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Relationship between HER-2, COX-2, p53 and clinicopathologic features in gastric adenocarcinoma. Do these biomarkers have any prognostic significance?

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Original Article

ABSTRACT

Background/Aims: The aim of this study was to examine the expression of HER-2, p53 and COX-2 in gastric adenocarcinoma and to investigate whether these markers were useful in predicting the clinicopathologic features.

Materials and Methods: Primary gastric adenocarcinoma specimens were obtained from 56 patients who underwent total/subtotal gastrectomy with extended lymphadenectomy between December 2011 – December 2012. We investigated the association between the expression of these markers and clinicopathologic factors by immunohistochemistry.

Results: COX-2 positive cases were detected in 28 (50%) of the 56 patients and COX-2 expression was significantly correlated with presence of perineural invasion (p=0,032). 37 cases (66.1%) were defined positive for p53. The expression of p53 was significantly correlated with increasing age (p=0,003), but there was no correlation with other clinicopathological variables. Among the 56 primary gastric cancers, 9 (16.1%) cases showed intermediate (2+) positive expression and 7 (12.5%) cases showed (3+) positive expression for HER-2. No significant correlations were determined between HER-2 and the other variables.

Conclusion: Although, this study failed to show any relationship between HER-2 and clinicopathological factors, but our results demondtrated that COX-2 expression might serve as a powerful indicator for estimating perineural invasion, which is an independent worse prognostic factor for survival in gastric adenocarcinoma. Additionally, detecting the expression of p53 can assist with the treatment options for elderly patients with gastric adenocarcinoma. A better understanding of HER-2, COX-2 and p53 expression in gastric adenocarcinoma may improve the staging strategies and influence new treatment modalities.

Keywords: HER-2, p53, COX-2, clinicopathologic features, gastric adenocarcinoma

INTRODUCTION

In the latter half of the twentieth century, gastric adenocarcinoma (GA) was the second most common cause of cancer-related death after lung carcinoma (1). GA continues to be a significant and important health problem because the incidence in Asia is decreasing very slowly, and in Western countries, the mortality rate associated with GA is high (2). The important prognostic factors for patients with resected GA are the depth of the tumor in the stomach wall, lymph node status and distant metastases (3). Key prognostic factors have been defined to account for the staging and proper therapeutic strategies in GA. However, the factors are imprecise, and patients with similar GA stage prognosis may actually be at different stages. Therefore, studies are needed to constitute new prognostic factors.

Human epidermal growth factor receptor 2 (HER-2) is a growth factor receptor that encodes a transmembrane tyrosine kinase receptor that drives tumorigenesis pathways, such as cell growth, differentiation, invasion, metastasis and angiogenesis (4). Consequently, HER-2 positive tumors likely have a poor prognosis compared with HER-2 negative tumors. Various reports have shown that HER-2-positive breast tumors demonstrate aggressive characteristics compared with HER-2 negative breast tumors (5). HER-2 is overexpressed in approximately 10-30% of gastric cancers (6). Recent reports

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have demonstrated that the immunoreactivity of HER-2 was not correlated with histopathological parameters (7) or survival (8).

p53 is a tumor suppressor gene that is located on the short arm of chromosome 17. p53 is involved in regulating cell proliferation, inducing apoptosis, and promoting chromosomal stability. The disruption of these functions appears to play an important role in the carcinogenesis of many tumor types, including gastric adenocarcinoma, colon cancer, ovarian cancer, and lung cancer (9). Some reports have shown that the immunoreactivity of p53 ranges from 13 to 54% in gastric adenocarcinoma (9).

Cyclooxygenase (COX) is a central, rate-limiting enzyme that is important in the biosynthesis of prostaglandins from arachidonic acid. Two distinct isoforms of COX have been identified, COX-1 and COX-2. COX-2 constitutes the basis for various processes, including cellular differentiation, growth, inflammation and carcinogenesis. COX-2 expression has been shown to be especially prominent in gastrointestinal cancers, such as colorectal adenocarcinomas, and its overexpression has been associated with a poor prognosis (10). One recent study showed that COX-2 expression was elevated in gastric carcinomas and their precursor lesions (11).

In this study, we performed immunohistochemical analyses in 56 primary gastric adenocarcinoma cases and analyzed the relationship between HER2, p53, and COX-2 expressions as well as clinicopathologic features.

