Original Article

Expression of human epidermal growth factor receptor 2 and p53 in gastric cancer patients: Clinical and prognosis relevance

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ABSTRACT

Background: Increased evidence showed that human epidermal growth factor receptor 2 (HER2) and p53 play an important role in gastric cancer development and could have a prognostic relevance. We aimed to study the expression of HER2 and p53 in gastric adenocarcinomas and to investigate the correlation with clinicopathological parameters as well as the overall survival in Tunisian patients. **Patients and Methods:** A total of 95 patients who had undergone gastrectomy for gastric adenocarcinoma at Sfax University Hospital of Tunisia were included in this study. The expression of HER2 and p53 in tumor tissues was performed by immunohistochemistry. **Results:** Positive expression of HER2 was observed in 17.89% of cases while p53 nuclear expression was negative in 40%, moderate in 38.94%, and intense in 21.05% of tumor tissues. The expression of HER2 correlated significantly with patient's age ($P = 0.011$), histological type ($P = 0.005$), tumor differentiation ($P = 0.009$), and tumor node metastasis (TNM) stage ($P = 0.048$). On the other hand, the expression of p53 correlated with patient's age ($P = 0.028$), gender ($P = 0.022$), and TNM stage ($P = 0.029$). With regard to the prognostic value, negative nuclear expression of p53 alone or associated with loss of HER2 expression correlated significantly with prolonged overall survival (P log rank = 0.019 and 0.002, respectively). **Conclusion:** Our findings highlight the importance of HER2 and p53 proteins as predictive markers for prognosis allowing a better management of patients with gastric adenocarcinoma.

Key words: Gastric adenocarcinoma, human epidermal growth factor receptor 2, immunohistochemistry, p53, prognostic

INTRODUCTION

Despite the overall incidence of gastric cancer (GC) has declined in recent decades, GC still represents the second cause of cancer-related death worldwide.[1] Among the prognostic factors commonly used for GC, the Union for International Cancer Control tumor node metastasis (TNM) stage is the most important one. Nevertheless, there is a wide heterogeneity among individuals with the same tumor stage, which led for searching of biomarkers to identify subgroups of patients with different biological profiles that correlate more closely with a prognosis and/or response to treatment. In recent years, several biomarkers have been described and tested for their clinical relevance in GC management including oncogenes, tumor suppressor, growth factors, and receptors.[2,3] The oncogene human epidermal growth factor receptor 2 (HER2) codes for a transmembrane glycoprotein of 185 kDa composed of an extracellular ligand-binding domain, a membrane-spanning region, and a cytoplasmic tyrosine kinase domain. HER2 is one of the members of the HER family which plays an important role in normal development, differentiation, and

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apoptosis.[4] Overexpression of HER2 has been reported in many cancer types, including that of lung[5] and prostate.[6] In breast cancer, it has been reported that the overexpression of HER2 was detected in about 10%–34% of invasive breast cancers and was associated with poor prognosis.[7-9] The prognostic relevance of HER2 remains controversial in GC since conflicting data are reported in the literature.[10-14] The tumor suppressor gene p53 is involved in cell cycle regulation and has antiproliferative and antitransforming activities.[15,16] In GC, the p53 gene is inactivated essentially by missense mutations in the DNA binding domain affecting its function as transcription factor.[17] Mutations in p53 gene appear to be a crucial event in tumor development, and there is evidence indicating the association of mutated p53 and its overexpression in tumors.[18,19] Indeed, it is well documented that the mutant p53 protein has a prolonged half-life and can be therefore detected by immunohistochemistry contrary to the wild-type p53 protein whose level is controlled strictly by MDM2 through a feedback loop process.[20,21]

In this study, we analyze by immunohistochemistry the expression of HER2 and p53 in GCs specimens and next to correlate their expression levels with clinicopathological parameters and patients' survival.

PATIENTS AND METHODS

Retrospective analysis was performed on 95 nonconsecutive patients with gastric adenocarcinoma, surgically treated at the department of visceral surgery, at University Hospital Habib Bourguiba of Sfax, between 2009 and 2014. Histological data were reviewed from the corresponding hematoxylin and eosin stained slides. Clinicopathological parameters including gender, age, anatomical site, histological type, pathological stage, and tumor size were evaluated by reviewing medical charts and pathological records. The study was approved by the Local Ethical Committee of University Hospital Habib Bourguiba of Sfax.

