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ORIGINAL PAPER

The prognostic significance of p21 and Her-2 gene expression and mutation/polymorphism in patients with gastric adenocarcinoma

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Abstract Analyses of gene expression status and genetic polymorphisms are methods to identify novel histopathological prognostic factors. In patients with gastric cancer, some cell cycle regulators p53, p21, p27 and Her-2 oncogene have been proposed as prognostic factors. We aimed to investigate the expression and mutation/polymorphism of p21 and Her-2 and also relationship between that genes status and histopathological factors and prognosis in patients with gastric cancer. Forty-four patients with locally advanced gastric cancer were analyzed in this study from January 2000 to December 2008. Clinicopathological parameters, expression and mutation/polymorphism of p21 and Her-2 results were used to predict disease-free survival and overall survival. The positive expression of p21 and Her-2 was observed in 61.4 % ($n = 27$) and 9.1 % ($n = 4$) of all 44 tumors, respectively. p21 gene mutation and Her-2 gene polymorphism were detected in 20 % ($n = 11$) and 2.3 % ($n = 1$, II phenotype) of cases, respectively. The negative expression of p21 was correlated significantly with diffuse and undifferential type histologies, whole gastric involvement and positive vascular/neural invasion. The median survival rate of patients with negative expression was significantly poorer than that of patients with positive expression of p21 (17 vs. 27 months, $p = 0.01$, cox regression). p21 mutation was significantly higher in patients with diffuse ($p = 0.03$) and undifferential ($p = 0.02$) type histologies. There was no statistically significant association between histopathological parameters and Her-2 gene polymorphism/expression. The negative expression of p21 correlates with disease survival and may be a poor prognostic factor in patients with resected gastric cancer treated with adjuvant chemotherapy.

Keywords p21 expression · p21 gene mutation · Her-2 expression · Her-2 gene polymorphism · Gastric adenocarcinoma

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Introduction

Current guidelines recommended that patients with clinical stage II/III gastric adenocarcinoma should be treated with subtotal or total gastrectomy followed by adjuvant chemoradiotherapy. After R0 resection, patients with T1-2, N0 tumors may be observed and fluoropyrimidine-based adjuvant chemoradiotherapy is recommended in some of the selected patients with T2, N0 tumors with high-risk features such as poorly differentiated, lymphovascular and neural invasion [1].

The stage is the most important prognostic factor in gastric cancer. However, gastric cancer is not a uniform disease, and therefore, prognosis varies even among patients in the same stage. For this reason, in order to better identify the biological subset, more classification parameters need to be defined in addition to TNM and classic histopathological characteristics. The expressions of epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) and cErbB-2 (Her-2/Neu) have been found associated with poor prognosis in patients with gastric cancer [2, 3]. On the contrary, the absence of p21 expression has been associated with poor prognosis in patients with gastric cancers [4–6].

We aimed to investigate the expression and mutation/polymorphism of p21 and Her-2 proteins and also relationship between that genes status and histopathological factors and prognosis in patients with pathologically staged T3-4, N0-3(+) gastric adenocarcinoma after subtotal or total gastrectomy, with the goal of identifying factors related to predicting outcome.

Materials and methods

Patient selection

For this retrospective analysis, subjects were selected from 382 patients with gastric cancer treated at the Trakya University Medical Faculty Hospital in Edirne, Turkey, from January 2000 to December 2008. For the present analysis, the patient selection criteria were as the following: (1) without any preoperative therapy; (2) undergone total or subtotal gastrectomy; (3) pathologically confirmed T3-4, N0-3(+) gastric adenocarcinoma; (4) no evidence of distant metastases. After the medical and pathologic records were carefully reviewed, 44 patients with locally advanced gastric adenocarcinoma were selected.

