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• GASTRIC CANCER •

Expression of p21^{WAF1} and p53 and polymorphism of p21^{WAF1} gene in gastric carcinoma

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Abstract

AIM: To investigate the relationship between expression of p21^{WAF1} and p53 gene, and to evaluate the deletion and polymorphism of p21^{WAF1} gene in gastric carcinoma (GC).

METHODS: Expression of p21 and p53 proteins, and deletion and polymorphism of p21 gene in GC were examined by streptavidin-peroxidase conjugated method (SP) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) respectively.

RESULTS: The expression of p21 and p53 was found in 100% (20/20) and 0% (0/20) of normal gastric mucosae (NGM), 92.5% (37/40) and 15.0% (6/40) of dysplasia (DP) and 39.8% (43/108) and 56.5% (61/108) of GC, respectively. The positive rate of p21 in GC was lower than that in NGM and DP (P<0.05), while the positive rate of p53 in GC was higher than that in NGM and DP (P<0.05). p21 and p53 were significantly expressed in 63.3% (19/30) and 36.7% (11/30), 35.0% (14/40) and 77.5% (31/40), 26.7% (4/15) and 80.0% (12/15), 30.8% (4/13) and 30.8% (4/13), and 20.0% (2/10) and 30.0% (3/10) of well-differentiated, poorly-differentiated, undifferentiated carcinomas, mucoid carcinomas and signet ring cell carcinomas. The expression of p21 in well-differentiated carcinomas was significantly higher than that in poorly-differentiated, un-differentiated, mucoid carcinomas and signet ring cell carcinomas (P<0.05). Contrarily, The expression of p53 was increased from well-differentiated to poorly-differentiated and un-differentiated carcinomas (P<0.05). The expression of p21 and p53 in paired primary and metastatic GC (35.3% and 70.6%) was different from non-metastatic GC (62.5% and 42.5%) markedly (P<0.05). The expression of p21 in invasive superficial muscle (60.0%) was higher than that in invasive deep muscle or total layer (35.2%) (P<0.05) and was higher in TNM stages I (60.0%) and II (56.2%) than in stages III (27.9%) and IV (22.2%) (P<0.05), whereas the expression of p53 did not correlate to invasion depth or TNM staging (P>0.05). The exoression patterns of p53+/p21-, and of p53-/p21+ were found in 5.0% and 82.5% of DP. There was a significant correlation between expression of p21 and p53 (P<0.05). But there was no significant correlation between expression of both in GC (P>0.05). There was no deletion in exon 2 of p21 gene in 30 cases of GC and 45 cases of non-GC, but polymorphism of p21 gene at exon 2 was found in 26.7% (8/30) of GC and 8.9% (4/45) of non-GC, a significant difference was found between GC and non-GC (P<0.05). There was no significant relation between p21 expression of polymorphism (37.5%, 3/8) and non-polymorphism (45.5%, 10/22) in GC (P>0.05).

CONCLUSION: The loss of p21 protein and abnormal expression of p53 are related to carcinogenesis, differentiation and metastasis of GC. The expression of p21 is related to invasion and clinical staging in GC intimately. The expression of p21 protein depends on p53 protein largely in NGM and DP, but not in GC. No deletion of p21 gene in exon 2 can be found in GC. The polymorphism of p21 gene might be involved in gastric carcinogenesis.There is no significant association between polymorphism of p21 gene and expression of p21 protein.

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INTRODUCTION

Previous studies have shown that tumor suppressor genes play an important role in the progression of solid tumors. p21^{WAF1} is a recently identified gene. It encodes a nuclear protein of 21 ku, which inhibits cyclin-dependent kinase activity[1-3]. p21 protein has been reported to work as critical downstream effectors of p53 and a potential inhibitor of cyclin-dependent kinases. Thus, p21^{WAF1}gene is thought to play a central role in tumor suppression[4-9]. Alterations of p21 expression have been observed in a wide variety of human carcinomas by immunohistochemistry[10-15]. GC is common in China[16-27]. But the relationship between p21and p53 proteins expression and prognosis of GC is unclear.

