. 2000), and carcinoma of the salivary gland (Lim et al. 2003). VEGF expression is mediated by several factors such as hypoxia, cytokine levels (Takahashi et al. 1998; Ferrara 1999, 2000), and p53 mutations (Takahashi et al. 1998; Saito et al. 1999; Lim et al. 2003; Reinmuth et al. 2003).

The expression of p53 has been suggested to play an important role in the regulation of VEGF via angiogenesis

Table 2 Correlation between VEGF expression, p53 labeling index, and PCNA labeling index

VEGF	Total n (%)	p53 labeling index (mean $\pm$ SD)	P value	PCNA labeling index (mean $\pm$ SD)	P value
(−)	1(3.33)	12.54 ± 11.66	0.0001[a]*	0 ± 0	<0.0001[b]*
(+)	29(96.7)	14.14 ± 15.22		22.03 ± 17.10	

* Statistically significant

[a] ANOVA Kruskal–Wallis test

[b] Student's t test

Table 3 Correlation between p53 expression and PCNA-labeling index

p53	Total *n* (%)	PCNA labeling index (mean ± SD)	*P* value
(−)	2(6.67)	9.83 ± 15.92	0.0016[a]*
(+)	28(93.33)	15.25 ± 15.60	

* Statistically significant

[a] Student's *t* test

Table 4 Correlation between VEGF and p53 expression and PCNA-labeling index

	Total *n* (%)	PCNA labeling index (mean ± SD)	*P* value
VEGF (+) and p53 (−)	0(0.00)	0.00 ± 0.00	0.2202[a]
VEGF (+) and p53 (−) or VEGF (−) and p53 (+)	3(10.00)	21.45 ± 14.79	
VEGF (−) and p53 (+)	27(90.00)	17.51 ± 16.88	

[a] Student's *t* test

and protein kinase C induction (Maeda et al. 1998; Takahashi et al. 1998; Saito et al. 1999; Maehara et al. 2000, 2001; Lim et al. 2003; Kondo et al. 2000; Hasan et al. 2002). In our study, the positive immunostaining of p53 was detected in 28(93.55%) of 30 GITC cases, and a strong immunoreaction was mainly localized in the nucleus of tumor cells, which suggests that p53 gene mutations are frequent and critical in these tumors, similar to esophageal (Saeki et al. 2002), gastric (Saito et al. 1999), urinary tract (Furihala et al. 2002) and colorectal tumors (Smith et al. 1996; Kaklamanis et al. 1998; Takahashi et al. 1998). The cytoplasmic diffuse p53 immunostaining in GITC might indicate that p53 is chaperoned to the cytoplasm by the negative regulator Mdm2, an ubiquitin ligase related to upregulation in the presence of active p53, where Mdm2 polyubiquinates p53 for proteasome targeting avoiding its binding to DNA (Marine et al. 2006; Rosa et al, 2003). The polyclonal antibody FL-393 that we used recognize both, wild type and mutant p53, but given the short half-time of wild type p53 protein (~5 min), immunostaining is likely to represent abnormal accumulation of the mutant type only (Smith et al. 1996; Saito et al. 1999; Woods and Vousden 2001; Okuyama et al. 2002; Fenoglio-Preiser et al. 2003; Rosa et al. 2003; Marine et al. 2006; Qiu et al. 2007). Recently, Capello et al. (2006) observed a heterogeneous topographical distribution of the mutant p53 in prostate cancer. In addition, the positive immunostaining to p53 in normal cells could be due to the anti-p53 antibody FL-393, which recognizes the 395 amino acids, representing the full length of p53, resulting in the detection of not degraded wild type protein, as previously found in esophageal (Saeki et al. 2002), and gastric tissues (Okuyama et al. 2002).

