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The expressions of p16 and p21 to predict the clinicopathological behavior and prognosis of gastric cancer

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ABSTRACT

In this study, the associations of expression of two cell cycle proteins, p16 and p21, with conventional prognostic parameters and survival were investigated in gastric carcinomas. The expression of p16 and p21 were investigated by immunohistochemical method in 84 cases who had undergone gastrectomy. P16 and p21 expressions were classified as positive and negative according to the intensity of staining. The associations of p16 and p21 expressions with age, gender, localization, WHO histologic type, Lauren's histologic type, WHO differentiation grade, Goseki grade, lymphovascular invasion, TNM stage, lymph node metastasis and depth of invasion and survival were also investigated. For p16 expression, nuclear immunoreactivity was traced as 10% and over in 50 cases (59.5%) and as under 10% in 34 cases (40.5%). For p21 expression, nuclear immunoreactivity was traced as 10% and over in 47 cases (55.9%) and as under 10% in 37 cases (44.1%). P16 and p21 expressions were determined to have no significant correlations with age, gender, localization, WHO histologic type, Lauren's histologic type, WHO differentiation grade, Goseki grade and lymphovascular invasion. However, p21 expression was found to have significant correlations with TNM stage, lymph node metastasis and depth of invasion (p=0.006, p=0.013, p=0.003, respectively) and P16 expression was found to have significant correlations with survival and lymph node metastasis (p=0.012, p=0.017, respectively). As a conclusion, gastric cancer is still a significant cause of morbidity and mortality in our region, as well as all over the world. Although TNM staging system has been one of the most significant prognostic parameters, studies on new prognostic parameters are in progress. Our results show that p16 and p21 expressions may be helpful in predicting the clinicopathologic behavior of gastric cancer.

1. Introduction

Gastric carcinomas, which have aggressive behavior, are frequently encountered tumors worldwide. Despite the reduction observed in frequency in recent years in United States and some European countries, gastric carcinoma is still reported as the second most common cause for cancer-related deaths, following lung cancer (Stadtlander et al., 1999; Washington, 2010).

Despite improved protocols for diagnosis and treatment, gastric carcinoma still has a poor prognosis. Following curative surgical resection, 5-year survival is between 10-15% (Stacey, 2010).

Various conventional prognostic parameters have been in use for predicting the prognosis in gastric cancers. The most important prognostic factors are stage of the tumor and total surgical excision. Other frequently used prognostic parameters are histological grade and histopathological type of tumor. In addition to these parameters, additional prognostic predictors, which can be evaluated more objectively and can lead to development of new treatment strategies, are necessary.

In numerous studies performed on this subject, results have been found supporting the suggestion of p16 inactivation as a probable factor for malignant transformation in gastric mucosa. Also, in various studies, p16 and p21 gene

differentiations were claimed to be closely associated with the degree of differentiation and metastasis in gastric cancer (Eliseo et al., 2007). Ficorella et al. (2003) have claimed that hypermethylation of the p16 locus and p53 mutation play an integral role in the pathogenesis of gastric cancer.

p21 protein, coded as WAF1/CIP1 gene, is a very important member of CDKIs family and is located on the short arm of chromosome 6. This protein not only inhibits the function of CDKs, but also inhibits the DNA replication and progression of cell cycle by interacting with proliferating cell nuclear antigen (PCNA), bcl-2 and c-myc. For this reason, p21 plays a central role in the regulation of cell cycle. p21 gene mutations have been shown in prostate cancers (Michael et al., 1986). Also, abnormal p21 expression was claimed to be associated with many brain and colon tumors (Michael et al., 1986; Ruan et al., 1998; Che et al., 2000). In numerous studies, the association of this protein with gastric cancers was also investigated. In some of these studies, p21 was shown to be expressed more in tumoral regions when compared to adjacent tissues and normal mucosa. Also, in some studies, p21 expression was shown to be associated with the size, differentiation, invasion and metastasis of the tumor in gastric cancer (Armando et al., 2007; Juan et al., 2010). In our study, the association of p16 and p21 expressions with clinicopathological findings and prognosis was investigated immunohistochemically in cases who underwent gastric resection for gastric cancer.

