Clinicopathological correlations with p53 expression in gastric cancer patients with curative resection

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Purpose: Mutations in the p53 gene and accumulation of p53 protein are the most common genetic events in gastric carcinomas. This study evaluates the association of p53 gene expression with clinicopathologic findings and prognosis of gastric cancer patients who underwent curative resection.

Methods: Retrospective analysis was performed on 236 consecutive patients with gastric cancer who underwent surgery at the Kosin University Gospel Hospital between December 2011 and September 2012. Nuclear p53 expression was detected by immunohistochemistry.

Results: p53 expression was detected in 213 patients (90.3%). There was no significant association between positivity for p53 and clinicopathological features. Expression of p53 (P < 0.001), tumour, node, metastasis (TNM) stage (P < 0.001), T stage (P < 0.001), N stage (P < 0.001), Lauren classification (P = 0.016), neural invasion (P = 0.001), lymphatic invasion (P < 0.001), vascular invasion (P < 0.001), and recurrence (P < 0.001) were identified as independent risk factors for overall survival (OS) in univariate analysis. However, on multivariate regression analysis, there was no statistically significant association with OS.

Conclusion: In this study, there was no correlation between expression of p53 and clinicopathological features. Although p53 expression was identified as a risk factor for OS in univariate analysis, it had no statistical significance on multivariate regression analysis.

Keywords: Gastric cancer, p53, Immunohistochemistry, Clinicopathological characteristics, Prognosis

INTRODUCTION

Gastric carcinoma is a major cause of morbidity and mortality worldwide. The most reliable prognostic factors are tumor stage and completeness of excision, although tumor grade and histological type may also be useful.

METHODS

This descriptive analytic study included a total of 236 consecutive
patients with gastric cancer who were treated by curative resection without any preoperative therapy in the Department of Surgery of Kosin University College of Medicine from 2011–2012. The medical records and surgical specimens of these patients were retrospectively evaluated after obtaining approval from the Investigational Review Board of Kosin University Gospel Hospital. The disease stage was determined according to the (American Joint Committee on Cancer)-tumour, node, metastasis (TNM) classification (seventh edition) [4]. Patients with pathologic confirmation of gastric cancer were selected. All participants signed an informed written consent for use of their biopsy samples. Clinicopathological parameters, including age, gender, tumor location, histological classification, pathological TNM stage, neural invasion, lymphatic invasion, and vascular invasion status were retrieved from medical charts or pathology reports. Histological classification was determined according to Lauren’s classification and the World Health Organization (WHO) classification [5].

**Immunohistochemistry**

Tumor tissues were fixed in 10% formalin and embedded in paraffin. Immunohistochemical staining was performed using antibody for p53 (DO-7 1:100; Dako, Glostrup, Denmark). After preparing slices using a microtome, 4-µm tissue sections were immersed in xylene solution to remove residual paraffin and hydrated in an alcohol series. Sections were boiled in citrate buffer (pH 6.0) for 5 minutes to retrieve antigenicity and incubated for 30 minutes at room temperature. After exhausting endogenous peroxidase by incubation with H2O2 in methyl alcohol for 10 minutes, sections were washed three times with phosphate-buffered saline (PBS) and then blocked for 30 minutes with blocking solution (HistostainTM kit, Zymed Company, San Francisco, CA, USA) at room temperature. Sections were incubated with antibody specific for p53 at room temperature, rinsed three times with PBS, and incubated with biotinylated anti-mouse IgG (1:300; Zymed). After washing, the sections were incubated with avidin-alkaline phosphatase for 7 minutes at 40°C. Sections were visualized by incubation with red chromogen at 40°C and counterstained using the Mayer hematoxylin method. Expression of p53 was regarded as positive when more than 10% of the tumor cells displayed nuclear immunostaining (Fig. 1).

**Patient follow-up and statistical analysis**

According to the study protocol, patients were asked to return for follow up and oncological assessment every 6 months.

Statistical evaluation was performed using Spearman’s correlation test to analyze rank data. Survival analysis was carried out using the Kaplan–Meier method and multivariate survival analysis was performed using Cox proportional hazards regression models. The significance tests were two-sided and P-values < 0.05 were considered statistically significant. PASW ver. 18.0 (SPSS Inc., Chicago, IL, USA) was applied to analyze all data and P < 0.05 was considered to indicate a statistically significant difference.

**RESULTS**

**Demographic characteristics**

Patient demographics and tumor-related factors are summarized in Table 1. This study included 236 patients with gastric cancer. Of these, 164 (69.5%) were men and 72 (30.5%) were women and the mean age of all patients was 60.74 years (range, 34–85 years). The location of the tumor within the stomach was in the lower third in 125, in the middle third in 66, and in the upper third in 38 cases. In accordance with the WHO classification standards, 121 patients were incubated with antibody specific for p53 at room temperature, rinsed three times with PBS, and incubated with biotinylated anti-mouse IgG (1:300; Zymed). After washing, the sections were incubated with avidin-alkaline phosphatase for 7 minutes at 40°C. Sections were visualized by incubation with red chromogen at 40°C and counterstained using the Mayer hematoxylin method. Expression of p53 was regarded as positive when more than 10% of the tumor cells displayed nuclear immunostaining (Fig. 1).

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**Fig. 1.** Expression of p53 in gastric cancer cell by immunohistochemistry. (A) Diffuse (×200). (B) Intestine (×200).
(51.3%) had well- or moderately-differentiated tumors, and 115 patients (48.7%) had poorly-differentiated carcinoma. According to the pathological depth of tumor, 148 patients (62.7%) were pT1a, 28 (11.9%) were pT1b, 0 (0.0%) were pT2, 35 (14.8%) were pT3, and 25 (10.6%) were pT4a.
25 (10.6%) were pT4a. Regarding tumor stage, 163 (69.1%) were stage I, 32 (13.6%) were stage II, and 41 (17.4%) were stage III.