Immunohistochemistry

Before immunostaining, pathologists (Najla Abid, Afef Khanfir) reviewed hematoxylin and eosin stained slides in each case and blocks representing invasive adenocarcinoma were selected. Briefly, a 4-µm section from each specimen was stained with H and E for histological staining evaluation, and representative specimens were chosen for immunohistochemical study. Tissues sections were attached on silanized slides, dewaxed in xylene, rehydrated in graded ethanol, and covered with 10 mM citrate buffer (pH = 6). After endogenous peroxidase blocking, sections were incubated for 45 min at room temperature with of primary mouse antihuman p53 monoclonal antibody (clone: DO-7, isotype IgG2b; Dako, dilution 1:50), then immunostained with secondary antibody and finally counterstained with hematoxylin. Scoring system of p53 was used to evaluate the immunoreactivity as previously reported.[22] Briefly, 0 = ≤5%, 1 = 6%–25%, 2 = 26%–75%, and 3 = 76%–100%, and the intensity of immunostaining was graded as follows: 0 (negative), 1 (weak), 2 (moderately positive), and 3 (strongly positive). The overall immunostaining score (IS) was calculated as follows: percentage score × intensity score. Cases were considered as negative when the IS = 0–1, moderate when the IS = 2–4, and intense when the IS >4. Only nuclear immunostaining was considered.

For HER2 immunoexpression, we used the Food and Drug Administration approved HercepTest™ kit (Dako) according to the manufacturer's protocol. The HER2 immunoscore was calculate as reported previously;[19] the immunostaining was assessed as follow –0: no reactivity or membranous reactivity in <10% of tumor cells, 1+: weak membranous reactivity in ≥10% of tumor cells, 2+: moderate complete or basolateral membranous reactivity in ≥10% of tumor cells, and 3+: strong complete or basolateral membranous stain in ≥10% of tumor cells. Only a membranous stain, either complete or incomplete, was considered meaningful. The HER2 immunoscore was considered as negative (score 0) and positive (score 1+, 2+, and 3+).

Statistical analysis

All statistical analyses were conducted using SPSS 20 software (SPSS, Inc., Chicago, IL) of windows, and $P < 0.05$ was considered as significant. The correlations between expression of both proteins and clinicopathological features were analyzed using Chi-square test. The Kaplan–Meier method with the log-rank test was used for univariate analysis of the correlation between protein expression and overall survival. Cox proportional hazard models were used to carry out multivariate survival analyses. $P < 0.05$ was considered significant.

RESULTS

Membranous expression of HER2 was positive in 17.89% of cases while 78 out of 95 tumors were negative [Figure 1a and b]. Nuclear immunostaining for p53 was negative in 40%, moderate in 38.94%, and intense in 21.05% of tumor specimens [Figure 1c and d]. As shown in Table 1, tumor differentiation and TNM stage correlated significantly with expression of HER2 ($P = 0.009$, $P = 0.005$, and $P = 0.048$, respectively). In addition, positive expression of HER2 was more frequent in tumors of older patients compared to those of patients < 60 years old ($P = 0.011$) [Table 1].

With regard to p53 expression, significant associations were seen with patient's age, gender, and TNM stage ($P = 0.028$, $P = 0.022$, and $P = 0.029$, respectively) [Table 1].

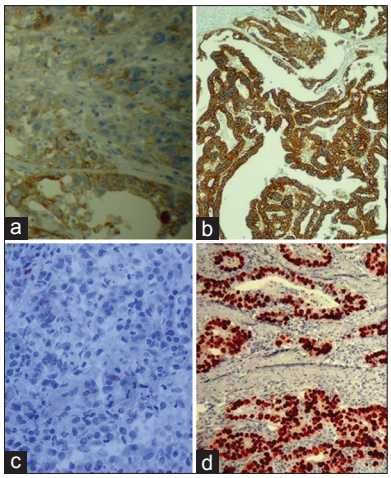

Figure 1: Immunohistochemical analysis of human epidermal growth factor receptor 2 and p53 protein expression in gastric adenocarcinoma. (a) Positive human epidermal growth factor receptor 2 immunostaining in tumor cell membrane (score 1+). (b) Positive human epidermal growth factor receptor 2 immunostaining in tumor cell membrane (score 3+). (c) Negative p53 nuclear immunostaining. (d) Intense p53 nuclear immunostaining

Within our patient cohort, the survival data were available for 70 out of 95 patients. The Kaplan–Meier plot showed that negative expression of p53 is significantly related to the overall survival (*P* log rank = 0.019) [Figure 2a] as well as for the expression of HER2. In fact, patients with negative expression of HER2 tend to have a prolonged survival time compared to those with positive HER2 expression (*P* log rank = 0.043) [Figure 2b]. The association becomes more statically significant if we consider the group of patients who are both negative for HER2 and p53 expression. Indeed, among patients who displayed negative expression for p53, those who are also negative for HER2 have a prolonged survival time compared to the group of patients p53−/HER2+ (*P* log rank = 0.002) [Figure 2c]. In addition, the effects of parameters associated with prognosis were studied by multivariate analysis using the Cox model. As a result, tumor site (*P* = 0.033), p53 expression (*P* = 0.04), and coexpression of p53/HER2 (*P* = 0.05) were revealed as independent prognostic factors [Table 2].