2. Patients, materials, and methods

A total of 84 cases, who had been diagnosed with gastric adenocarcinoma at Atatürk University, Medical Faculty, Department of Pathology and had undergone total gastrectomy in the 5-year period, between March 2003-June 2008 were included in the study. All of H&E-stained sections were reexamined. During determination of histological types of cases, Lauren and WHO criteria were taken into consideration (Lauren, 1965; Uta et al., 2002). Histological grade was evaluated according to the conventional grading and Goseki classification (Songun et al., 1999). In addition to these, the presence of lymphovascular invasion and perineural invasion were also investigated. In all of the surgical resection specimens, the depth of invasion (pT), lymph node involvement (pN) and pathological stage were determined according to AJCC TNM classification (Washington, 2010). In this study, immunohistochemistry, which is an effective method for investigating the expression of p16 and p21 genes, was performed (Geradts et al., 1995; Sakaguchi et al., 1996).

Immunohistochemistry

We performed immunohistochemistry using a Leica Bond -max automated immunostainer (Leica Microsystems, Newcastle, UK) on the basis of the manufacturer's protocol. Nuclear staining for both antibodies was considered positive, regardless of staining intensity and distribution, in all sections. All stained sections were reexamined by two pathologists. For p16 and p21, nuclear staining 10% or over was considered positive (+), and staining under 10% as negative (-) (Hye et al., 2003; Young et al., 2003; Maria et al., 2012).

Statistical analysis

PSS 15.0 software was used for statistical analysis. In order to compare immunohistochemical and clinicopathological parameters, the chi-square test, Mann-Whitney U test, Kruskal-Wallis test and ANOVA were used. Survival was assessed according to the Kaplan-Meier method by using the log-rank test and Breslow test. Results with p value less than 0.05 were considered significant.

3. Results

In the survey, it was determined that 26 cases (30.9%) out of 84 lived for 5 years and over and 58 cases (69.1%) lived less than 5 years. Regarding p16 studied immunohistochemically in 84 patients with gastric cancer, 50 cases (59.5%) showed 10% or more nuclear immunoreactivity (Fig. 1) and 34 cases (40.5%) showed less than 10% immunoreactivity. Regarding p21, 47 cases (55.9%) showed 10% or more nuclear immunoreactivity (Fig. 2) and 37 cases (44.1%) showed less than 10% immunoreactivity.

Table 1 summarizes the evaluation of histopathological findings of the cases according to their expressions of p16 and p21.

Immunoreactivities of p16 and p21 were analyzed in terms of pT. While no significant association was found between pT and p16 immunoreactivity (p=0.083), the correlation was statistically significant between pT and p21 immunoreactivity (p=0.003).

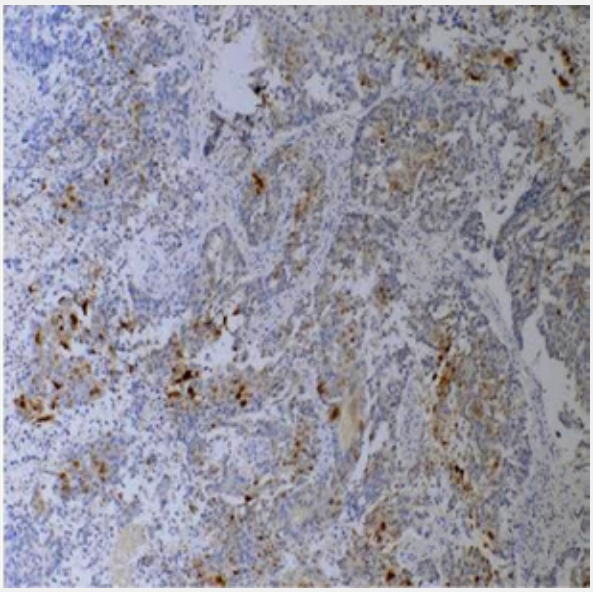

Fig. 1. p16, nuclear reactivity over 10% (100x)

When p16 and p21 immunoreactivities were analyzed in terms of lymph node metastasis, TNM stage and lymphovascular invasion, a significant correlation was found between immuno reactivities of p16 and immunoreactivities of p21 with lymph node metastasis (p=0.017, p=0.013, respectively).