Immunohistochemical staining for biomarkers

All information on biomarker staining was extracted from the pathologic records. All dissected tumor specimens were routinely fixed in buffered formalin and embedded in paraffin. Sections of 5-mm thickness were stained with hematoxylin and eosin for histologic diagnosis and then labeled with two primary antibodies: anti-P21 (Clone NCC-RAS-001, code M0367, DAKO; dilution 1:100) and anti-Her 2 (Clone: PN2A, code M7269, DAKO; dilution 1:400). For antigen retrieval, sections were treated with 10 mM citrate at pH 6.0 in a 750-W microwave oven for three 5-min cycles. Then, the sections were immunostained with horseradish peroxidase (HRP) polymer (DAKO) in accordance with manufacturer's specifications. Diaminobenzidine was used to develop the stains and hematoxylin for the counterstaining. Negative controls consisted of substituting normal mouse serum for the primary antibodies. Expression of two markers' was quantified by using a visual grading system based on staining intensity on a scale from 0 to 3.

DNA extraction and polymerase chain reaction (PCR–RFLP and PCR-SSCP) analysis

Genomic DNA was isolated from paraffin-embedded tissues using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) in compliance with the manufacturer's protocol. All PCR amplifications were performed in a total volume of 50 μL containing 100 ng of extracted DNA, 20 pmol/L of each forward and reverse primer, and 25 μL of HotStarTaq Master Mix [containing 2.5 units of HotStarTaq DNA polymerase, 1× PCR buffer with 1.5 mmol/L MgCl2, and 200 μmol/L of each dNTP (Qiagen, Hilden-Germany)].

SNP in Her-2 transmembrane segment involving codon 655 was analyzed by PCR–RFLP. The 148-bp transmembrane segment of Her-2 was PCR-amplified using the gene-specific primers, that is, forward (Her-2/F) 5′-AGAGCGC-CAG CCCTCTGACGTCCAT-3′, and reverse (Her-2/R) 5′-TC CGTTTCCTGCAGCAGTCTCCGCA-3′. Amplification was performed with initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C, annealing at 62 °C, and extension at 72 °C for 1 min each and a final extension at 72 °C for 10 min. The PCR product was digested with 5 units of BsmAI restriction enzyme (New England Biolabs, Ipswich, MA-USA) at 55 °C for 2 h and run on 3 % agarose gel, stained with Et-Br and visualized under UV light. BsmAI digestion gave fragments of 116 and 32 bp for the Val (GTC) allele and a single 148-bp fragment for the Ile (ATC) allele.

SSCP analysis was performed by PCR amplification of exon 3 of the p21 gene. Exon 3 was amplified with oligonucleotides 5′-GCCCCCCACTGTCTTCCT and 5′-GC GCTTCCAGGACTGCAGG. Amplification was consisted in 10 min, denaturation step at 95 °C followed by 37 cycles of 30 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C. Totally, 20 μl solution [containing 10 μl of each PCR product was diluted in 10 μl of denaturing loading buffer (98 % formamide; 0.05 % bromphenol blue; and 0.05 % xylene cyanol FF)] was electrophoresed on 8 % non-denaturing polyacrylamide gels. Gels were run in 1× Tris–borate-EDTA (TBE), respectively, at 200 V for 7–15 h. After electrophoresis, silver staining was performed by a staining kit (Promega, USA).

All SSCP analyses were repeated twice each under electrophoresis conditions. Negative assay controls consisted of water and extraction buffer blanks for PCR.

Definition of treatment failure

All treatment failures were verified through review of the medical records. Local-regional failure was defined as recurrence within pelvis, including the tumor bed, regional lymph nodes and anastomosis. Distant failure was indicated as disease recurrence detected in the liver, lung, brain and other organs.

Statistical analyses

The data used for this analysis included patient age and sex, cellular differentiation, the presence or absence of lymphatic, vascular or perineural invasion, the number of dissected lymph nodes, and whether adjuvant therapy had been administered. Two potential predictive molecular markers (p21 and Her-2), detected by immunohistochemical staining polymerase chain reaction of surgical specimens, were also evaluated as potential prognostic factors. Characteristics were described in terms of frequency for the categorical variables, means and standard deviations for continuous data, and medians for non-normally distributed continuous data. Survival time was calculated from the date of surgery to the date of event or the last follow-up.