Table 1: Relationship between clinicopathological parameters and immunohistochemical expression of human epidermal growth factor receptor 2 and p53 in gastric adenocarcinoma

Parameters	*n*=95	p53 expression, *n* (%)			HER2 expression, *n* (%)	
		Negative (40)	Moderate (38.94)	Intense (21.05)	Negative (82.10)	Positive (17.89)
Gender						
Male	59	30 (50.8)	19 (32.2)	10 (16.9)	46 (78)	13 (22)
Female	36	8 (22.2)	18 (50)	10 (27.8)	32 (88.9)	4 (11.1)
P			0.022		0.17	
Age						
<60	49	22 (44.9)	22 (44.9)	5 (10.2)	45 (91.8)	4 (8.2)
≥60	46	16 (34.8)	15 (32.6)	15 (32.6)	33 (71.7)	13 (28.3)
P			0.028		0.011	
Differentiation						
Moderate well	40	16 (40)	12 (30)	12 (30)	28 (70)	12 (30)
Poor	55	22 (40)	25 (45.5)	8 (14.5)	50 (90.9)	5 (9.1)
P			0.132		0.009	
TNM						
I-II	37	15 (40.5)	16 (43.2)	6 (16.2)	27 (73)	10 (27)
III	48	20 (41.7)	20 (41.7)	8 (16.7)	44 (91.7)	4 (8.3)
IV	10	3 (30)	1 (10)	6 (60)	7 (70)	3 (30)
P			0.029		0.048	
Lauren type						
Intestinal	49	21 (42.9)	15 (30.6)	13 (26.5)	35 (71.4)	14 (28.6)
Diffuse	46	17 (37)	22 (47.8)	7 (15.2)	43 (93.5)	3 (6.5)
P			0.178		0.005	
Anatomical site						
Antrum	50	22 (44)	20 (40)	8 (16)	38 (76)	12 (24)
Body	28	9 (32.1)	11 (39.3)	8 (28.6)	26 (92.9)	2 (7.1)
Cardia	15	5 (33.3)	6 (40)	4 (26.7)	13 (86.7)	2 (13.3)
P			0.67		0.15	
Tumor size						
≤5	44	20 (45.5)	17 (38.6)	7 (15.9)	34 (77.3)	10 (22.7)
>5	46	16 (34.8)	19 (41.3)	11 (23.9)	40 (87)	6 (13)
P			0.49		0.23	
HP						
Negative	39	17 (43.6)	11 (28.2)	11 (28.2)	31 (79.5)	8 (20.5)
Positive	26	12 (46.2)	12 (46.2)	2 (7.7)	20 (76.9)	6 (23.1)
P	0.094				0.805	

HER 2: Human epidermal growth factor receptor 2, TNM: Tumor node metastasis, HP: Helicobacter pylori

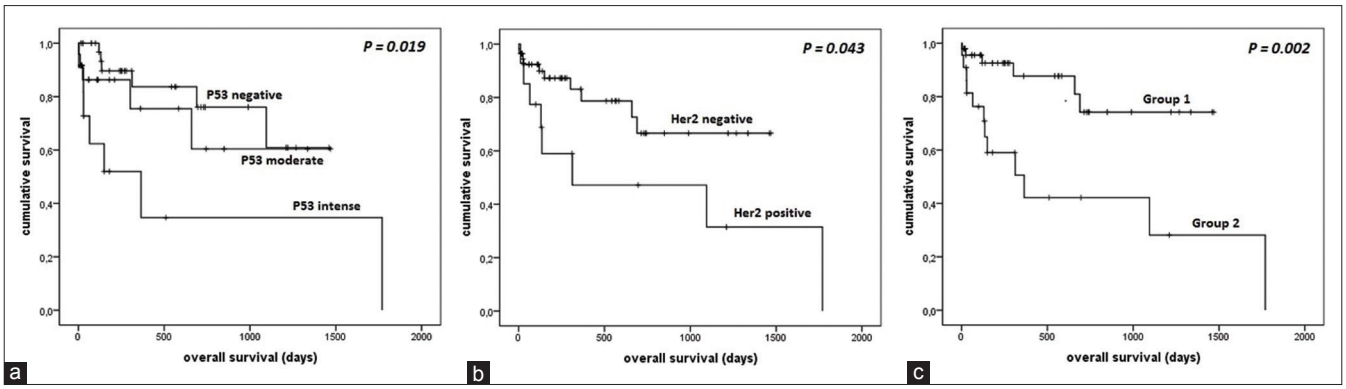

Figure 2: Kaplan–Meier survival analysis. (a) Overall survival curves of seventy gastric cancer patients according to p53 expression ($P = 0.019$). (b) Overall survival curves of patients according to human epidermal growth factor receptor 2 expression ($P = 0.043$). (c) Overall survival curves of patients showing negative p53 expression combined with negative human epidermal growth factor receptor 2 (Group 1) or positive human epidermal growth factor receptor 2 (Group 2) expression ($P = 0.002$)