While there was a significant correlation between TNM stage and p21 immunoreactivity (p=0.006), no significant correlation was found between TNM stage and p16 immunoreactivity (p=0.077). Additionally, there were no significant relationships of p16 and p21 immunoreactivities with lymphovascular invasion (p=0.063, p=0.130, respectively). This may have related to inadequate sampling.

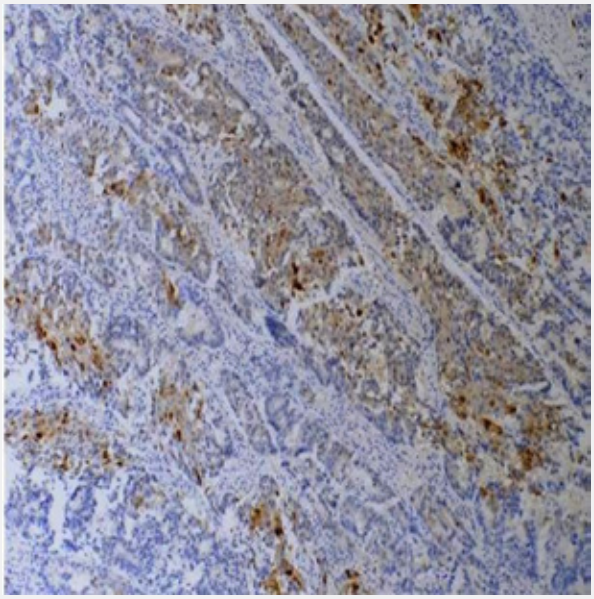

Fig. 2. p21, nuclear reactivity over 10% (100x)

When survival times were analyzed according to TNM stage, lymphovascular invasion and Lauren histological type, a highly significant correlation was found between survival and TNM stage (Fig. 3) (p=0.001). Likewise, there were significant correlations of survival with lymphovascular invasion and Lauren histological type (p=0.043, p=0.012, respectively).

When Goseki grades, conventional histological grades and pT were analyzed according to survival of the cases, significant correlations between survival time and, Goseki grade, conventional grade and pT were found (p=0.007, p=0.040, respectively). Statistically, the most significant correlation with survival of the cases was found to be with the conventional histological grade (p=0.002).

When conventional histological type, lymph node metastasis and tumor localization were analyzed according to survival of the cases, significant correlation was found between survival of the cases and lymph node metastasis (p=0.001). While the relationship between survival and conventional histological type was not significant (p=0.270), a significant correlation was found between survival and tumor localization (p=0.007).

When p21 immunoreactivity was analyzed in terms of survival, in 10 (38.5%) cases out of 26 who survived 5 years and over, 10% or more immunoreactivity was detected; in 16 (61.5%) cases, p21 immunoreactivity was less than 10%. In 37 (63.8%) cases out of 58 who survived less than 5 years, 10% or more immunoreactivity was detected; in 21 (36.2%) cases, p21 immunoreactivity was less than 10%. There was no significant relationship between survival and p21 immunoreactivity in this case series (p=0.073).

When p16 immunoreactivity was analyzed in terms of survival, in 9 (34.6%) cases out of 26 who survived 5 years and over, 10% or more immunoreactivity was detected; in 17 (65.4%) cases, p16 immunoreactivity was less than 10%. In 41 (70.7%) cases out of 58 who survived less than 5 years, 10% or more immunoreactivity was detected; in 17 (29.3%) cases, p16 immunoreactivity was less than 10%. There was significant relationship between survival and p16 immunoreactivity in this case series (p=0.012).

