ORIGINAL ARTICLES

P53 and Vascular Endothelial Growth Factor Expressions are two Important Indices for Prognosis in Gastric Carcinoma

Rende Guo[1], Qiang Li[1], Lingxin Meng[2], Yuqing Zhang[2], Chuan Gu[3]

ABSTRACT

Aim: *This study examined the correlation between P53 and vascular endothelial growth factor (VEGF) expression together with tumour vascularity and investigated their clinical significance in the prognosis of gastric carcinoma.*

Subjects and Methods: *Ninety-five patients with gastric carcinoma who underwent curative surgical resection were studied using immunohistochemical staining. Correlation between the expression of p53, VEGF, microvessel count (MVC) and various clinicopathologic factors were studied.*

Results: *No significant correlation was found between p53 expression and clinicopathologic factors. The rate of VEGF positivity was significantly higher in patients with haematogenous metastasis than in those without haematogenous metastasis. Both p53 and VEGF expression were associated with MVC. The MVC in p53 positive tumours was significantly higher than that in p53 negative tumours. Similarly, the same trend was seen between VEGF expression and MVC. The p53 and VEGF were co-expressed in 61 of 95 tumours (64.2%), and a significant (*$p < 0.01$*) association between p53 and VEGF expressions was demonstrated. The rate of VEGF positivity was significantly (*$p < 0.01$*) higher in the patients with disease recurrence than in those without recurrence, whereas no significant correlation was found between disease recurrence and the expression of p53.*

Conclusions: *The p53 expression may play an important role in controlling angiogenesis by regulating VEGF expression and VEGF expression is associated closely with disease recurrence. In addition, both p53 and VEGF expression might be useful in indicating the prognosis in patients with gastric carcinoma.*

West Indian Med J 2008; 57 (1): 2

Las Expresiones del P53 y del Factor de Crecimiento del Endotelio Vascular Constituyen dos Índices Importantes para la Prognosis del Carcinoma Gástrico

Rende Guo[1], Qiang Li[1], Lingxin Meng[2], Yuqing Zhang[2], Chuan Gu[3]

RESUMEN

Objetivo: *Este estudio examinó la correlación entre el P53 y la expresión del factor de crecimiento del endotelio vascular (VEGF) junto con las vascularidad del tumor, e investigó su importancia clínica en la prognosis del carcinoma gástrico.*

Sujetos y métodos: *Noventa y nueve pacientes con carcinoma gástrico que fueron sometidos a resección quirúrgica curativa, fueron estudiados usando teñido inmunohistoquímico. Se estudió la*

From: [1]Department of Hepatobiliary Surgery, Cancer Hospital of Tianjin Medical University, Huanhu Western Road, Hexi District, Tianjin 300060, [2]Pathological Department, Tianjin Medical University Cancer Hospital. Tianjin, 300201, [3]Department of General Surgery, Tianjin First Central Hospital, Tianjin, 300192, China.

Correspondence: Dr Qiang Li, Department of Hepatobiliary Surgery, Cancer Hospital of Tianjin Medical University, Huanhu Western Road, Hexi District, Tianjin 300060, China PR, Fax: 86-22-23359984, e-mail: liqiang_2644@sina.com.

correlación entre la expresión de p53, VEGF, el conteo de microvasos (MVC) y varios factores clínico-patológicos.

Resultados: *No se halló una correlación significativa entre la expresión de p53 y los factores clínico-patológicos. La tasa de positividad de VEGF, fue significativamente más alta en pacientes con metástasis hematogénica que en pacientes sin metástasis hematogénica. Tanto la expresión de p53 como la de VEGF estuvieron asociadas con el conteo MVC. El MVC en tumores p53 positivos fue significativamente más alto que en tumores p53 negativos. De manera similar, la misma tendencia se observó entre la expresión de VEGF y MVC. El p53 y el VEGA fueron co-expresados en 61 de 95 tumores (64.2%), y se demostró una asociación significativa (*$p < 0.01$*) entre las expresiones de p53 y VEGA. La tasa de positividad VEGA fue significativamente más alta (*$p < 0.01$*) en los pacientes con recurrencia de la enfermedad que en aquellos sin recurrencia, en tanto que no se halló una correlación significativa entre la recurrencia de la enfermedad y la expresión de p53.*

Conclusiones: *La expresión p53 puede desempeñar un importante papel en el control de la angiogénesis mediante la regulación de la expresión de VEGF y la expresión de VEGF está estrechamente asociada con la recurrencia de la enfermedad. Además, tanto la expresión de p53 como la de VEGF podrían ser útiles para indicar la prognosis de pacientes con carcinoma gástrico.*