Several studies have analyzed the prognostic significance of VEGF and p53 expression in human malignant tumors. This association may further contribute to the understanding of the gastrointestinal tumor biology. In our study, a significant correlation between VEGF and p53 expression suggest the existence of a pathway from p53 to regulate angiogenesis induced by VEGF, comparable to the findings in other gastric (Maeda et al. 1998a; Saito et al. 1999; Maehara et al. 2000), colorectal (Kondo et al. 2000; Takahashi et al. 1998), head and neck (van Houten et al. 2002), and salivary gland tumors (Lim et al. 2003). The close relationship between VEGF expression and p53 protein accumulation suggest that p53 status might be a key event to the angiogenesis of GITC (Maeda et al. 1998a; Takahashi et al. 1998; Saito et al. 1999; Kondo et al. 2000; Maehara et al. 2000, 2001; Lanz et al. 2002; Lim et al. 2003), since it is known that p53 protein accumulation may induce angiogenesis through upregulation of VEGF (Saito et al. 1999). The VEGF and p53 expression in GITC was correlated with the tumor size, infiltration, vascular invasion, and gastritis, suggesting an important association with a poor prognosis. The VEGF expression has been considered an important prognostic factor in human cancers of gastrointestinal tract, breast carcinoma and hepatocellular carcinoma (Lim et al. 2003; Maeda et al. 1998a, 1999; Niu et al. 2000; Okuyama et al. 2002; Uehara et al. 2004). VEGF also plays an essential role in tumor angiogenesis and metastasis (Takahashi et al. 2003) of hepatocellular carcinoma (Niu et al. 2000). The p53 expression has been reported as a prognostic indicator of the invasion (Maeda et al. 1998a; Saito et al. 1999; Maehara et al. 2000), infiltration and metastasis (Smith et al. 1996; McLeod and Murray 1999; Buglioni et al. 2001; Capello et al. 2006). The most significant predictor of poor prognosis is a large tumor size and metastasis (Uehara et al. 2004). However, in the present study no correlation was found between VEGF or p53 expression and metastasis probably related to the small number of patients (Takahashi et al. 2003; Lim et al. 2003). Similarly, in other reports also, no correlation between VEGF or p53 expression and lymph node metastasis was found in lung cancer, oral squamous cell carcinoma (Maeda et al. 1998b; Saito et al. 1999; Uehara et al. 2004), and gastric cancer (Maeda et al. 1998a; Saito et al. 1999).

In this study, the nuclear positive immunostaining of PCNA in tumor gastrointestinal cells was similar to other carcinomas (Linden et al. 1992; McLeod and Murray 1999; Okuyama et al. 2002). The immunoreaction in non-tumor cells could be due to secretion of growth factors inducing the synthesis of PCNA, and the accumulation of this protein in normal cells (Linden et al. 1992), as demonstrated in gastric tissue (Maeda et al. 1999). The PCNA expression was correlated with histological grade, infiltration, vascular

invasion, gastritis and metastasis. These results suggest that the increase of the proliferative activity could be related to the local tumor invasion and metastasis spread. Cell proliferation has been associated to tumor aggressiveness (Medri et al. 2003). The aggressive behavior of tumor in patients with metastasis considered in this study was similar to other neoplasias including gastric (Maeda et al. 1998a), and colorectal (Sumiyoshi et al. 2000). On the contrary, in our cases no statistically significant correlation was found between the tumor size and the PCNA index labeling score, possibly related to the size of a lesion measured one-time during its growth and not reflecting its growth rate as a proliferative score (Vesselle et al. 2000).

We found a positive correlation between VEGF and PCNA expression in GITC. Recently, a similar association was found in salivary gland carcinomas (Lim et al. 2003) and breast cancer (Kushlinskii et al. 2004); however, an inverse correlation between VEGF expression and PCNA labeling index was found in endometrial cancer (Fujiwaki et al. 1999). Giatromanolaki et al. (1998) using Ki-67 showed no correlation between VEGF expression and tumor proliferation in non-small cell lung cancer.