4. Discussion

WHO classification and Lauren classification are also frequently used in histopathological classification of gastric cancer (Adachi et al., 2000; Miao et al., 2013). In his first article on this subject, Lauren classified gastric cancers into two major groups named as intestinal and diffuse types. In his study, Lauren reported that gastric cancers of the intestinal type were more common and had better prognosis than cancers of diffuse type (Lauren, 1965; Adachi et al., 2000; Miao et al., 2013). Similarly, in the study conducted on 200 cases, Anna et al. (2012) determined that 58% of their cases were of intestinal type and 25% were of diffuse type, according to Lauren classification (Davessar et al., 1990; Anna et al., 2012). In our study, contrary to the reports in the literature, 44% of the patients were of intestinal type and 56% were of diffuse type. Additionally, average survival of the cases having diffuse type was determined to be significantly shorter, which was consistent with the literature (p=0.012).

In addition to conventional histological grading, Goseki grading system, which has been defined in recent years, shows a close relationship with WHO classification and histopathological classification of Lauren (Dixon et al., 1994). Moreover, in various performed studies, the prognostic importance of Goseki grading system was shown, together with conventional TNM staging (Martin et al., 1994; Songun et al., 1999). Guglielmi et al. (1997) in their study conducted on 89 cases, showed that Goseki grading system had a strong correlation with Lauren classification, but stated that Goseki grading system did not have any effect on survival. In our study, when survival of the cases were compared in terms of their Goseki grades, a statistically significant correlation was found (p=0.007). In addition to this, no significant relationship was found between Goseki grade and TNM stage (p=0.781).

In addition to those conventional prognostic parameters mentioned above, studies are being conducted on more objective and easily applicable prognostic factors. In recent years, the relationships of p16 and p21 expressions, known especially as cell cycle markers, with prognosis in gastric carcinomas were suggested as worth investigating.

Loss of p16 expression is usually because of hypermethylation of p16INK4a promoter region (Ficorella et al., 2003; Daya et al., 2006; Mir et al., 2012). Jing et al. (2012), in their study on 119 cases with gastric carcinoma, reported that p16 gene hypermethylation is associated with tumoral differentiation, lymph node metastasis and survival (Jing et al., 2012). Nahye et al. (2000), in their study on 76 patients with gastric cancer, determined increased Rb expression together with decreased expressions of p16, p27, cyclin D1, cyclin E and CDK4. They stated that loss of p16 and p27 were strongly correlated with development of gastric cancer. In the study conducted by Guo et al. (2003) on 40 patients, p16 gene deletion was found to be associated with the degree of tumor differentiation and gastric cancer metastasis.

Consistent with our data, Xiu et al. (2005), in their study conducted on 122 cases, stated that loss of p16 expression was significantly correlated with lymph node metastasis. However, contrary to our results, they also determined that loss of p16 expression was correlated significantly with histological subtypes. Similar to our study, they found no significant correlation between p16 expression and pT (Xiu et al., 2005). Teiichiro et al. (2005), in their study on 274

Table 1. Relationship between p16, p21 expression and clinicopathological findings

Variable	Group	p16 positive	p16 negative	p21 positive	p21 negative
Sex	Male	31	28	31	28
	Female	19	6	16	9
Tumor location	Cardia	7	7	5	9
	Antrum	14	9	12	11
	Fundus	4	6	6	4
	Corpus	22	11	20	13
	Diffuse involvement	3	1	1	3
WHO histological type	Tubular	35	27	31	31
	Mucinous	9	2	8	3
	Signet ring cell	6	5	8	3
Lauren's type	Intestinal	23	14	20	17
	Diffuse	27	20	27	20
WHO histological grade	Well	3	4	2	5
	Moderate	21	12	18	15
	Poor	26	18	27	17
TNM stage	IA	0	3	0	3
	IB	3	4	1	6
	IIA	2	4	1	5
	IIB	6	6	7	5
	IIIA	21	8	21	8
	IIIB	18	9	17	10
				(p 0.006)	
Goseki grade	1	15	14	15	14
	2	4	2	4	2
	3	25	16	21	20
	4	6	2	7	1
Tumor size (cm)	5 cm above	35	17	31	21
	5 cm below	15	17	16	16
Bormann type	Type 1 (Polypoid)	11	5	8	8
	Type 2 (Fungating)	9	9	9	9
	Type 3 (Ulcerated)	27	19	26	20
	Type 4 (Diffusely infiltrative)	3	1	4	0
pT	Lamina propria or submucosa	0	3	0	3
	Muscularis propria or subserosa	7	6	3	10
	Penetrates serosa	43	25	44	24
				(p 0.003)	
Lymph node metastasis	Absent	5	8	2	11
	(1-2)	3	8	6	5
	(3-6)	22	8	20	10
	7 and above	20	10	19	11
		(p 0.017)		(p 0.013)	
Lymphovascular invasion	Absent	19	12	14	17
	Present	31	22	33	20
Survey	Over 5 years	9	17	10	16
	Under 5 years	41	17	37	21
Total		50	34	47	37