West Indian Med J 2008; 57 (1): 3

INTRODUCTION

It is well known that malignant tumours depend on neovascularization for their growth and metastasis (1). It has also been suggested that the degree of tumour angiogenesis is related to clinical outcome in gastric carcinoma, suggesting that angiogenic properties correlate with tumour aggressiveness. Many investigators have demonstrated that vascular endothelial growth factor (VEGF) plays a crucial role in the formation of neovasculature and correlates strongly with tumour angiogenesis in gastric carcinoma (2, 3).

P53 is a well known tumour suppressor gene. Mutations of the P53 gene are the most common genetic alterations and are known to occur in a wide range of human malignancies. Recent studies have shown that P53 may play a crucial role in the control of angiogenesis through regulation of VEGF expression (4).

In this study, immunohistochemical staining was used to examine the correlation between P53 and VEGF expression together with tumour vascularity and investigate their clinical significance in gastric carcinoma.

SUBJECTS AND METHODS

Clinical Materials

Ninety-five patients with gastric carcinoma who underwent curative surgical resection at the TianJin Cancer Hospital between May 1996 and May 2001 were studied. The patients ranged in ages from 26 to 79 (average 60.95) years, and there were 67 males and 28 females. No patient had received chemotherapy or radiation therapy before surgery. Throughout this study, the Japanese classification of gastric carcinoma was used for the pathologic diagnosis and classification of variables (5). Tumours were divided into two histologic subgroups: differentiated type, which consisted of papillary and tubular adenocarcinoma; and undifferentiated type which included poorly differentiated adenocarcinomas, signet ring cell carcinoma and mucinous adenocarcinomas. All patients were observed for at least five years after surgery and routinely monitored by diagnostic imaging (computed tomography, ultrasonography or magnetic resonance imaging) once a year and serum levels of carcinoembryonic antigen (CEA). Patients who died of other diseases were excluded from the study. The type of recurrence was established by diagnostic imaging, serum levels of CEA, cytology, biopsy or open surgery, and was classified as hepatic recurrence, peritoneal recurrence, or other type of recurrence. Almost all patients had only one site of recurrence. In patients who had multiple sites of recurrence, the mode of recurrence was determined at the time of first relapse. But it is difficult to distinguish second primary carcinomas in the gastric stump from synchronous tumours overlooked at the time of first surgery; tumours in the residual stomach were excluded from the study.

The patients were examined endoscopically prior to surgery and several samples from various portions of the tumour were obtained by routine forceps biopsy. The specimens were fixed with 10% formalin and embedded in paraffin. Sections were cut at 4 μm and mounted on glass slides.

Antibodies and Reagent

As primary antibodies, a rabbit polyclonal antibody A-20 which recognizes VEGF and a mouse monoclonal antibody DO-7 which recognizes P53 protein were used (Santa Cruz Biotecnology, Inc, Santa Cruz, CA). Given the extremely short half-life of wild-type P53 protein, the DO-7 staining almost certainly represents abnormal accumulation of only the mutant-type P53 protein. F8/86, a mouse monoclonal antibody was used to detect factor VII-related antigen which localizes to vascular endothelium. Normal rabbit, normal goat serum, biotinylated rabbit antimouse immunoglobulin G (IgG), biotinylated goat antirabbit IgG, streptavidin-peroxidase reagent and diaminobenzidine were purchased from Zhong Shan Corporation (Peking, China).

Immunohistochemical Techniques

Immunohistochemical staining was performed by the streptavidin-biotin method. Briefly, sections were deparaffinized and incubated with 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Then sections were washed in phosphate-buffered saline (PBS) and incubated in 10% normal rabbit serum for 10 minutes to reduce nonspecific antibody binding. Specimens were then incubated with primary antibodies (5 μg/ml) for 90 minutes at room temperature, followed by three washes with PBS. Afterwards, sections were incubated with biotinylated rabbit antimouse IgG at a dilution of 1:100 for 30 minutes followed by 3 washes and were treated with streptavidin – peroxidase regent for 30 minutes at a dilution of 1:100, then followed by three washes with PBS. Finally, slides were incubated in PBS containing diaminobenzidine and 1% hydrogen peroxide for 5 minutes, counterstained with Mayer haematoxylin and mounted. Normal mouse IgG was substituted for primary antibody as the negative control.