Correlation of p53 status with clinicopathological features
The correlation between p53 and clinicopathological features is shown in Table 2. Positivity for p53 was detected in 213 (90.3%) patients. There was no significant association between p53 expression and clinicopathological features.

Correlation of p53 expression with survival
According to the study protocol, all 236 patients were successfully followed for survival analysis. The median follow-up time was 27.09 months (range, 9–32 months); 12 patients (5.1%) died of cancer whereas the remaining 224 patients (94.9%) were still alive at the end of the study period. Expression of p53 (P < 0.001), TNM stage (P < 0.001), T stage (P < 0.001), N stage (P < 0.001), Lauren classification (P = 0.016), neural invasion (P = 0.001), lymphatic invasion (P < 0.001), vascular invasion (P < 0.001), and recurrence (P < 0.001) were identified as an independent risk factors for overall survival (OS) in univariate analysis. On multivariate regression analysis, there was a statistically significant association between OS and TNM stage (P < 0.001) (Table 3).

DISCUSSION
The p53 gene is a tumor suppressor gene located on chromosome 17p13.1 and the single most common target for genetic alterations in human cancer that are induced in response to genotoxic and non-genotoxic insults to cells [6]. Mutation of p53 results in a metabolically stable abnormal protein that accumulates in the nucleus and deregulates the cell cycle regulation required for DNA repair [7]. The reported incidence of p53 mutations in invasive carcinomas ranges from 0% to 76.9% [8,9]. More than one mutation may be present in a single tumor [10] and there can be heterogeneity of the p53 mutational status within a given tumor [11].

The mutated nuclear p53 protein can be detected by immunohistochemistry. In our study, p53 expression was detected in 90.3% of patients. In previous studies the percentage of patients with gastric cancer that showed p53 positivity was 54.05% [12], 60% [9], and 43% [13].

Under normal conditions, wild-type protein, which is stabilized via a mechanism other than mutation—for example, by binding with Simian virus 40 T antigen, which greatly increases the half-life of the protein [16]. Varied range of results has been reported for p53 expression in gastric cancer and its correlations with clinicopathological features in several studies. We demonstrated p53 expression in 90.3% of cases, which is more frequent than previous studies ranging from 25.2% to 57.5%. Genetic studies for the confirmed detection of p53 mutations were not conducted in this study, only its expression was considered positive.

Al-Moundhri et al. [17] reported that the prevalence of p53 expression in their patients with gastric cancer was 54% and also noticed that expression of p53 was associated with more aggressive tumor behavior and was an independent prognostic factor. A study by Tzanakis et al. [18] also suggested that more pronounced expression of p53 was a prognostic factor. However, a study by Petersson et al. [19] found no significant association between p53 expression and sex, age, disease stage, and pathologic type of tumor. Our study also showed no significant association between p53 expression and clinicopathological features of the patients.

Goncalves et al. [7] noted that p53 expression was more frequent among gastric intestinal-type, differentiated, and macroscopically elevated cancers. Significantly shorter survival times were observed in p53-positive patients compared with p53-negative patients. Tzanakis et al. [18] demonstrated that more marked expression of p53 was associated with a tumor size > 5 cm, and that p53 expression in advanced stage disease was significantly lower in poorly differentiated adenocarcinoma compared with well-differentiated or moderately differentiated adenocarcinoma.

The degree of p53 expression correlates with the proliferative rate of the tumors [20], possibly explaining the higher incidence of p53 positivity in intestinal versus diffuse gastric cancer (diffuse gastric cancer tends to have low proliferative rates).
ities seem to occur earlier in intestinal type cancers than in diffuse types and there is a tendency for p53 [21-23].

Young patients (aged < 40) have a lower incidence of p53 mutations than older individuals [24]. Some studies show that advanced gastric cancers tend to have a higher rate of p53 mutation and that there is a relationship between the presence of p53 mutations and aneuploidy [25], although not all studies report similar associations [26]. However, most studies agree that p53 mutations are more common in tumors arising in the cardia than in tumors arising in the antrum [10,24].

It has been reported that p53 mutations are more common in metastatic than in primary gastric carcinomas and that the percentage of mutations in gastric cancer cell lines in general is much higher than that seen in primary gastric cancer [8,27,28]. Furthermore, gastric cancers containing p53 mutations are much more likely to metastasize than those without mutations [29,30]. The risk of metastasis is further magnified if the mutations are at hot spots (codons 175, 248, and 243) and at non-CpG sites [30]. The presence or absence of mutation combined with the immunohistochemical score may also serve as a prognostic marker. After adjusting for depth of invasion and nodal status, Shiao et al. [30] found that p53 mutations of any type combined with the lowest or highest level of protein accumulation (scores of 0 or 4, respectively) independently predicted regional metastasis in gastric cancer.

In the present study, we found that p53 expression had no correlation with clinicopathological features. p53 expression was identified as a risk factor for OS in univariate analysis, but did not retain statistical significance on multivariate regression analysis. These data are not concordant with a previous findings that p53 overexpression is an independent adverse prognostic factor for survival [18].

The limitations of this study include the relatively short period follow-up, particularly with respect to addressing oncologic issues. Therefore, long-term follow-up in a larger study population is needed.

In summary, we assessed p53 expression in 236 samples from consecutive surgical cases of gastric cancer. The overall p53 positivity rate was 90.3%. There was no correlation between expression of p53 and clinicopathological features of patients. Although p53 expression was identified as a risk factor for OS in univariate analysis, it had no statistical significance on multivariate regression analysis.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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