In this study, expression of p21 and p53 proteins was detected by streptavidin- peroxidase conjugated method (SP). PCR and PCR-RFLP methods were used to analyze the deletion and polymorphism of p21^{WAF1} gene in GC. The correlation between the expression of p21^{WAF1} gene and carcinogenesis, differentiation, invasion and metastases, and the deletion and gene polymorphism of exon 2 in p21^{WAF1} gene in GC were investigated.

MATERIALS AND METHODS

Materials

One hundred and eight cases of GC and 20 cases of normal gastric mucosa (NGM) and 40 cases of dysplasia (DP) with gastric ulcer at the First Affiliated Hospital of Nanhua University, between April 1990 and December 1997 were used for this study, including 30 cases of well-differentiated, 40 cases of poorly-differentiated, 15 cases of undifferentiated GC, 13 cases of signet ring cell carcinoma and 10 cases of mucoid carcinoma. Eigty-one cases were men and 27 cases women, with a mean age of 58 years at diagnosis. Sixty-eight cases had lymph node metastasis and 40 cases had no lymph node metastasis.

Fifteen cases were stage I, 32 cases stage II, 43 cases stage III and 18 cases stage IV according to TNM staging. None of them had received any therapy before surgery. Freshly resected specimens including carcinoma, pericarcinoma tissues and normal mucosas located far from carcinoma, were cut into 2-4 blocks under sterile conditions. Each block was 1.5 mm×1.5 mm×1.0 mm and stored at -80 ℃ for PCR and PCR-RFLP analysis. The rest tissues were fixed in 100 mL/L neutral formalin, dehydrated, cleaned and paraffin-embedded. All paraffin embedded tissues were cut into 5 μm thick sequential slices and mounted onto glass slides previously processed by polylys.

Methods

Reagents and instruments Moues anti-human $p21^{WAF1}$ and p53 monoclonal antibody, SP kit and DAB were all purchased from Maxim Company, USA. Protease K (Merk, USA), ALW261 agar gel, propylene acrylamide, N-N-sulmethyl bipropylene acrylamide, ammonium persulfate, xylene nitrile, and bromophenol blue were purchased from Shanghai Sangon Company. PCR primers were synthesized by Shanghai Sangon. Primer sequences of $p21^{WAF1}$ gene at exon 2, Sense: 5' -CGGGATCCGGCGCCATGTCAGAACCGGC-3' , antisense:5' -CCAGACAGGTCAGCCCTTGG-3' . The amplification fragment length was 536 bp, and 309 bp. Primer sequences of β-actin served as an internal control, Sense: 5' -TCCGTGGAGAAGAGC TACGA-3' , antisense:5' -GTACTTGCGCTCAG- AAGGAG-3' . The amplification fragment length was 309 bp.

SP immunohistochemical staining All immunohistochemical analyses were performed on routinely processed, formalin- fixed, paraffin-embedded tissues using SP. Briefly, sections were dewaxed, rehydrated endogenous peroxidase was blocked, first antibody was added, then antibody bridge and enzyme labeled SP were added, colorized by DAB, stained by hematoxylin, dehydrated, cleaned and paraffin-embedded, observed under a microscope. According to Seta' standard with a slight modification[15], nuclei were considered positive when they showed a distinct brown color in the absence of background staining. A breast carcinoma with known positive immunostaining for p21 served as a positive control. Positive and negative control slides were included within each batch of slides. (+) indicates the cells were stained weakly or the number of stained cells was less than 25%, (++) indicates the cells were stained moderately or the stained cells were covered about 26-50%, (+++) indicates the cells were stained strongly or the number of stained cells was more than 50%.

Genomic DNA extraction High molecular weight DNA was isolated from tumors by standard protease K digestion and phenol-chloroform extraction. Frozen tissue of 0.5 g was put into liquid nitrogen and powdered immediately, 10×buffer (10 mmol/L Tris-HCl pH 8.0, 0.1 mol/L EDTA pH 8.0, 5 g/L SDS) was added and spanned in 37 ℃ water for 1 h at the same time. Protase K was added to the mixture at a final concentration of 100 mg/L in 50 ℃ water for 3 h and protease K was readjusted as possible reaction. After the mixture was lysed completely, 20 mg/L RNase reacted in 37 ℃ water for 1 h.Then saturated phony was put together and bugged slightly for 10 min, centrifuged and extracted. The supernant was transferred to a cleaned plastic tube, saturated phony was processed 3 times. 1/10 volume 3 mol/L NaAc and 2-2.5 times cold ethyl were added, DNA was precipitated by centrifugation. Ethyl was removed, DNA was washed by 700 mL/L ethyl, and centrifuged 3 times, dried, resolved with TE, A_{260}/A_{280}:1.8-1.9, and stored at 0 ℃ for use.