Events were defined: local-regional failure, distant metastasis and death. Survival curves were estimated by using the Kaplan–Meier method and compared with log-rank test in the univariate analysis. Cox proportional hazards regression was used for multivariate modeling and for examining the prognostic significance of the variables identified in the models. p values of less than 0.05 were taken to indicate statistically significant differences.

Results

Patient characteristics

Patient characteristics are presented in Table 1. Thirty-four patients were men and 10 patients were women; the median age was 63 years (range, 26–75). Nineteen patients (43.2 %) were diffuse histology and sixteen patients (36.4 %) were intestinal histology. Thirty-six patients (81.6 %) were T3, and in thirty patients (68.2 %), lymph node involvement was positive. Surgical margin was positive in six patients (13.6 %).

Thirty-four patients (77.3 %) were treated with chemo-radiotherapy, and 10 patients (14.7 %) were treated with only chemotherapy or radiotherapy. Main chemotherapy regimen was 5-FU plus folinic acid.

Table 1 Patient characteristics

Clinicopathological factors	*n* (%)
Gender	
Male	34 (77.3)
Female	10 (22.7)
Age (year)	
Mean ± SD	60 ± 12
Surgery	
Subtotal gastrectomy	21 (47.7)
Total gastrectomy	23 (52.3)
Histopathology	
Diffuse	19 (43.2)
Intestinal	16 (36.4)
Differentiation	
Differentiated	26 (59.1)
Undifferentiated	14 (31.8)
T	
T3	38 (86.3)
T4	6 (13.7)
Lymph node involvement	
Yes	30 (68.2)
No	14 (31.8)
Surgical resection type	
R0	38 (86.43)
R1	6 (13.7)
Vascular invasion	
Yes	27 (61.4)
No	16 (36.4)
Neural invasion	
Yes	30 (68.2)
No	14 (31.8)

The results of immunohistochemical staining and polymerase chain reaction

Her-2 expression was negative in forty patients (90.9 %), and p21 expression was negative in seventeen patients (38.6 %). II type Her-2 gene polymorphism was positive in only one patient (2.3 %). p21 gene mutation was positive in 9 (20.5 %) patients (Table 2).

Correlation of molecular markers and clinicopathological factors

For all clinicopathological factors, there was statistically significant correlation between negative p21 expression and diffuse-type histology, whole gastric invasion, vascular/neural invasion and undifferentiated histology. There was significant correlation between p21 gene mutation and diffuse type, and undifferentiated histology (Table 3). We

Table 2 Immunohistochemical staining and polymerase chain reaction results

		n (%)
Immunohistochemical staining (*IHC*)		
Her-2 expression	(−)	40 (90.9)
	(+/++/+++)	4 (9.1)
p21 expression	(−)	17 (38.6)
	(+ /++ /+++)	27 (61.4)
Polymerase chain reaction (*PCR*)		
Her-2 gene polymorphism	II	36 (81.8)
	IV	7 (15.9)
	VV	1 (2.3)
p21 gene mutation	Positive	9 (20.5)
	Negative	35 (79.5)

Table 3 Correlation between p21 expression/mutation and clinicopathological factors

	n	p21 expression			*n*	p21 gene mutation		
		(−)	(+/++/+++)	*p*		(−)	(+)	*p*
Gender								
Male	34	20	14	0.401	34	27	7	0.672
Female	10	7	3		10	8	2	
Histopathology								
Diffuse	19	12	7	**0.008**	19	12	7	**0.032**
Intestinal	16	2	14		16	15	1	
T								
T3	38	24	14	0.426	38	30	8	0.644
T4	6	3	3		6	5	1	
Lymph node involvement								
Yes	30	20	10	0.457	30	24	6	0.523
No	3	1	3		3	2	1	
Localization								
Cardia	5	2	3	**0.031**	5	4	1	0.997
Corpus	15	11	4		15	12	3	
Antrum	20	14	6		20	16	4	
Whole gastric	4	4	0		4	3	1	
Differentiation								
Differentiated	26	19	7	**0.024**	26	23	3	**0.024**
Undifferentiated	14	7	7		14	8	6	
Vascular invasion								
Yes	16	13	3	**0.032**	27	19	8	0.071
No	27	13	14		16	15	1	
Neural invasion								
Yes	14	13	1	**0.003**	30	22	8	0.135
No	30	14	16		14	13	1	

could not find any correlation between any clinicopathological factors and Her-2 polymorphism/expression.