Staining Analysis

VEGF staining

The degree of immunostaining for VEGF was considered postive when unequivocal staining of the membrane or cytoplasm was observed in tumour cells regardless of the number of cells stained.

P53 staining

P53 immunoreactivity was assessed positive when tumour cells showed a complete nuclear staining pattern, diffuse or granular, regardless of the number of cells stained.

Microvessel Counting

The methods of microvessel counting were as described previously (6). In brief, any single brown-stained cell or cluster of endothelial cells that was clearly separate from adjacent microvessels, tumour cells and other connective tissue element was considered a vessel. Branching structures were counted as a single vessel unless there was a discontinuity in the structure. The stained sections were screened at x 5 magnification to identify the areas of the highest vascular density within the tumour. These high neovascular areas could occur anywhere within the tumour but were most frequent at the margin of the carcinoma. Vessels were counted in the five areas of highest vascular density at x 200 magnification. Microvessel count (MVC) was assessed as the mean number of vessels in these areas.

Characterization of VEGF and P53 expression and microvessel counting were performed by two investigators who had no knowledge of other clinicopathologic features or clinical outcomes.

Statistical Methods

The relationship between P53 or VEGF expression and the MVC was evaluated by Wilcoxon rank-sum test and the correlation between P53 expression, VEGF expression and the various clinicopathologic factors was examined by Chi-square test. The accepted level of significance was $p < 0.05$.

RESULTS

No immunoreactivity of VEGF was detected in normal mucosal cell. Vascular endothelial growth factor was mainly localized to the cytoplasm or the membrane of the carcinoma cell (Fig. 1). Tumour cells that were strongly immuno-

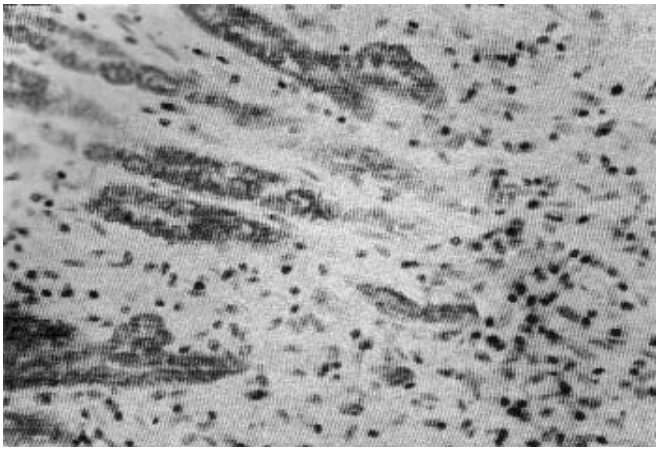

Fig 1: Immunohistochemical staining for VEGF in tumour tissue of the stomach (x 400).

positive for VEGF were observed more often at the invasive front than in the centre of the tumour. VEGF expression was detected in 52 of 95 (54.7%) tumours. p53 immunoreactivity was localized in the nuclei of tumour cells (Fig. 2) and

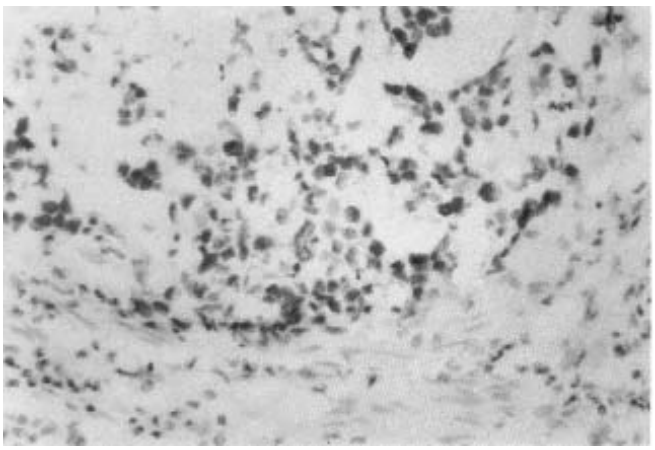

Fig. 2: Immunohistochemical staining for p53 in tumour tissue of the stomach (x 400).

positive p53 protein accumulation was detected in 40 of 95 (42.1%) tumours.

Table 1 shows the correlation between p53 or VEGF expression and various clinicopathologic factors. No significant correlation was found between p53 expression and clinicopathologic factors. The rate of VEGF positivity was significantly higher in patients with haematogenous metastasis than in those without haematogenous metastasis.