PCR amplification PCR was performed in 100 μL reactive volume containing 0.5 μg DNA template, 200 μmol/L each of dCTP, DATP, dGTP, dTTP, 0.25 μmol/L primer, PCR buffer (Tris- HCl 10 mmol/L, pH 8.3, $MgCl_2$ 1.5 μmol/L, KCl 50 mmol/L, gelatin 100 mg/L) pre-denatured at 95 ℃ for 5 min and 1.5 μL of Taq DNA polymerase and 75 μL of mineral oil were added. These samples were subjected to 30 cycles: at 95 ℃ for 1 min, at 68 ℃ for 1 min, at 72 ℃ for 1 min, and a final extention at 72 ℃ for 5 min. Five μL of PCR product and appropriate bromophenol blue were added to the sample point container and electrophoresed on 20 g/L agarose gel containing 0.5 mg/L ethidium bromide at a tank with 0.5×TBE liquid of electrophoresis, then they were observed and photographed with ultraviolet radiography.

PCR- RFLP A 50 μL PCR product was purified by ethanol, dissolved in 17 μL sterilized water, mixed with 2 μL 10×buffer and Alw261 2u, digested at 37 ℃ for 3 h. A 10 μL of the digested product and 10 μL loading buffer were added to the gel containing 80 g/L polypropylene acrylamide. Then it was vertically electrophoresed at 100 V for 4 h and gel was stained with silver in the following order: fixed in 100 mL/L alcohol for 10 min, oxidized in 100 g/L nitric acid for 3 min, washed in drip for 1 min with double distilled water, stained in 12 mmol/L silver nitric acid for 20 min, washed in drip for 1 min with double distilled water, colored in 0.028 mol/L anhydrous sodium carbonate and 0.19 g/L formaldehyde, ended by adding 100 mL/L glacial acetic acid, washed in drip with double distilled water and photographed.

Statistical analysis Statistical analysis was performed using Chiq-square test and analysis of variance. A P value less than 0.05 was considered statistically significant.

RESULTS

Expression of 21^{WAF1} and p53 protein in gastric carcinoma

The positive expression rate of p21 and p53 protein was 100% (20/20) and 0% (0/20) in NGM, 92.5% (37/40) and 15.0% (6/40) in DP and 39.8% (43/108) and 56.5% (61/108) in GC, respectively (Figure1) (Table 1). p21 expression could only be seen in mucosal epithelial and adenoepithelial cells in NGM, no staining in matrix fibrocytes, lymphocytes and smooth myocytes was observed. The positive rate of p21 in GC was lower than that in NGM and DP (P<0.05), and positive expression of p53 in GC was higher than that in NGM and DP (P<0.05). But there was no significant difference between NGM and DP (P>0.05). Among the 108 cases of GC, the positive rate of p21 and p53 proteins was 63.3% (19/30) and 36.7% (11/30), 35.0% (14/40) and 77.5% (31/40), 26.7% (4/15) and 80.0% (12/15) , 30.8% (4/13) and 30.8% (4/13), and 20.0% (2/10) and 30.0% (30.0/100) in well-differentiated, poorly-differentiated, undifferentiated GC, mucoid carcinomas and signet ring cell carcinomas. The positive rate of p21 in well-differentiated GC was significantly higher than that in poorly- differentiated, undifferentiated, mucoid carcinomas and signet ring cell carcinomas (P<0.05). Contrarily, the expression of p53 was increased from well-differentiated to poorly-differentiated and undifferentiated GC (P<0.05). The positive rate of p21 and p53 in paired primary and lymph node metastatic GC (35.3% and 70.6%) was significantly different from that in non-metastasis carcinomas (62.5% and 42.5%) (P<0.05). The expression of p21 in invaded superficial muscle (60.0%) was higher than that in invaded deep muscle or total layer (35.2%) (P<0.05), and was higher in TNM stages I (60.0%) and II (56.2%) than that in stages III (27.9%) and IV (22.2%) (P<0.05). Whereas the expression of p53 did not correlate wth invasion depth and TNM stages (P>0.05). There was no association between age and sex and expression of p21 and p53 (P>0.05) (Table 2).