Recently, Cristi et al. (2005) suggested that the VEGF protein might be an indirect mediator of tumor growth in colon cancer by microvessel proliferation induction, although the direct impact of VEGF expression on tumor growth kinetics is not clearly defined. The role of PCNA in the regulation of VEGF is currently being investigated (Niu et al. 2000).

In the present study, p53 expression was associated with tumor proliferation, indicating that p53 accumulation may confer a proliferative advantage for gastrointestinal cells, in correspondence with other malignancies in breast (Sangrajrang et al. 2003) and prostate (Capello et al. 2006).

We found in our cases a positive association between the mitotic index and the PCNA expression and several clinicopathological factors. The mitotic index has been implicated in the proliferate activities of tumor, and considered as a variable to determine the histological grade of the tumor (van Diest et al. 1998) in stomach cancer (Takahashi et al. 2003). Kontogianni et al. (2003) found that tumor size, necrosis, mitoses, metastasis and PCNA labeling index are correlated as independent poor prognostic factors in GITC. The mitotic activity as an independent prognostic variable is currently used in the tumor-grading system (Medri et al. 2003).

In conclusion, VEGF and p53 expression are related as independent prognostic factors with tumor proliferation in human GITC. Further studies including biochemical and molecular analysis will be necessary to elucidate the role(s) of VEGF and p53 expression and their correlation with cell proliferation in GITC, as well as the implications in tumor angiogenesis and possible selection of therapy.

Acknowledgments This work was supported by Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH-UCV) Grant (03-10-5113-2003 to PT).

References

Buglioni S, D'Agnano I, Vasselli S, Perrone R, D'Angelo C, Brenna A, Benevolo M, Cosimelli M, Zupi G, Mottolese M (2001) p53 nuclear accumulation and multiploidy are adverse prognostic factors in surgically resected stage II colorectal cancers independent of fluorouracil-based adjuvant therapy. Am J Clin Pathol 116:360–368

Capello F, Ribbene A, Campanella C, Czarnecka AM, Anzalone R, Bucchieri F, Palma A, Zummo G (2006) The value of immunohistochemical research on PCNA, p53 and heat shock proteins in prostate cancer management: a review. Eur J Histochem 50:25–34

Constantinou C, Bushell M, Jeffrey, Tilleray V, West M, Frost V, Hensold J, Clemens J (2003) p53-induced inhibition of protein synthesis is independent of apoptosis. Eur J Biochem 270:3122–3132

Cristi E, Perrone G, Toscano G, Verzì A, Nori S, Santini D, Tonini G, Vetrani A, Fabiano A, Rabitti C (2005) Tumour proliferation, angiogenesis, and ploidy status in human colon cancer. J Clin Pathol 58:1170–1174

Doussis-Anagnostopoulou I, Talks K, Turley H, Debnam P, Tan D, Mariatos G, Gorgoulis V, Kittas C, Gatter C (2002) Vascular endothelial growth factor (VEGF) is expressed by neoplastic Hodgkin–Reed–Sternberg cells in Hodgkin's disease. J Pathol 97:677–683

Ellis LM, Takahashi Y, Liu W, Shaheen RM (2000) Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. Oncologist 1:11–15

Fenoglio-Preiser C, Wang J, Stemmermann G, Noffsinger A (2003) TP53 and gastric carcinoma: a review. Hum Mutat 21:258–270

Ferrara N (1999) Role of vascular endothelial growth factor in regulation of angiogenesis. In: Teicher BA (ed) Antiangiogenic agents in cancer therapy. Humana Press, Totowa, New Jersey, pp 119–141

Ferrara N (2000) The role of vascular endothelial growth factor in angiogenesis. In: Rubanyi GM (ed) Angiogenesis in health and disease. Marcel Dekker, New York, Basel, pp 47–73

Ferrara N (2004) Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 25:581–611

Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285: 1182–1186

Folkman J, Merler E, Abernathy C, Williams G (1971) Isolation of a tumor factor responsible for angiogenesis. J Exp Med 133:275–288