Table 1: Expression of p53 protein and VEGF and clinicopathological features

Variable	p53 expression positive cases/ total cases	VEGF expression positive cases/ total cases
Gender		
Male	26/67	36/67
Female	14/28	16/28
Age		
< 60 years	10/24	14/24
$ 60 years	30/71	38/71
Histology		
Differentiated	8/17	6/17
Undifferentiated	32/78	46/78
Lymph mode metastasis		
Negative	19/51	18/51
Positive	21/44	34/44
Hematogenous metastasis		
Negative	30/73	36/73[a]
Positive	10/22	16/22[b]
Stage		
II	20/53	21/53
III	18/39	29/39
IV	2/3	2/3

Table 2 shows the correlation between p53, VEGF expression and MVC; both p53 and VEGF expression were associated with MVC. The MVC in p53 positive tumours was significantly higher than that in p53 negative tumours. Similarly, the same trend was seen between VEGF expression and MVC.

Table 2: Correlation between p53/VEGF expression and MVC

p53/VEGF	n	MVC	*p* value
P53 expression			
Positive	40	23.74 ± 4.61	
Negative	55	17.23 ± 5.47	< 0.05
VEGF expression			
Positive	52	25.83 ± 6.32	
negative	43	16.41 ± 5.39	< 0.05

The p53 and VEGF were co-expressed in 61 of 95 tumours (64.2%) and a significant ($p < 0.01$) association between p53 and VEGF expression was demonstrated (Table 3).

Table 3: Correlation between p53 and VEGF expression

	VEGF expression		
	Positive	Negative	*p* value
P53 expression			
Positive	29	11	
Negative	23	32	< 0.01

The correlation between disease recurrence and expression of VEGF and p53 is shown in Table 4. The rate of VEGF positivity was significantly ($p < 0.01$) higher in the patients with diseases recurrence than in those without recurrence, whereas no significant correlation was found between disease recurrence and the expression of p53.

Table 4: Correlation between disease recurrence and VEGF, p53 expression

Variable	No of patients With recurrence	Without recurrence (n = 36)	*p* value (n = 59)
VEGF expression			
Positive	25	27	
Negative	11	32	< 0.01
P53 expression			
Positive	17	23	
Negative	19	36	NS

DISCUSSION

Mutations of the p53 gene are the most common genetic alteration known to occur in a wide range of human malignancies (7). In this study, the abnormal accumulation of p53 protein by immunohistochemistry in 40 of 95 (42.1%) gastric carcinomas could be detected. Some correlations between p53 abnormality and clinical features have been suggested in gastric carcinomas and results in this study were in line with former reports (8–10). We observed no correlation between p53 expression and histologic type, lymph node metastasis, haematogenous metastasis or tumour stage.

Angiogenesis is thought to be regulated by growth factors that are secreted by host and tumour cells. Among these regulators, VEGF is thought to be a major tumour angiogenesis regulator. Vascular endothelial growth factor is a selective mitogen for endothelial cells and may directly stimulate the growth of new blood vessels (11). It has been reported that VEGF expression is correlated with tumour angiogenesis and prognosis in patients with gastric carcinoma (12). But Chen *et al* (13) reported that VEGF expression level was not significantly correlated with MVC. In the current study, the MVC in VEGF positive tumours was significantly higher than in VEGF negative ones. Furthermore, the haematogenous metastasis in VEGF positive tumours was significantly higher than VEGF negative ones.

Recently, it was reported that p53 plays an important role in controlling angiogenesis by regulating VEGF expression. Kieser *et al* (14) reported that VEGF expression was induced through protein kinase C pathway stimulation in the presence of p53 tumour suppressor gene mutation. Volpert *et al* (15) reported that loss of wild-type p53 cause a four-fold induction of VEGF. Kiyoshi *et al* (16) reported that there was a significant correlation between p53 and VEGF expression in gastric carcinoma. However, Joo *et al* (17) reported that the expression of p53 did not correlate with VEGF expression and the relationship between the status of p53 expression and MVC had no statistically significant differences. In the current study, the VEGF expression signi-

ficantly elevated as the expression of p53 increased. Furthermore, both p53 and VEGF status were significantly associated with MVC. Thus, we can conclude that nuclear p53 accumulation may correlate with tumour angiogenesis through a VEGF upregulation in gastric carcinoma.