MATERIALS AND METHODS

Clinical materials

Primary gastric adenocarcinoma specimens were obtained from 56 patients who underwent total/subtotal gastrectomy with extended lymphadenectomy between December 2011 – December 2012. Additionally, at least 15 lymph nodes were resected and examined from each case. None of the patients received chemotherapy or radiotherapy prior to surgery. The group of patients consisted of 40 males and 16 females with ages ranging between 18 and 88 years old (mean age: 63 years old). There were at total of 26 (46.4%) tumors from the distal stomach and 30 (53.6%) tumors from the proximal stomach. Histological types were divided into well differentiated (G1), moderately differentiated (G2) and undifferentiated (G3). Signet-ring cell carcinomas and poorly differentiated adenocarcinomas were classified as the undifferentiated type. Two (3.6%) tumors were classified as G1, 23 (41.1%) tumors were classified as G2 and 31 (55.4%) tumors were classified as G3. According to the seventh edition of the classification TNM-UICC/AJCC, depth of tumor invasion was recorded using T classification. Mucosal or submucosal tumor invasion was defined as T1, muscularis propria or subserosal tumor invasion was defined as T2, subserosal connective tissue without involvement of visceral peritoneum tumor invasion was defined as T3, and invasion of adjacent structures was defined as T4 (12). The existence of intestinal metaplasia and H. pylori, the extent of lymphatic and perineural invasion and lymph node involvement, were also recorded and marked as present or absent.

Immunohistochemistry

All specimens were fixed in formalin and embedded in paraffin. Serial sections (4 µm) were prepared, and one section was stained with hematoxylin and eosin, whereas the additional sections were utilized for immunohistochemistry. HER-2, p53 and COX-2 immunohistochemistry were performed using an automated slide stainer (Ventana Benchmark XT, Arizona,USA). The primary antibodies used were e2-4001-3B5 (Thermo Scientific, UK, 1:500) for HER-2, SP21 (Cell Marque, USA, 1:250) for COX-2 and Clone BP-53-12 (Genemed Biotechnologies, USA, 1:150) for p53. The sections were counter-stained with hematoxylin.

Assessment of staining

All the slides were evaluated without clinical information by two independent pathologists (NU, OY). The DAKO Hercep Test Protocol System (13) was used to grade the degree of HER-2 membrane staining. The extent of staining was scored negative (-) when there was no membrane staining or the membrane staining showed less than 10% of the tumor cells. The extent of staining was scored intermediate (2+) if there was moderately complete basolateral membrane staining in more than 10% of the tumor cells and scored as strongly positive (3+) if there was intensely complete basolateral membrane staining in more than 10% of the tumor cells.

Furthermore, the expression of p53 was evaluated by assessing nuclear staining. Nuclear positivity in more than 10% of the tumoral cells was considered positive.

The accepted staining pattern of COX-2 was cytoplasmic and perinuclear. Overall, the immunoreactivity of the tumor cells without a cut-off point was considered positive.

Statistical analysis

Continuous variables were expressed with median (minimum-maximum) values, whereas categorical variables were expressed with frequency and related percentage values. Continuous variables between group comparisons were performed using a Kruskal Wallis and Mann Whitney test. For categorical variables, a Fisher exact test was performed, and for between group comparisons a generalized Fisher exact test (Freeman-Halton test) was implemented. Statistical significance was set at $p<0.05$and SPSS 20.0 was used for performing statistical analysis.

RESULTS

We studied 56 patients with primary gastric adenocarcinoma. Five (8.9%) patients had tumors with invasion of mucosa or submucosa (T1), 6 (10.7%) patients had tumors with invasion of muscularis propria (T2), 39 (69.6%) patients had tumors with

invasion of subserosal (T3) and 6 (10.7%) patients had tumors with invasion of adjacent structures (T4).

Lymph node metastases were proven histologically in 40 (71.4%) patients, and the median number of lymph nodes was 4 (1-35). The median tumor size was 26 (1.5-240). Lymphatic invasion was seen in 30 (53.6%) patients, and perineural invasion was present in 27 (48.2%) patients. H. pylori were detected in 11 (19.6%) patients, and 29 patients exhibited intestinal metaplasia (51.8%).