Fontanini G, Boldrini L, Vignati S, Chine S, Basolo F, Silvestri V, Lucchi M, Mussi A, Angeletti CA, Bevilacqua G (1998) Bcl2 and p53 regulate vascular endothelial growth factor (VEGF)-mediated angiogenesis in non-small cell lung carcinoma. Eur J Cancer 34:718–723

Fontanini G, Faviana P, Lucchi M, Boldrini L, Mussi A, Camacci T, Mariani MA, Angeletti CA, Basolo F, Pingitore R (2002) A high vascular count and overexpression of vascular endothelial growth factor are associated with unfavourable prognosis in operated small cell lung carcinoma. Br J Cancer 86:558–563

Fujiwaki R, Hata K, Iida K, Maede Y, Watanabe Y, Koike M, Miyazaki K (1999) Co-expression of vascular endothelial growth factor and thymidine phosphorylase in endometrial cancer. Acta Obstet Gynecol Scand 78:728–734

Furihala M, Kurabayashl A, Matsumoto M, Sonobe H, Ohtsuki Y, Lerao N, Kuwahara M, Shuin T (2002) Frequent phosporylation at serine 392 in overexpressed p53 protein due to missense mutation in carcinoma of the urinary tract. J Pathol 197:82–88

Giatromanolaki A, Koukourakis M, Kakolyris S, Turley AH, O`Byrne K, Scott P, Pezzella F, Georgoulias V, Harris A, Gatter K (1998) Vascular endothelial growth factor, wild-type p53, angiogenesis in early operable non-small cell lung cancer. Clin Cancer Res 4:3017–3024

Guan Y-H, He Q, La Z (2006) Roles of p53 in carcinogenesis, diagnosis and treatment of hepatocellular carcinoma. J Cancer Mol 2:191–197

Gunsilius E, Tschmelitsch J, Eberwein M, Schwelberger H, Spizzo G, Kähler C, Stockhammer G, Lang A, Petzer A, Gastl G (2002) In vivo release of vascular endothelial growth factor from colorectal carcinomas. Oncology 62:313–317

Hasan J, Byersm R, Jayson GC (2002) Intratumoral microvessel density in human solid tumors. Cancer Res 86:1566–1577

Haupt Y, Robles A, Prives C, Rotter V (2002) Deconstruction of p53 functions and regulation. Oncogene 21:8223–8231

Kaklamanis L, Savage S, Whitehouse R, Doussis-Anagnostopoulou I, Biddolph S, Tsiotos P, Mortensen N, Gatter KC, Harris AL (1998) Bcl-2 protein expression: association with p53 and prognosis in colorectal cancer. Br J Cancer 77:1864–1869

Kondo Y, Arii S, Furutani M, Isigami S, Mori A, Onodera H, Chiba T, Imamura M (2000) Implication of vascular endothelial growth factor and p53 status for angiogenesis in non-invasive colorectal carcinoma. Cancer 88:1820–1827

Kontogianni K, Demonakou M, Kavantzas N, Lazaris ACh, Lariou K, Vourlakou C, Davaris P (2003) Prognostic predictors of gastrointestinal stromal tumors: a multi-institutional analysis of 102 patients with definition of a prognostic index. Eur J Surg Oncol 29:548–556

Kushlinskii NE, Orinovskii MB, Gurevich LE, Kazantseva IA, Talaeva ShZh, Shirokii VP, Ermilova VD, Dvorova EK, Ozherelev AS (2004) Expression of biomolecular markers (Ki-67, PCNA, Bcl-2, BAX, BclX, VEGF) in breast tumors. Bull Exp Biol Med 137:182–185

La Rosa S, Uccella S, Finzi G, Albarello L, Sessa F, Capella C (2003) Localization of vascular endothelial growth factor and its receptors in digestive endocrine tumors: correlation with microvessel density and clinicopathologic features. Hum Pathol 34:18–27