According to recurrence, it most likely arises from occult metastasis already existing at the time of surgery. Therefore, the identification of specific indicators for the metastatic potential of primary tumours would allow for better prognostic stratification of patients and thus more effective treatment. Thus, it could be considered that tumours with VEGF expression would produce an environment suitable for metastasis by recruiting VEGF-induced angiogenesis in the primary tumour (18). Fondervila *et al* (19) reported that p53 and VEGF expression were independent prognostic factors in patients with curatively resected gastric cancer. In the current study, we investigated the correlation between disease recurrence and VEGF expression together with p53 abnormality. Only VEGF expression was found to be associated significantly with disease recurrence. Disease recurrence was observed in 48.1% (25 of 52) of the patients with VEGF positive tumours and was significantly higher compared with VEGF negative tumours (25.6%, 11 of 43). Therefore, VEGF can be considered a sensitive marker for disease recurrence.

In conclusion, this study demonstrated that p53 expression may play an important role in controlling angiogenesis by regulating VEGF expression and VEGF expression is associated closely with disease recurrence. In addition, both p53 and VEGF expression might be useful in assessing the prognosis of patients with gastric carcinoma.

REFERENCES

1. Bicknell R, Harris AL. Novel growth regulatory factors and tumour angiogenesis. Eur J Cancer 1991; **27:** 781–4.
2. Dvorak HF, Siossat TM, Brown LF, Berse B, Nagy JA, Sotrel A et al. Distribution of vascular permeability factors in tumours: concentration in tumour blood vessels. J Exp Med 1991; **174:** 1275–8.
3. Du JR, Jiang Y, Zhang YM, Fu H. Vascular endothelial growth factor and microvascular density in esophageal and gastric carcinomas. World J Gastroenterol 2003; **9:** 1604–6.
4. Maeda K, Kang SM, Onoda N, Ogawa M, Sawada T, Nakata B et al. Expression of p53 and vascular endothelial growth factor associated with tumour angiogenesis and prognosis in gastric cancer. Oncology 1998; 55**:** 594–9.
5. Japanese Research Society for Gastric Cancer. The general rules for gastric cancer study. Jpn J Surg 1985; **11:** 127–39.
6. Saito H, Tujitani S, Ikeguchi M, Maeta M, Kaibara N. Neoangiogenesis and relationship to nuclear p53 accumulation and vascular endothelial growth factor expression in advanced gastric carcinoma. Oncology 1999; **57:** 164–72.
7. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992; **70:** 523–6.
8. Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F. p53 expression and prognosis in gastric carcinoma. Int J Cancer 1993; **50:** 859–62.
9. Fonseca L, Yonemura Y, De Aretxabala X, Yamaguchi A, Miwa K, Miyazaki I. p53 detection as a prognostic factor in early gastric cancer. Oncology 1994; **51:** 485–90.
10. Maeda K, Ogawa M, Chung YS. Over expression of p53 associated with tumour angiogenesis, tumour cell proliferation, and prognosis in gastric carcinoma. Oncol Rep 1997; **4:** 765–8.
11. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989; **246:** 1306–9.
12. Jiao ZY, Gou CZ, Cao N, Li YM. Correlation of tissue factor expression to angiogenesis of gastric carcinoma and its clinical significance. Ai Zheng 2005; **24:** 880–4.
13. Chen CN, Hsieh FJ, Cheng YM, Cheng WF, Su YN, Chang KJ, et al. The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. Cancer Lett 2004; **213:** 73–82.
14. Kieser A, Weich HA, Brandner G, Marmé D, Kolch W. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene 1994; **9:** 963–9.
15. Volpert OV, Dameron KM, Bouck N. Sequential development of an angiogenic phenotype by human fibroblast progressing to tumourigenicity. Oncogene 1997; **14:** 1495–502.
16. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M et al. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer 1996; **77:** 858–63.
17. Joo YE, Sohn YH, Joo SY, Lee WS, Min SW, Park CH et al. The role of vascular endothelial growth factor (VEGF) and p53 status for angiogenesis in gastric cancer. Korean J Intern Med 2002; **17:** 211–9.
18. Maeda K, Kang SM, Onoda N, Ogawa M, Kato Y, Sawada T et al. Vascular endothelial growth factor expression in preoperative biopsy specimens correlates with disease recurrence in patients with early gastric carcinoma. Cancer 1999; 86(4):566–71.
19. Fondevila C, Metges JP, Fuster J, Grau JJ, Palacin A, Castells A et al. p53 and VEGF expression are independent predictors of tumour recurrence and survival following curative resection of gastric cancer. Br J Cancer 2004; **90:** 206–15.