Features of expression and the relationship with clinicopathological factors

The expression of COX-2 was detected at very low levels in normal gastric mucosa. In gastric cancer, COX-2 was expressed in the cytoplasm and membrane of the cancer cells (Figure 1a). COX-2 positive cases were detected in 28 (50%) of the 56 patients. There was a significant relationship between COX-2 expression and the presence of perineural invasion (p=0,032). However, COX-2 expression was not associated with additional variables, including gender, age, histological grade, lymphatic invasion, lymph node involvement, tumor size, location, existence of H. pylori and intestinal metaplasia (Table 1).

The p53 tumor suppressor gene was expressed in the nuclei of tumor cells (Figure 1b). In total, 37 cases (66.1%) were positive for p53. The median age value of the p53 positive group was higher compared with the p53 negative group (p=0.003). However, p53 expression was not associated with additional variables (Table 2).

HER-2 immunoreactivity was negative in the normal gastric tissue surrounding the cancerous lesions. Among the 56 primary gastric cancers, 9 (16.1%) cases showed intermediate (2+) positive expression (Figure 1c), and 7 (12.5%) cases showed strong (3+) positive expression for HER-2. The staining pattern was in-

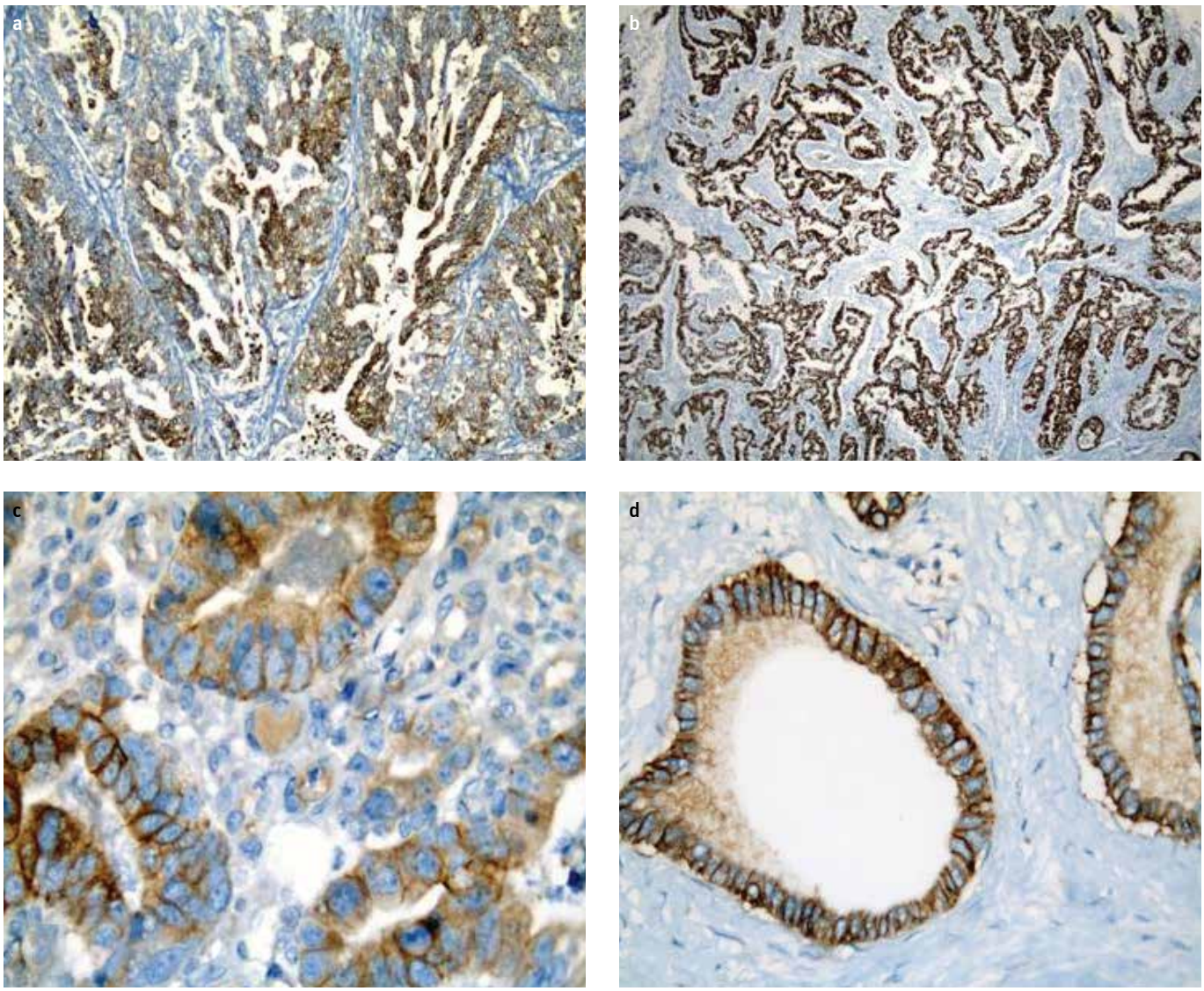

Figure 1. a-d. Representative images of immunohistochemical staining for HER-2, p53 and COX-2 in gastric adenocarcinoma (original magnification, X200). Diffuse COX-2 observed in cytoplasm and perinuclear area of tumor cells **(a)**. Strong p53 expression in nuclei **(b)**. Score +2 basolateral membrane staining for HER-2 **(c)**. Score +3 strong membrane staining for HER-2 **(d)**.

Table 1. Correlation between COX-2 expression and clinicopathological features

		Negative (n=28)	Positive (n=28)	p value
Gender	Female	19 (67.9)	21 (75)	0.088
	Male	9 (32.1)	7 (25)	
Age		62 (18-84)	63 (32-88)	0.768
Depth of invasion	T1	1 (3.6)	4 (14.3)	0.542
	T2	3 (10.7)	3 (10.7)	
	T3	20 (71.4)	19 (67.9)	