Lanz C, Süsse S, Wies Müller L (2002) p53 and recombination intermediates: role of tetramerization at DNA junctions in complex formation and exonucleotic degradation. Oncogene 21:2130–2140

Lappi-Blanco E, Soini Y, Kinnula V, Pääkkö P (2002) VEGF and bFGF are highly expressed in intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia. J Pathol 196:220–227

Lim J, Kang S, Llee M, Pal H, Yoon H, Lee J, Hong S, Lim C (2003) Expression of vascular endothelial growth factor in salivary gland carcinomas and its relation to p53, Ki-67 and prognosis. J Oral Pathol Med 32:552–561

Linden M, Torres F, Kubus J, Zarbo R (1992) Clinical application of morphologic and immunocytochemical assessments of cell proliferation. Am J Clin Pathol 97:S4–S13

Maeda K, Chung Y-S, Takatsuka S, Onoda N, Ogawa M, Sawada T, Kato Y, Nitta A, Arimoto Y, Kondo Y, Sowa M (1995) Tumor angiogenesis and tumor cell proliferation as prognostic indicators in gastric carcinoma. Br J Cancer 72:319–323

Maeda K, Kang S-M, Onoda N, Ogawa M, Sawada T, Nakata B, Kato Y, Chung Y-S, Sowa M (1998a) Expression of p53 and vascular endothelial growth factor associated with tumor angiogenesis and prognosis in gastric cancer. Oncology 55:594–599

Maeda T, Matsumura S, Hiranuma H (1998b) Expression of vascular endothelial growth factor in human oral squamous cell carcinoma: its association with tumor progression and p53 status. J Clin Pathol 51:771–775

Maeda K, Kang S-M, Onoda N, Ogawa M, Sawada T, Kato Y, Chung Y-S (1999) Vascular endothelial growth factor expression in preoperative biopsy specimens correlates with disease recurrence in patients with early gastric carcinoma. Am Cancer Soc 86:566–571

Maehara Y, Tomoda M, Hasuda S, Kabashima A, Tokunaga E, Kakeji Y, Sugimachi K (1999) Prognostic value of p53 protein expression for patients with gastric cancer—a multivariate analysis. Br J Cancer 79:1255–1261

Maehara Y, Kabashima A, Koga T, Tokunaga E, Takeuchi H, Kakeji Y, Sugimachi K (2000) Vascular invasion and potential for tumor angiogenesis and metastasis in gastric carcinoma. Surgery 128:408–416

Maehara Y, Kakeji Y, Oda S, Baba H, Sugimachi K (2001) Tumor growth patterns and biological characteristics of early gastric carcinoma. Oncology 61:102–112

Marine J-C, Francoz S, Maetens M, Wahl G, Toledo F, Lozano G (2006) Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. Cell Death Differ 13:927–34

Mattioli E, Vogiatzi P, Sun A, Abbadessa G, Angeloni G, D'ugo D, Trani D, Gaughan J, Vecchio F, Cevenini G, Persiani R, Giordano A, Claudio PP (2007) Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, $p16^{INK4A}$, $P27^{KIP1}$, $P21^{WAF1}$, KI-67 expression patterns in gastric cancer. J Cell Physiol 210:183–191

McLeod HL, Murray GI (1999) Tumour markers of prognosis in colorectal cancer. Br J Cancer 79:191–203

Medri L, Volpi A, Nanni O, Vecci AM, Mangia A, Schittulli F, Padovani F, Giunchi DC, Zito A, Amadori D, Paradiso A, Silvestrini R (2003) Prognostic relevance of mitotic activity in patients with node-negative breast cancer. Mod Pathol 16:1067–1075

Nayak B, Das G (2002) Stabilization of p53 and transactivation of its target genes in response to replication blockade. Oncogene 21:7226–7229

Niu Q, Tang ZY, Ma ZC, Qin LX, Zhang LH (2000) Serum vascular endothelial growth factor is a potential biomarker of metastatic recurrence after curative resection of hepatocellular carcinoma. World J Gastroenterol 6:565–568