Relationship between expression of $p21^{WAF1}$ and p53

Five precent cases had p53 positivity and p21 negativity simultaneously, whereas 82.5% cases had p53 negativity and p21 positivity simultaneously in 40 cases of DP. There was a significant correlation between expression of p21 and p53 proteins (P<0.05). Meanwhile, 32.4% of p21 negativity and p53 positivity and 15.7% of p21 positivity and loss of p53 protein were detected in GC, but there was no significant correlation between expression of p21 and p53 proteins in GC (P>0.05) (Table 3).

Table 1 Expression of p21 and p53 proteins in normal gastric mucosa, dysplasia and gastric carcinoma

Tissue type	Cases number	p21					p53				
		Negativity (%)	Positivity (%)				Negativity (%)	Positivity (%)			
			+	++	+++	total		+	++	+++	total
NGM	20	0 (0)	6	7	7	20 (100)	20 (100)	0	0	0	0 (0)
DP	40	3 (7.5)	8	11	18	37 (92.5)	34 (85.0)	1	3	2	6 (15.0)
GC	108	65 (60.2)	9	14	20	43 (39.8)	47 (43.5)	17	19	25	61 (56.5)

NGM: normal gastric mucosa; DP: dysplasia; GC: gastric carcinoma.

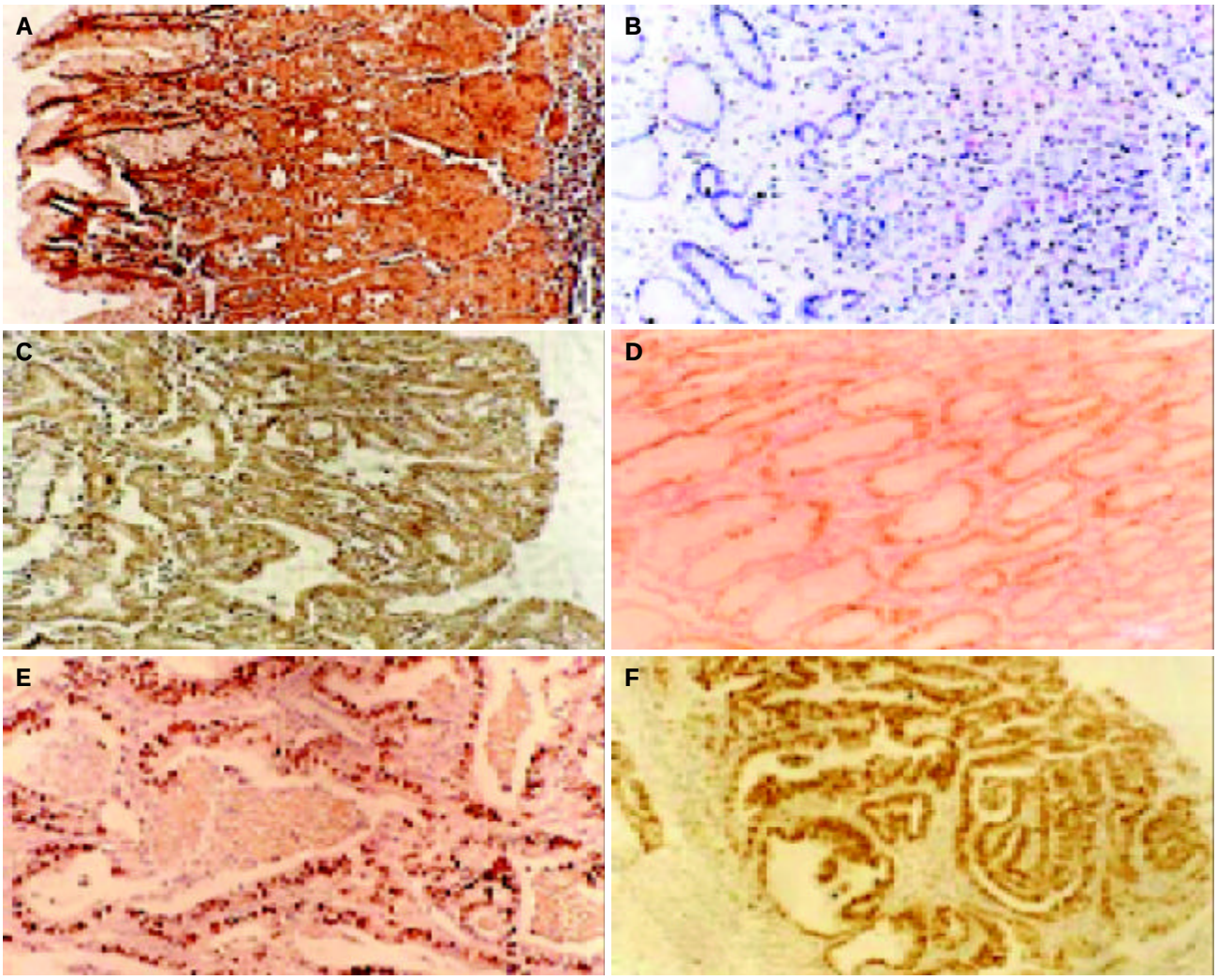

Figure 1 The results of immunohistochemical staining. A: p21 (left) and p53 (right) in NGM.×100, B: p21 (left) and p53 (right) in DP. ×100, C: p21 (left×200) and p53 (right×100) in GC.

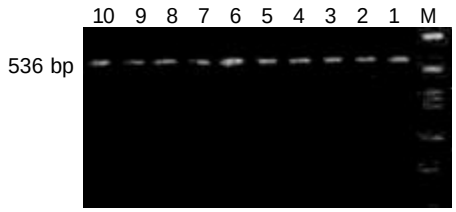

Figure 2 PCR amplified products at exon 2 of p21^{WAF1} gene. Lines 2, 4, 6, 8, 10: GC. Lines 1, 3, 5, 7, 9: tumor adjacent tissue. M: PCR marker.