	T4	4 (14.3)	2 (7.1)	
Histological Grade	G1	0 (0)	2 (7.1)	0.523
	G2	11 (39.3)	12 (42.9)	
	G3	17 (60.7)	14 (50)	
Perineural invasion	Absent	10 (35.7)	19 (67.9)	0.032
	Present	18 (64.3)	9 (32.1)	
Vascular invasion	Absent	10 (35.7)	16 (57.1)	0.180
	Present	18 (64.3)	12 (42.9)	
Intestinal Metaplasia	Absent	15 (53.6)	12 (42.9)	0.593
	Present	13 (46.4)	16 (57.1)	
H. Pylori	Absent	24 (85.7)	21 (75)	0.501
	Present	4 (14.3)	7 (25)	
Location	Proximal	12 (42.9)	18 (64.3)	0.180
	Distal	16 (57.1)	10 (35.7)	
Lymph node involvement	Absent	6 (21.4)	10 (35.7)	0.375
	Present	22 (78.6)	18 (64.3)	
Tumor size		28 (1.50-240)	21.25 (4-240)	0.743

Values were expressed as median (minimum-maximum) or n (%)

Table 2. Correlation between p53 expression and clinicopathological features

		Negative (n=19)	Positive (n=37)	p value
Gender	Female	13 (68.4)	27 (73)	0.964
	Male	6 (31.6)	10 (27)	
Age		52 (32-84)	64 (18-88)	0.013
Depth of invasion	T1	2(10.5)	3 (8,1)	0.488
	T2	3 (15.8)	3 (8,1)	
	T3	11 (57.9)	28 (75,7)	
	T4	3 (15.8)	3 (8,1)	
Histological Grade	G1	0 (0)	2 (5,4)	0.898
	G2	8 (42.1)	15 (40,5)	
	G3	11 (57.9)	20 (54,1)	
Perineural invasion	Absent	9 (47.4)	20 (54,1)	0.848
	Present	10 (52.6)	17 (45,9)	
Vascular invasion	Absent	7 (36.8)	19 (51,4)	0.455
	Present	12 (63.2)	18 (48,6)	
Intestinal Metaplasia	Absent	8 (42.1)	19 (51,4)	0.709
	Present	11 (57.9)	18 (48,6)	
H. Pylori	Absent	13 (68.4)	6 (31,6)	0.156
	Present	32 (86.5)	5 (13,5)	
Location	Proximal	8 (42.1)	22 (53,6)	0.342
	Distal	11(57.9)	15 (40,5)	
Lymph node involvement	Absent	4 (21.1)	12 (32.4)	0.562
	Present	15(78.9)	25 (67.6)	
Tumor size		26 (5.75-70)	26 (1.50-240)	0.467

Values were expressed as median (minimum-maximum) or n (%).

tense mostly on the cell basolateral membrane (Figure 1d). No significant relationships were determined between HER-2 and additional variables, such as age, gender, depth of invasion, histological grade, lymphatic and perineural invasion, lymph node involvement, tumor size, location, existence of H. pylori and intestinal metaplasia (Table 3).