Table 2: Multivariate survival analysis using the Cox proportional hazards model

Covariates	*P*	HR*	95.0% CI* (lower-upper)
Differentiation	0.936	0.956	0.314-2.909
Age	0.152	2.317	0.733-7.319
Metastasis	0.06	3.676	0.919-14.712
N-stage	0.629	0.687	0.15-3.149
pT-stage	0.324	2.89	0.35-23.844
Tumor localization	**0.033**	2.289	1.07-4.937
Expression of p53	**0.04**	0.144	0.024-0.939
Expression of HER2	0.21	0.33	0.06-1.858
Coexpression of p53/HER2	**0.05**	2.257	0.974-5.231

Bold characters indicate significant *P* value. *HR: Hazard ratio, 95% CI: 95% confidence interval, HER 2: Human epidermal growth factor receptor 2

DISCUSSION

HER2 amplification and overexpression have been reported in various cancers including GC.[23] Determining the HER2 status has an important impact for therapy since patients with advanced GC overexpressing the oncogene are eligible for target treatment with trastuzumab combined with conventional chemotherapy.[24] According to the published data, HER2 overexpression/amplification ranges between 8.2% and 53.4% in GC.[11,25-27] In our study, we found that 17.89% of tumor tissues showed are positive for expression which is in agreement with previous studies. In one large series conducted on 1414 cases of whole tissue sections and 595 cases of tissue microarrays (TMAs), HER2 positivity was detected in 12.3% of whole tissue sections and 17% of TMAs.[25] It has been generally reported that HER2 overexpression is correlated with aggressive biological behavior and poor prognosis.[5,28-31]

In our study, no relationship was observed between HER2 positivity and gender, tumor site, and size of lesion ($P > 0.05$). However, intestinal-type and moderate to well-differentiated tumors showed a higher HER2-positive rate than diffuse-type and poorly differentiated tumors which is in concordance with previous studies.[32-34] Moreover, in our cohort, significant associations were seen with age at diagnosis and TNM stage.

The survival analysis according to HER2 positivity revealed that the prognostic outcome of HER2-positive cases is poor compared to those with negative expression of HER2; nevertheless, we should confirm this finding on larger cohort since the follow-up is available for only 70 out of 95 patients. The value of HER2 as a prognostic factor in GC has been controversial; however, recent studies indicate that HER2 is a poor prognostic factor in GC patients.[11,12,27-29]

In gastric carcinogenesis, p53 mutations are frequent and appear, from the early stage of the malignancy.[35,36] It is well known that wild-type p53 protein induces cell apoptosis, whereas the accumulation of the mutant form promotes uncontrolled cell proliferation, resulting in tumor development. The intracellular accumulation of p53 protein is generally mutant forms, in comparison with the wild-type p53 which is usually negative in normal tissues due to its very short half-life.[18] In our present study, moderate and intense expression of p53 was observed, respectively, in 38.94% and 21.05% of gastric adenocarcinoma cases and correlated positively with gender and patient's age. On the other hand, a significant association was seen between intense expression of p53 and advanced TNM stage. Indeed, 60% of tumors at stage IV displayed a nuclear intense p53 staining. However, unlike previous studies, we did not find any relationship between p53 expression and biological behavior of tumors such as tumor size, differentiation, and histological types.[37-39]

The prognostic value of p53 expression in GC remains controversial. While some authors support the relationship of p53 with survival, others report that p53 overexpression is not related to patients outcome in GC.[40,41] In our study, we showed that p53 expression is inversely correlated with the survival since negative p53 nuclear immunostaining confers to patients a longer survival rate compared to those with

positive p53 expression. When we consider the expression of both proteins, no association is seen between p53 and HER2 ($P = 0.11$) contrary to previous studies that reported strong correlation between p53 expression and HER2 positivity.[42,43] On the other hand, in the group of patients displaying negative p53 expression, those with negative expression of HER2 have a significant better overall survival time compared to those showing positive expression of HER2. This finding suggests that p53 and HER2 expression have prognosis relevance in GC; nevertheless, we should confirm this result on larger cohort.

CONCLUSION

Our study indicated that intense expression of p53 was more frequent in tumors at advanced TNM stage while HER2 expression was significantly less frequently observed in diffuse type and poor differentiated tumors. Overexpression of HER2 and p53 was related to poor survival rate, and in the group of patients with negative p53 expression, those positive for HER2 still have a shorter survival rate. Altogether, our findings emphasize the prognosis relevance of HER2 and p53 in GC; nevertheless, a long-term follow-up is needed to confirm this association.

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Conflicts of interest
There are no conflicts of interest.

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