Deletion and polymorphism of p21^{WAF1} gene at exon 2 in gastric carcinoma

PCR amplification showed target products in all GC and NGM. All experiments were performed three times. The results were identical (Figure 2). The 536-bp PCR amplified fragment at exon 2 of p21 gene was digested with the ALW261 restriction enzyme. Digestion of the wild type allele produced DNA fragments with a length of 124, 174, and 238 bp. The C→A polymorphism created an extra enzyme recognition site, resulting in ALW261 digested fragments of 76, 98, 124, and 238 bp in length[28] (Figure 3). There was no deletion at exon 2 of p21 gene in 30 cases of GC and 45 cases of non-GC, but polymorphism of p21gene at exon 2 was found in 26.7% (8/30) of GC and 8.9% (4/45) of non-GC. A significant difference was found between GC and non-GC (P<0.05). There was no significant relation between p21 expression of polymorphism (37.5%, 3/8) and non- polymorphism (45.5%, 10/22) in GC (P>0.05).

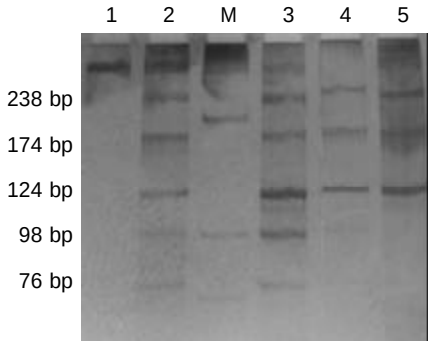

Figure 3 RFLP analysis of exon 2 of $p21^{WAF1}$ gene and ALW261 digestion analysis of codon 31. Digestion of a 536 bp polymerase chain reaction fragment containing wild type exon 2 yielded fragments of 124, 174, and 238 bp in length (lanes 2, 3). The C→A polymerase polymorphism created an extra recognition site resulting in DNA fragments of 76, 98, 124, 174, and 238 bp in the heterozygote 9 (Lanes 4, 5). M: PCR marker. Lane 1: undigested fragment.