DISCUSSION

Gastric adenocarcinoma is a multifactorial and heterogeneous disease with a poor prognosis that has remained substantially unchanged over recent decades, even after progress in surgical, chemo- and radiotherapy. Currently, factors that have different molecular pathways are considered predictive and prognostic factors for GA. These factors include growth factors, adhesion molecules, oncogenes, tumor suppressor genes, proteolytic molecules, angiogenic factors, chemokines and cytokines. Nonetheless, the relevance of these molecular markers is not consistent with the results of altered reports, which have shown discordant and differing conclusions. In the current study, we have chosen three molecules from several prior studies to attempt to understand which of these molecules are the most advantageous clinicopathologically (7-11).

Previous case-control and epidemiologic cohort studies have demonstrated that the orderly use of non-steroidal anti-inflammatory drugs decreased digestive tract malignancy mortalities, including colorectal and gastric adenocarcinoma (14,15). Consequently, the COX-2 enzyme is considered a potential therapeutic marker for the prevention and treatment of cancer. COX-2 reactivity has been studied to relate the expression of COX-2 with clinicopathological features in gastric adenocarcinoma. Perineural invasion, which is also called neurotropic carcinomatous spread or perineural spread, is the infiltration of neural fascicles or perineurium by tumor cells. Recent studies have proven that the presence of perineural invasion is an independent prognostic factor for survival and may contribute to the understanding of relapse or metastasis (16). A number of reports have related COX-2 expression to clinicopathological data from gastric can-

Table 3. The association between HER-2 expression and clinicopathologic features

		Negative (n=19)	Positive (n=37)	p value	
Gender	Male	28 (70)	6 (66.7)	6 (85.7)	0.801
	Female	12 (30)	3 (33.3)	1 (14.3)	
Age		60 (18-84)	64 (44-78)	63 (52-88)	0.342
Depth of invasion	T1	3 (7.5)	2 (22.2)	0	0.341
	T2	6 (15)	0	0	
	T3	25 (62.5)	7 (77.8)	7 (100)	
	T4	6 (15)	0	0	
Histological	G1	1 (2.5)	0(0)	1 (14.3)	0.196
Grade	G2	14 (35)	6 (66.7)	3 (42.9)	
	G3	25 (62.5)	3 (33.3)	3 (42.9)	
Perineural invasion	Absent	18 (45)	6 (66.7)	5 (71.4)	0.274
	Present	22 (55)	3 (33.3)	2 (28.6)	
Vascular invasion	Absent	18 (45)	6 (66.7)	2 (28.6)	0.368
	Present	22 (55)	3 (33.3)	5 (71.4)	
Intestinal Metaplasia	Absent	18 (45)	5 (55.6)	4 (57.1)	0.761
	Present	22 (55)	4 (44.4)	3 (42.9)	
H. Pylori	Absent	34 (85)	6 (66.7)	5 (71.4)	0.371
	Present	6 (15)	3 (33.3)	2 (28.6)	
Location	Proximal	20 (50)	6 (66.7)	4 (57.1)	0.762
	Distal	20 (50)	3 (33.3)	3 (42.9)	
Lymph node	Absent	11 (27.5)	3 (33.3)	2 (28.6)	0.899
involvement	Present	29 (72.5)	6 (66.7)	5 (71.4)	
Tumor size		27 (1.50-174)	13.30 (5.75-80)	28 (7.50-240)	0.432

Values were expressed as median (minimum-maximum) or n (%).

cers. Our results revealed that the expression of COX-2 was significantly correlated with perineural invasion (p=0,032). Furthermore, this outcome resembles the results from a previous study by Rehab et al. (17). In addition, this study indicated that gastric carcinoma cases that were positive for COX-2 expression were also associated with deeper invasion, lymph node involvement and vascular invasion, which was inconsistent with our results (17). Previous reports have shown that the expression of COX-2 was significantly correlated with deeper depth of invasion, vascular invasion, lymphatic invasion, and lymph node metastasis (18,19). We further demonstrated that COX-2 expression was useful as an independent predictor of perineural invasion.