Molecular markers and survival

The median follow-up interval was 23.23 months (range, 3.83–83.20 months). Univariate analysis showed that total gastrectomy, whole gastric invasion, positive vascular and neural invasion, negative p21 expression and recurrent disease were negative effects on overall survival ($p = 0.012$, 0.026, 0.047, 0.005, 0.038 and 0.001, respectively). Cox multivariate analysis revealed only two factors to be associated with overall survival: negative p21 expression and recurrent disease ($p = 0.01$ and 0.002). The median survival rate of patients with negative expression was significantly poorer than that of patients with positive expression of p21 (17 vs. 27 months, $p = 0.01$, cox regression).

The 3-year disease-free survival (DFS) and overall survival rates for all patients were 18.8 and 30.45 %. The median overall survival was 26.52 months (range, 5.23–83.60 months), and median DFS was 19.45 months (range, 1.17–83.60 months). During the follow-up period, 4 patients developed recurrent disease and fourteen patients developed with distant metastasis.

We could not find any relation between DFS and any parameter.

Discussion

The function of p21 protein was investigated in cell cultures, and it was showed that it provides DNA repair or apoptosis of cell by stopping at the G1 phase checkpoint in irradiated cells [7–9]. In addition, direct inhibition of cell proliferation in tumor cells and healthy fibroblasts was showed in different studies [10, 11]. In these studies, this effect of p21 protein has been advocated tumor suppressor p53 gene-dependent effect. In two different studies, p53-independent effect of p21 protein was investigated in cell culture and inducing effect of p21 gene was showed after DNA damage in cases of suppressed p53 gene [12, 13].

In the healthy gastric mucosa while epithelial cells are progressing in the cell cycle, epithelial cells are going to the surface of gastric mucous and at the end of this period epithelial cells have been acquired much better differentiation, which cover the surface of the stomach. These cells in the stomach mucosa which are covering the surface have been shown p21 positivity, and as a result of this, it has been reported that p21 positive cells are well differentiated [14]. Despite being positive staining in all normal stomach tissue samples, Xie et al. [15] detected positive p21 staining rate only 39.8 % in gastric cancer tissue samples, and it

was significantly higher in patients with well-differentiated histology. Ogawa et al. [16] reported a statistically significant relationship between p21 expression and the well-differentiated histology. In our study, p21 expression was significantly higher in patients with well-differentiated histology.

Statistically significant relationship between p21 expression and histopathological parameters, such as tumor invasion, lymph node metastasis, lymphatic and venous invasion, has been reported in different studies [17, 18]. Ogawa et al. [16] reported a statistically significant reduction on the expression of p21 in patients with undifferentiated histology, deep tumor invasion, presence of venous and lymphatic invasion. Similarly, Kouraklis et al. [6] reported statistically significant reduction in the expression of p21 in patients with depth of tumor invasion, presence of venous and lymphatic invasion, and liver metastases. In our study, diffuse and undifferentiated histology, whole gastric involvement, the presence of vascular and neural invasion were found significantly higher in patients with negative expression of p21.

Ogawa et al. [16] reported significantly lower recurrence rate in patients with positive p21 expression, and they reported a longer overall survival in these patients. Gomyo et al. [19] reported significantly better overall survival rates in patients with the presence of p21 expression. Kouraklis et al. [6] reported a higher overall survival in patients with positive p21 expression. In contrast to these, Müller et al. [20] failed to demonstrate any relationship between the p21 expression and overall survival. In our study, there was no relation between p21 expression and recurrence, but there was statistically improved overall survival in the presence of p21 expression.