Table 2 Association of p21 and p53 expression with clinicopathologic parameters in gastric carcinoma

Parameters	Cases (*n*)	p21 positivity (%)	p53 positivity (%)
Age			
≤50	43	18 (41.9)	25 (58.1)
51-59	37	15 (40.5)	20 (54.1)
≥60	28	10 (35.7)	16 (57.1)
Sex			
Male	81	32 (39.5)	43 (53.1)
Female	27	11 (40.7)	18 (66.7)
Grade			
Well-differentiated	30	19 (63.3)	11 (36.7)
Poorly-differentiated	40	14 (35.0)	31 (77.5)
Undifferentiated	15	4 (26.7)	12 (80.0)
Mucoid	13	4 (30.8)	4 (30.8)
Signet ring cell	10	2 (20.0)	3 (30.0)
Invasion depth			
Superficial muscle	20	12 (60.0)	11 (55.0)
Total layer	88	31 (35.2)	50 (56.8)
Lymph node metastasis			
Absence	40	25 (62.5)	17 (42.5)
Presence	68	24 (35.3)	48 (70.6)
TNM stage			
I	15	9 (60.0)	7 (46.7)
II	32	18 (56.2)	17 (53.1)
III	43	12 (27.9)	26 (60.5)
IV	18	4 (22.2)	11 (61.1)

Table 3 Relationship between expression of p21 and p53 in gastric carcinoma and dysplasia

p21	p53		
	Positive (%)	Negative (%)	Total (%)
Dysplasia			
Positive	4 (10.0)	33 (82.5)	37 (92.5)
Negative	2 (5.0)	1 (2.5)	3 (7.5)
Total	6 (15.0)	34 (85.0)	40 (100)
Gastric carcinoma			
Positive	26 (24.1)	17 (15.7)	43 (39.8)
Negative	35 (32.4)	30 (27.8)	65 (60.2)
Total	61 (56.5)	47 (43.5)	108 (100)

DISCUSSION

$p21^{WAF1}$ gene is localized at chromosome 6p21.2. It could play an important role in the regulation of cell cycle transitions in normal cells, inhibit several cyclin/ CDK complexes and block cell cycle progression[1-3]. Moreover, expression of p21 protein could be induced by p53. p21 protein is an important tumor suppressor and has been proposed as a tumor suppressor gene and a potential mediator of p53-associated tumorigenesis[4-9]. However, unlike p53-deficient mice, p21-deficient mice could undergo normal development with no exhibition early tumorigenesis. On the other hand, it has been shown that p21 expression could be mediated through p53- independent pathways. Even in the presence of p53 alterations, p21 expression could be maintained. p21 might represent an independent marker of tumor behaviors[29].

It was reported that p21 expression was limited to foveola epithelial cells. p53 expression was confined to only a few regenerative epithelial cells of the mucous neck region in NGM. Increased p21 expression was limited to surface DP epithelium and weak p53 expression was in full thickness of DP epithelium[30]. It was found p53 was stained negatively, while p21 was stained positively in each NGM[34]. In regard to expression of p21 and p53 in GC, Seo *et al*.[4], Okuyama *et al*.[7], Baldus *et al*.[33], Oya *et al*.[36], Kaye *et al*.[37] and Xiangming *et al*.[64] separately demonstrated various expression levels of 63.7% and 33.3%, 42.6% and 37.9%, 58% and 45%, 75% and 40%, 75% and 40%, and 90.2% and 28.9%. However, Zolota *et al*.[31], Liu *et al*.[32], Ogawa *et al*.[35] and Gomyo *et al*.[43] observed that the positive rates of p21 and p53 were 32% and 51%, 40.5% and 42.1%, 37.2% and 50%, 32% and 65%, respectively. Our study showed that expression of p21 and p53 was found in 100% (20/20) and 0% (0/20) of NGM, 92.5% (37/40) and 15.0% (6/40) of DP and 39.8% (43/108) and 56.5% (61/108) of GC, respectively. p21 expression could only be seen in mucosal epithelial and adenoepithelial cells in NGM. Expression of p21 and p53 in GC was remarkably different from that in DP and NGM ($P<0.05$). The results indicated that gastric carcinogenesis was probably related to the loss of p21 expression and mutation of p53. This finding is consistent with other reports[7,31,34,35,38,45]. It was found that the expression rate of p53 was 0% in LGAs, 9% in HGAs, 39% in IMCs and 43% in SMCs, and expression of p21 was present in 100% of LGAs, 74% of HGAs, 46% of IMCs and 4% of SMCs. Whereas p53+/p21- lesions were observed in 0% of LGAs, 4% of HGAs, 11% of IMCs and 26% of SMCs[36].