The p53 gene is noted as the gate-keeper of the genome and acts as a tumor suppressor gene located on the 17p-chromosome, coding a protein of 53 kDa. This gene is known to be involved in cell cycle control and the regulation of programmed cell death. Functional inactivation or p53 mutations have been observed in approximately 50% of all human tumors, such as gastric carcinoma, colorectal carcinoma, breast carcinoma, and gallbladder carcinoma (20). In our study, the positive expression of p53 was shown in 66.1% of gastric adenocarcinoma cases. Some studies have detected p53 in 19-29% (21,22) of gastric adenocarcinomas, whereas others have demonstrated higher percentages of p53 in 34-65% (23,24) of gastric adenocarcinomas, similar to this study. Additionally, we have found that p53-positive tumors occur more frequently in elderly patients. Several studies have previously shown homogeneous results (9,23,25). Cancer accounts for more deaths than heart disease in people 85 years old and younger. Furthermore, cancer in older people remains a common problem due to the longevity of the general population. Thus, we suggest that p53 alterations play a critical role in gastric carcinogenesis in elderly patients.

The HER-2 protein is a 185 kDa glycoprotein encoded by a gene located in the long arm of chromosome 17. The main function of HER-2 is to mediate cell growth and differentiation (4). Trastuzumab, a humanized monoclonal antibody that targets HER-2, has recently become a chemotherapeutic agent for HER-2-positive breast cancers. HER-2-positive breast cancer patients who have received trastuzumab have been shown to exhibit a better prognosis compared with breast cancer patients that lack expression of HER-2 (26). Due to the importance of targeted adjuvant chemotherapy, previous studies have evaluated the HER-2 status in GA to correlate HER-2 expression with clinicopathological features and to predict prognosis (5-9). However, different results were obtained in these studies, but reached the same effect.

In the current study, we did not observe a statistically significant correlation between HER-2 overexpression and pathological features, such as age, gender, depth of invasion, histological grade, lymphatic and perineural invasion, lymph node involvement, tumor size, location, existence of H. pylori and intestinal metaplasia. Similar results were observed by Chua et al. (27) and Halon et al. (7). Chua et al. (27) performed a literature search from January 1990 to January 2011 and identified 49 published reports that investigated HER-2 immunoreactivity in gastric cancer tissue and the association of HER-2 with clinicopathologic characteristics and/or survival outcome. In the Chua et al. study, HER-2 protein expression was not associated with age (in 19 of 22 studies), gender (in 22 of 23 studies), tumor location (in 12 of 14 studies), tumor size (in 11 of 14 studies), tumor depth of invasion (in 15 of 22 studies), presence of lymphatic invasion (in 7 of 10 studies), presence of perineural invasion (in 4 of 4 studies) and presence of H. pylori infection (in 3 of 3 studies). Conversely, Lee et al. (9) showed a significant correlation between HER-2 immunoreactivity and older age, gender (males), and well-differentiated tumors. In addition, Wang et al. (28) revealed a relationship between HER-2 overexpression and older age, larger tumor size (≥5 cm), lymph node metastases, stage III/IV and the presence of lymphatic permeation. Therefore, various factors might explain these contradictory results, such as differences in the combination of

the cases, types of antibody detection methods, discrimination of the pathologist's score and the criteria for the evaluation of immunohistochemistry.

The current study demonstrated that COX-2 expression might serve as a powerful indicator for estimating perineural invasion, which is an independent worse prognostic factor for survival in gastric adenocarcinoma. Additionally, detecting the expression of p53 can assist with the treatment options for elderly patients with gastric adenocarcinoma. We did not observe a statistically significant correlation between HER-2 overexpression and pathological variables. However, recent studies have shown differing results for gastric cancer. This heterogeneity may originate from the use of different methods to determine HER-2 status, the subjectivity of the pathologist's observation, and the use of various scoring systems. Therefore, a common consensus needs to be established among pathologists. A better understanding of HER-2, COX-2 and p53 expression in gastric adenocarcinoma may improve the staging strategies and influence new treatment modalities.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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