In previous studies which are investigating the role of p21 in gastric cancer, it has been focused on especially p21 expression. There is only one study which investigates the role of p21 gene mutation in gastric cancer in the literature. In this study, Park et al. [21] investigated the presence of p21 gene mutation in gastric cancer tissue samples of 20 patients, but they failed to detect any mutation on exon 2 and exon 3. In our study, we studied p21 mutation on exon 3, and p21 gene mutation was identified in 9 (20.5 %) patients. In addition, p21 gene mutation was significantly higher in diffuse-type and undifferentiated patients. Our results which show this relation are similar with result of p21 expression studies.

Her-2 overexpression rate is 8–28 % in gastric cancer studies. Chariyalertsak et al. [22] reported Her-2 overexpression rate is in patient with early stage, advanced stage, recurrent and inoperable cases, respectively, 6.9, 15.9 and 28.6 %. In our study, Her-2 expression rate was 9.1 %.

The results of studies which are investigating the relationship between prognostic factors and Her-2 expression in gastric cancer patients show differences. There are some studies that show significantly relation between Her-2 expression and papillary histology, liver metastases, well differentiation, serosa invasion, lymph node metastasis and advanced stage according to the depth of invasion [23–25]. In addition, in our study, there was no significant correlation between Her-2 expression and histopathological parameters, and there was no significant relationship detected between Her-2 expression and overall survival, and DFS.

Satiroglu-Tufan et al. [26] reported Her-2 gene polymorphism rate in patients with gastric cancer (IIe/Val) is 20 %, and they reported significant relation with stage IV disease. In our study, Her-2 gene polymorphism was detected, VV phenotype, in only 1 patient. Significant relationship could not be identified between histopathological parameters and overall survival.

Conclusion

The negative expression of p21 correlates with disease survival and may be a poor prognostic factor in patients with resected gastric cancer treated with adjuvant chemotherapy. For clearly assessment of effect of p21 and Her-2 expression and mutation/polymorphism on overall survival, more studies which contain more patients are needed.

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Conflicts of interest There are no conflicts of interest.

References

1. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, Haller DG, Ajani JA, Gunderson LL, Jessup JM, Martenson JA. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med. 2001;345:725–30.
2. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Ann Oncol. 2008;19:1523–9.
3. Wagner AD, Moehler M. Development of targeted therapies in advanced gastric cancer: promising exploratory steps in a new era. Curr Opin Oncol. 2009;21:381–5.
4. Xiangming C, Hokita S, Natsugoe S, Tanabe G, Baba M, Takao S, Kuroshima K, Aikou T. p21 expression is a prognostic factor in patients with p53-negative gastric cancer. Cancer Lett. 2000;148:181–8.
5. Gamboa-Dominguez A, Seidl S, Reyes-Gutierrez E, Hermannstädter C, Quintanilla-Martinez L, Busch R, Höfler H, Fend F, Luber B. Prognostic significance of p21WAF1/CIP1, p27Kip1, p53 and E-cadherin expression in gastric cancer. J Clin Pathol. 2007;60:756–61.