Clinicopathologic and prognostic significances of p21 and p53 expression have been reported in various carcinomas[10-15,48-52]. In GC, expression of p21 correlated with advanced stage, lymph node metastasis and survival[4,6,32-34]. Univariate and multivariate survival analyses revealed that clinicopathological stage and expression status of p21 were independent prognostic factors[34]. But it was also reported that expression of p21 was not associated with clinicopathological features[64]. Expression of p53 correlated with depth of tumor invasion[4], lymph node metastasis[33] and poor prognosis[32]. However, no significant correlation could be observed between the status of p53 expression and survival[4]. Multivariate survival analysis revealed that TNM stage and lymph node state were independent prognostic factors[32]. In multivariate survival analyses, neither p21 nor p53 emerged as an independent prognostic factor[33]. There was no apparent correlation between the expression of p21 and p53 and tumor stage, depth of invasion or lymphnode metastases[31]. There were significant differences in the incidence of p53 expression, the loss of p21 expression, and the 5-year survival rate between Pen-A type and Super type in early GC[8]. Stratification of the carcinomas according to histological grade and growth pattern did not result in significant differences in p53 and p21 expression[38].

The present study found that the positive rate of p21 expression was 63.3% in well-differentiated carcinomas, which was significantly higher than that in poorly-differentiated (35.0%), undifferentiated GC (26.7%), mucoid carcinomas (30.8%) and signet ring cell carcinomas (20.0%) (P<0.05). Contrarily, expression of p53 was increased from well-differentiated (36.7%) to poorly-differentiated (77.5%) and undifferentiated GC (80.0%) (P<0.05). The positive rate of p21 and p53 in paired primary and metastatic GC (35.3% and 70.6%) was markedly different from that in non-metastasis GC (62.5% and 42.5%) (P<0.05). The expression of p21 in invaded superficial muscle (60.0%) was higher than that in invaded deep muscle or total layer (35.2%) (P<0.05). The expression of p21 in TNM stages I (60.0%) and II (56.2%) was higher than that in stages III (27.9%) and IV (22.2%) (P<0.05). Whereas the expression of p53 did not correlate with invasion depth and TNM stage (P>0.05). Our results indicated that loss of p21 expression and mutation of p53 were related to carcinogenesis, differentiation and metastasis, and loss of p21 expression was related intimately to invasion depth and TNM stage. It was suggested that combined examination of p21 and p53 expression was a reliable prognostic marker for GC.