cases, stated that loss of p16 expression was not correlated significantly with histological differentiation, but was seen more commonly in lymph node-negative gastric carcinomas.

In the study conducted by Masaki et al. (2000) on 80 cases with gastric cancer, a significant correlation between loss of p16 expression and poorly differentiated tumors was found, but no correlation was found with prognosis.

Ma et al. (2012), in their study on 65 patients with gastric cancer, showed that p16 expression was very much reduced in cancerous mucosa when compared to normal mucosa; patients with loss of p16 expression lived shorter and it was correlated with survival. In addition, they also found significant correlations of p16 expression with lymph node metastasis and tumor differentiation. However, they did not find any correlations of p16 expression with age, gender, tumor size and pT.

Contrary to most of the articles in the literature, Koriyama et al. (2004) in their study on 171 cases, found no significant correlations of TNM staging, age and prognosis with loss of p16 expression. Maria et al. (2010) in their study on 482 patients having gastric cancer, investigated the relationships of p16, p21, p27, Cyclin D1, Cyclin A and Cyclin B1 with age, gender, tumor localization, pN and prognosis. The only significant correlation they found was between Cyclin 1 and prognosis, and they identified no other significant relationships.

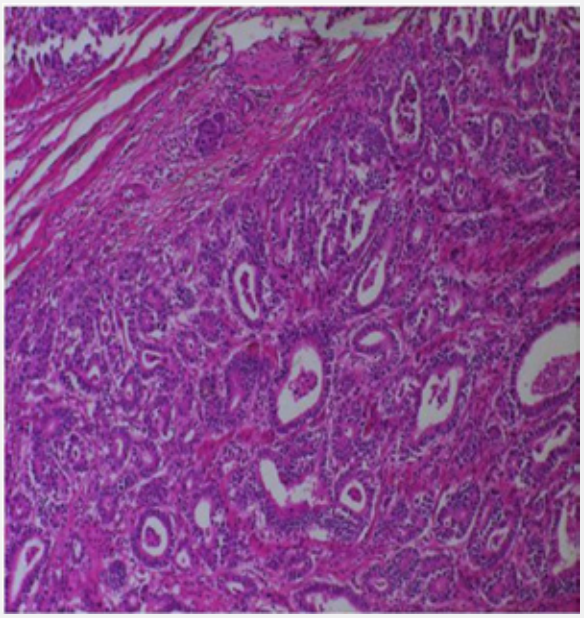

Fig. 3. Grade 1 Gastric Adenocarcinoma (100x)

In our study, p16 expression was found to have no significant correlations with age, gender, localization, WHO histological type, Lauren classification, WHO differentiation grade, Goseki grade, pT, lymphovascular invasion and TNM stage. However, the relationships with survival and lymph node metastasis were found to be significant (p=0.012, p=0.017, respectively).

P21 gene mutations were shown in prostate cancers. Also, abnormal p21 expression was stated to be associated with brain and colon tumors (Ruan et al., 1998; Che et al., 2000). In many studies, the role of this protein in gastric cancers was investigated. In some of these, p21 was found to be expressed more in tumoral regions when compared to the adjacent tissues and normal mucosa. Also, in some studies, p21 expression was found to be associated with size, differentiation, invasion and metastasis of the tumor in gastric cancer (Armando et al., 2007; Juan et al., 2010). In the study conducted by Czerniak et al. (1989) when compared to normal subjects without cancer, p21 expression was found to be increased in all types of early diagnosed gastric carcinomas and in areas showing metaplastic and dysplastic mucosal transformation accompanying gastric cancer; this overexpression of p21 was suggested to be an early indicator of gastric cancer. However, Sirak et al. (2009) investigated the effects of p16 and p21 expressions on preoperative chemoradiotherapy in patients with gastric cancer and found no significant correlation.