Okuyama T, Maehara Y, Kabashima A, Takahashi I, Kakeji Y, Sugimachi K (2002) Combined evaluation of expressions of p53 and p21 proteins as prognostic factors for patients with gastric carcinoma. Oncology 63:353–361

Qiu L-L, Hua P-Y, Ye LL, Wang YC, Qiu T, Bao HZ, Wang L (2007) The detection of serum anti-p53 antibodies from patients with gastric carcinoma in China. Cancer Det Prev 31:45–49

Reinmuth N, Parikh A, Ahmad SA, Liu W, Stoeltzing O, Takeda A, Akagi M, Ellis LM (2003) Biology of angiogenesis in tumors of the gastrointestinal tract. Microsc Res Tech 60:199–207

Rosa AR, Schirmer CC, Gurski RR, Meurer L, Edelweiss MI, Kruel CD (2003) Prognostic value of p53 protein expression and vascular endothelial growth factor expression in resected squamous cell carcinoma of the esophagus. Dis Esophagus 16:112–118

Saeki H, Ohno S, Miyazaki M, Araki K, Egashira A, Kawaguchi H, Watanabe K, Morita M, Sugimachi K (2002) p53 protein accumulation in multiple oesophageal squamous cell carcinoma: relationship to risk factors. Oncology 62:175–179

Saito H, Tujitani S, Ikegichu M, Maeta M, Kaibara N (1999) Neoangiogenesis and relationship to nuclear p53 accumulation and vascular endothelial growth factor expression in advanced gastric carcinoma. Oncology 57:164–172

Sangrajrang S, Arpornwirat W, Cheirsilpa A, Thisuphakorn P, Kalalak A, Sornprom A, Soussi R (2003) Serum p53 antibodies in correlation to other biological parameters of breast cancer. Cancer Det Prev 27:182–186

Scoazec J-Y (2000) Tumor angiogenesis: a pathologist's point of view. Elect J Oncol 2:23–32

Smith DR, Ji C-Y, Goh H-S (1996) Prognostic significance of p53 overexpression and mutation in colorectal adenocarcinomas. Br J Cancer 74:216–223

Sumiyoshi Y, Yamashita Y, Maekawa T, Sakai N, Shirakusa T, Kikuchi M (2000) Expression of CD44, vascular endothelial growth factor, and proliferating cell nuclear antigen in severe venous invasional colorectal cancer and its relationship to liver metastasis. Surg Today 30:323–327

Takahashi Y, Bucana CD, Cleary KR, Ellis LM (1998) p53, vessel count, and vascular endothelial growth factor expression in human colon cancer. Int J Cancer 79:34–38

Takahashi R, Tanaka S, Kitadai Y, Sumii M, Yoshihara M, Haruma K, Chayama K (2003) Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncology 64:266–274

Uehara M, Sano K, Ikeda H, Sekine J, Irie A, Yokota T, Tobita T, OHba S, Inokuchi T (2004) Expression of vascular endothelial growth factor and prognosis of oral squamous cell carcinoma. Oral Oncol 40:321–325

van Diest PJ, Brugal G, Baak JP (1998) Proliferation markers in tumours: interpretation and clinical value. J Clin Pathol 51:716–724

van Houten VM, Tabor MP, van den Brekel MW, Kummer JA, Denkers F, Dijkstra J, Leemans R, van der Waal I, Snow GB, Brakenhoff RH (2002) Mutated p53 as a molecular marker for the diagnosis of head and neck cancer. J Pathol 198:476–486

Vesselle H, Schmidt R, Pugsley JM, Li M, Kohlmyer SG, Vallires E, Wood DE (2000) Lung cancer proliferation correlates with (F-18) fluorodeoxyglucose uptake by positron emission tomography. Clin Cancer Res 6:3837–3844

Woods DB, Vousden KH (2001) Regulation of p53 function. Exp Cell Res 264:56–66