Many investigations showed that the mutation of p53 gene was common in various human carcinomas. Mutated p53 can act as a dominant oncogene. The immunoreactivity of p53 protein is a general indicator of tumors with altered p53 function. p21 performs a part of p53 function through the induction of p21 by wild type p53. However, p21 could be transcriptionally induced by wild type but not mutated p53[14,54]. It was found that p21 gene expression level was high in wild type p53 human breast cancer cells, but low in mutated p53 human breast cancer cells, indicating p21 gene expression at mRNA and protein levels are associated with p53 phenotype[55]. However, the expression of p21 protein was suppressed in neoplastic tissues with and without p53 gene alterations[56]. Western blot analysis revealed increased expression of p21 protein after infection with AxCA-p53 in all the cell lines. It was suggested that the apoptotic pathway dominated the growth arrest pathway after infection with AxCA-p53[40]. The level of p21 mRNA was very low or undetectable in all cell lines containing mutated p53 gene. But cell lines with wild-type p53 gene could express, it and the level of p21 mRNA was high in 8 GC cell lines[45]. Qian *et al*. used Northern and Western blot to analyze the expression of p21 and found that p21 mRNA was expressed in 2 cases of 15 p53+ and all of the p53- cases, indicating inactivation of p53 was closely related with the unexpression of p21 mRNA in GC[47]. However, some investigators believed p21 protein expression was unrelated with p53 protein expression[4,35,37,39]. Accumulation of mutated p53 protein might suppress the expression of p21 protein in GC, and cancer cells with overexpression of p53 might have a high proliferative activity[5]. Combined analysis of p21 and p53 showed that p21+/p53- GC displayed less aggressive characteristics, better survival and no recurrence after curative resection than other groups of GC[4,7,35,41,64]. Evaluation of expressions of p53 and p21 might aid in predicting clinical prognosis for surgical treatment in GC[7,32,35,38,41]. The p21 expression of non-neoplastic mucosae was most likely related to cell senescence and/or terminal differentiation. Moreover, p53-independent induction of p21 expression apparently occurred in a considerable proportion of early GC. It was suggested that defects downstream of p21 might cause this apparent discrepancy[39]. In our studies, 5.0% cases were p53+/p21-, 82.5% cases were p53-/p21+ in totally 40 cases of DP. There was a significant correlation between expression of p21 and p53 (P<0.05). Meanwhile, 15.7% of p53-/p21+, 32.4% of p53+/p21-, 24.1% of p53+/p21+ and 27.8% of p53-/p21- were detected in GC, respectively. There was no significant correlation between p21 and P53 protein expression. These findings suggested that p21 protein was expressed mostly in a p53-independent manner and in some other factors, such as platelet-derived growth factor (PDGF)[57].

Alterations in the integrity of human p21 gene were rare events in human tumors[58,59,61]. Mutation of p21 gene has not been detected[6]. The promoter of p21 gene was not methylated in GC[63]. However, recent reports have demonstrated an association of p21 gene polymorphisms with many carcinomas[28,60,61]. No abnormal bands of p21gene were found in all of samples or cell lines, but three major variants at exons 2 and 3 of the gene were found to be consistent with the existence of two different DNA polymorphisms. Sequence analysis of the amplified products producing these three variants at each exon from normal DNAs confirmed the presence of polymorphisms in p21 gene. Mutation within the coding portion of p21 gene was undetectable in a large series of human tumors, many of which had a normal p53 gene. This suggested that p21 alterations were generally caused indirectly by p53 mutation rather than by intragenic mutation of p21 itself[58]. Two polymorphisms have previously been characterized in p21 gene: a C→A transversion at codon 31 (ser→arg) and a C→T transition at 20 nucleotides downstream from the 3' end of exon 3[28]. The two polymorphisms were found in 18 of 96 tumor samples lacking p53 alterations (18.8%). Nine of 54 prostate adenocarcinoma samples (16.7%) contained both p21 variants, whereas 9 of 42 squamous cell carcinomas of the head and neck (21.4%) displayed both polymorphisms. Of the 110 controls examined, 10 (9.1%) had both alterations. Both p21 polymorphisms occurred in all samples examined and there was no indication of mutation in the coding region of p21 gene[28]. We analysed the deletion of p21gene at exon 2 with PCR and PCR-RFLP methods. No deletion of p21 gene was found in GC. The result suggested deletion at exon 2 of p21 gene was not the cause of loss expression of p21 gene. The induction of p21 was mediated by posttranscriptional mechanisms[62]. The polymorphism of p21gene at exon 2 was found in 26.7% (8/30) of GC and 8.9% (4/45) of non-GC. A significant difference was found between GC and non-GC (P<0.05). The results in this study indicated that the polymorphism of p21 gene might be involved in gastric carcinogenesis. But There was no significant relation between p21 expression of polymorphism (37.5%, 3/8) and non-polymorphism (45.5%, 10/22) in GC (P>0.05). Its mechanism is still unclear. It was found that p21 gene was activated only by histone deacetylase inhibitors, suggesting that formation of the inactive chromatins through histone deacetylation seems to be a general mechanism for inactivation of p21 gene in GC cells[63].

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