Masahide et al. (1998) in their study on 158 patients with gastric cancer, stated that p21 expression, p53 expression, tumor size and lymph node metastasis were independent prognostic predictors. Contrary to this statement, Gomyo et al. (1997) in their study on 93 cases with gastric cancer, found no correlation of p53 with prognosis but noted a significant correlation between p21 expression and prognosis. Ogawa et al. (1997) in their study on 172 patients with gastric cancer, found significant correlations between the loss of p21 expression differentiation and metastasis, pT, lymphovascular invasion and TNM stage of the tumor.

Young et al. (2003) in their study on 102 patients with gastric cancer, investigated the prognostic significance of p53 and p21. They found significant correlations of TNM stage, lymph node metastasis and survival with loss of p21 expression. Juan et al. (2010) in a similar study on 189 patients with gastric cancer, found no association of age and Borrmann classification with p21 expression; however, they showed that expression of p21 was in significant correlation with size and differentiation grade of the tumor, lymph node metastasis, Lauren classification, pT and prognosis. Eliseo et al. (2007) in their study on 47 patients with gastric cancer, did not find any significant correlation of p21 expression with clinicopathological parameters and stated that p21 expression was significantly correlated with differentiation grade of the tumor.

Liu et al. (2001) in their study on 140 cases with gastric cancer, stated that TNM stage, lymph node metastasis and p27 were independent prognostic predictors, but there was no correlation between p21 expression and prognosis. While Se et al. (1998) did not find any correlation of age, gender, TNM stage and lymph node metastasis with loss of p21 expression in their study on 84 patients with gastric cancer, they noted a significant correlation between loss of p21 expression and prognosis. Che et al. (2000) in their study on 256 cases with gastric cancer, stated that p21 expression was significantly associated with prognosis in p53-negative patients and they claimed that it can be used as an independent prognostic parameter in p53-negative patients.

In our study, consistent with the literature, p21 expression did not have any significant correlations with age, gender, localization, WHO histological type, Lauren classification, WHO differentiation grade, Goseki grade and lymphovascular invasion; however, it was significantly correlated with TNM stage, lymph node metastasis and pT (p=0.006, p=0.013, p=0.003, respectively). Contrary to the literature, no significant correlation could be identified between p21 expression and survival (p=0.073).

As a conclusion, gastric cancer is still a significant cause of morbidity and mortality in our region, as well as all over the world. Despite curative surgical approaches and chemotherapy, its prognosis is still quite poor. Although TNM staging system has been one of the most significant prognostic parameters, studies on new prognostic parameters are in progress in gastric cancer which is still a significant cause of mortality and morbidity. In our study, the correlations of p16 and p21 expressions, which are two cell cycle regulators, with conventional prognostic parameters and survival were investigated. In the literature, the prognosis is considered to be poorer in cases who manifest reduced p21 expression and increased p16 expression. Results of our study were generally consistent with the literature reporting that the prognosis is considered to be poorer in cases who manifest reduced p21 expression and increased p16 expression. However, among patients with increased p21 expression and reduced p16 expression, some were determined to have poor prognosis. Similar contrary results have also been obtained like in some other studies. Therefore, it is quite difficult to explain tumor development, prognosis and other clinicopathological parameters with alteration in only one tumor suppressor gene. Various genetic and epigenetic changes, including alterations of tumor suppressor genes, should be considered during this process. In conclusion, expressions of p16 and p21 may be helpful in predicting the clinicopathological behavior of

gastric cancer. However, in order to evaluate the effects of these parameters on prognosis more accurately, prospective clinical trials with larger sample groups should be performed in addition to retrospective studies.

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