6. Kouraklis G, Katsoulis IE, Theocharis S, Tsouroufl is G, Xipolitas N, Glinavou A, Sioka C, Kostakis A. Does the expression of cyclin E, pRb, and p21 correlate with prognosis in gastric adenocarcinoma? Dig Dis Sci. 2009;54:1015–20.
7. El-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y. WAF1/CIP1 is induced in p53 mediated G1 arrest and apoptosis. Cancer Res. 1994;54:1169–74.
8. Sheikh MS, Rochefort H, Garcia M. Overexpression of p21/Waf1/Cip1 induced growth arrest, giant cell formation and apoptosis in human breast carcinoma cell lines. Oncogene. 1995;11:1487–98.
9. Chen CY, Faller DV. Direction of p21 generated signals towards cell growth or apoptosis is determined by protein kinase C and Bcl-2. Oncogene. 1995;11:1675–83.
10. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledges SJ. The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993;75:805–16.
11. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell. 1993;75:817–25.
12. Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE, Givol D. Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res. 1994;54:3391–5.
13. Akagi M, Yasui W, Akama Y, Yokozaki H, Tahara H, Haruma K, Kajiyama G, Tahara E. Inhibition of cell growth by transforming growth factor beta 1 is associated with p53-independent induction of p21 in gastric carcinoma cells. Jpn J Cancer Res. 1996;87:377–84.
14. Yasui W, Akama Y, Kuniyasu H, Yokozaki H, Semba S, Shimamoto F, Tahara E. Expression of cyclin-dependent kinase inhibitor p21WAF1/CIP1 in non-neoplastic mucosa and neoplasia of the stomach: relationship with p53 status and proliferative activity. J Pathol. 1996;180:122–8.
15. Xie HL, Su Q, He XS, Liang XO, Zhou JG, Song Y. Expression of p21WAF1 and p53 and polymorphismof p21WAF1gene in gastric carcinoma. World J Gastroenterol. 2004;10:1125–31.
16. Ogawa M, Maeda K, Onoda N, Chung Y-S, Sowa M. Loss of p21 espression correlates with disease progression in gastric carcinoma. Br J Cancer. 1997;75:1617–20.
17. Seo YH, Joo YE, Choi SK, Rew JS, Park CS, Kim SJ. Prognostic significance of p21 and p53 expression in gastric cancer. Korean J Intern Med. 2003;18:98–103.
18. Mizokami K, Kakeji Y, Oda S, Maehara Y. Relationship of hypoxia-inducible factor 1α and p21WAF1/CIP1 expression to cell apoptosis and clinical outcome in patients with gastric cancer. World J Surg Oncol. 2006;4:94–101.
19. Gomyo Y, Ikeda M, Osaki M, Tatebe S, Tsujitani S, Ikeguchi M, Kaibara N, Ito H. Expression of p21, but not p53 protein, is a factor in the survival of patients with advanced gastric carcinoma. Cancer. 1997;79:2067–72.
20. Müller W, Noguchi T, Wirtz HC, Hommel G, Gabbert HE. Expression of cell-cycle regulatory proteins cyclin D1, cyclin E, and their inhibitor p21 WAF1/CIP1 in gastric cancer. J Pathol. 1999;189:186–93.
21. Park YE, Choi KC, Choi YH. p21 expression and mutation in gastric carcinoma: analysis by immunohistochemistry and PCR-SSCP. J Korean Med Sci. 1998;13:507–12.
22. Chariylertsak S, Sugano K, Ohkura H, Mari Y. Comparison of c-erbB-2 oncoprotein expression in tissue and serum of patients with stomach cancer. Tumour Biol. 1994;15:294–303.
23. Yonemura Y, Ninomiya I, Yamaguchi A. Evalution of immünreactivity for c-erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res. 1991;51:1034–8.
24. Yonemura Y, Ninomiya I, Ohoyama S, Kimura H, Yamaguchi A, Fushida S, Kosaka T, Miwa K, Miyazaki I, Endou Y. Expression of c-erbB-2 oncoprotein in gastric carcinoma. İmmünreactivity for c-erbB-2 protein is an indipentent indicatör of poor short–term prognosis in patients with gastric carcinoma. Cancer. 1991;67:2914–8.
25. Mizutani T, Onda M, Tokunaga A, Yamanaka N, Sugisaki Y. Relationship of c-erbB-2 protein expression and gene amplification to invasion and metastasis in human gastric cancer. Cancer. 1993;72:2083–8.
26. Satiroglu-Tufan NL, Bir F, Calli-Demirkan N. Investigation of HER-2 codon 655 single nucleotide polymorphism frequency and c-ErbB-2 protein expression alterations in gastric cancer patients. World J Gastroenterol. 2006;12:3283–7.