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Mutant pattern of p53 predicts local recurrence and poor survival rate in gastric cancer

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Summary. Background. *TP53* mutation is a poor prognostic factor for various organ malignancies such as colorectal cancer, breast cancer, ovarian cancer, hepatocellular carcinoma, lung adenocarcinoma and clinical pathologists previously evaluated it using immunohistochemistry for p53. The clinicopathologic significance of p53 expression in gastric cancer remains unclear due to inconsistent classification methods.

Methods. Immunohistochemistry for p53 protein was performed using tissue microarray blocks generated from 725 cases of gastric cancer, and p53 expression was divided into three staining patterns using a semi-quantitative ternary classifier: heterogeneous (wild type), overexpression, and absence (mutant pattern).

Results. Mutant pattern of p53 expression had a male predominance, greater frequency in cardia/fundus, higher pT stage, frequent lymph node metastasis, local recurrence clinically, and more differentiated histology microscopically compared with wild type. In survival analysis, p53 mutant pattern was associated with worse recurrent-free survival and overall survival rates, and significance was maintained in subgroup analysis of early versus advanced gastric cancers. In Cox regression analysis, p53 mutant pattern was a significant predicting factor for local recurrence (relative risk (RR=4.882, $p<0.001$)) and overall survival (RR=2.040, $p=0.007$). The p53 mutant pattern remained significant for local recurrence (RR=2.934, $p=0.018$) in multivariate analyses.

Conclusions. Mutant p53 pattern on immunohistochemistry was a significant prognostic factor for local recurrence and poor overall survival in gastric cancer.

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Introduction

TP53 is a well-known critical tumor suppressor gene, and mutation at this site has been reported to be a poor prognostic factor for various organ malignancies including breast cancer, colorectal cancer, lung adenocarcinoma, hepatocellular carcinoma, and ovarian carcinoma (Munro et al., 2005; Russo et al., 2005; Olivier et al., 2006; Kobel et al., 2010, 2016; Li et al., 2019). In clinical pathology, *TP53* gene mutation status was previously evaluated by immunohistochemistry (IHC), because IHC is widely used and inexpensive compared with molecular diagnostic techniques such as polymerase chain reaction. Several studies to determine *TP53* mutation status using IHC for p53 were attempted, and p53 expression patterns on IHC have correlated well with molecular evaluations of *TP53* mutations, which were mainly performed for gynecologic cancers (Yemelyanova et al., 2011; Kobel et al., 2016; Singh et al., 2020). A study of ovarian carcinomas found that heterogeneous nuclear staining for p53 could be classified into wild type, diffuse strong staining, and complete loss (Kobel et al., 2016). Recently, *TP53* mutation evaluated by p53 IHC has been included as a major factor in the molecular risk classifier for endometrial cancer (Alexa et al., 2021).

In gastric cancer, *TP53* is one of the most important factors in the molecular subgroup (Cristescu et al., 2015). Based on The Cancer Genome Atlas (TCGA) project, gastric cancers have the following molecular subtypes: Epstein-Barr virus (EBV)-positive, microsatellite instability (MSI), genomic stability (GS), and chromosomal instability (CIN) subtypes (Cancer Genome Atlas Research, 2014), and these molecular subtypes also have prognostic significance. Among these molecular subtypes, EBV and GS have been reported as

the best and worst prognostic subgroups, respectively, and TCGA risk score was reported as an independent prognostic factor in multivariate analysis (Sohn et al., 2017). CIN subtype also has notable clinical significance due to adjuvant chemotherapy having the greatest benefit for this subtype, and the *TP53* tumor suppressor gene is an important player as shown by the frequency of *TP53* mutation (71%) (Cancer Genome Atlas Research, 2014; Sohn et al., 2017).

Two meta-analyses were published in 2015 that evaluated the clinicopathological significance of *TP53* mutation in gastric cancer. These papers concluded that p53 overexpression on IHC was a poor prognostic factor (Wei et al., 2015; Yildirim et al., 2015). However, studies included in these meta-analyses used various criteria to measure p53 expression, mainly a binary classifier with a 10% cut-off value. Recently, a few studies attempted to apply various reading criteria for p53 IHC to predict *TP53* mutation status in gastric cancer (Hwang et al., 2020; Schoop et al., 2020). However, there is still no consensus classifier for p53 expression on IHC that accurately reflects *TP53* mutation status and serves as a prognostic factor. Therefore, in this study, we aimed to identify the clinicopathologic significance of p53 expression pattern on IHC by applying a semi-quantitative ternary classifier already validated in gynecological cancers.

Materials and methods

Patients and gastric cancer specimens

This study was approved by the regional Institutional Review Board of Kangbuk Samsung Hospital (approval no. 2020-11-033; Seoul, South Korea). We enrolled 725 patients who underwent gastrectomy or endoscopic resection due to gastric cancer at Kangbuk Samsung Hospital between January 2011 and December 2014. Clinical data including patient age, sex, and follow-up findings were obtained from electronic medical records.

Pathologic diagnosis and tissue microarray generation

The specimens were obtained by total gastrectomy, subtotal gastrectomy, and proximal gastrectomy and endoscopic submucosal resection in 110 (15.2%), 409 (59.4%), 2 (0.3%), and 204 (28.1%) cases, respectively. The specimens were fixed in 10% neutrally buffered formalin and the representative sections were submitted for paraffin blocks. Two pathologists (Y. Chung and K. Kim) reviewed all glass slides and recorded microscopic features including tumor type by WHO and Lauren classifications, pT and pN stages, tumor invasion to lymphovascular space and perineural tissue, and peritumoral dysplasia. For tissue microarray construction, one representative tumor core with a 2-mm diameter was obtained from each case. In the cases with synchronous tumors, one tumor was selected considering the following conditions: 1) highest pT stage and 2) largest size if the pT stage was the same.

Immunohistochemistry and interpretation

Immunohistochemistry was performed with TMA array blocks using primary antibody for p53 (clone DO-7, 1:200 dilution; DakoCytomation, Glostrup, Denmark) and aBOND III autostainer (Leica Biosystems Nussloch GmbH, Nussloch, Germany) according to the manufacturer's protocol.

For measuring p53 expression, a semi-quantitative ternary classifier was used a previously reported method and cut-off value determined through consensus by two pathologists (Y. Chung and K. Kim). The three observed patterns were 1) heterogeneous (wild type), nuclear staining with various intensity and percentage; 2) overexpression, diffuse strong staining in more than 90% of tumor cells; 3) absence, no staining in more than 90% of tumor cells (Kobel et al., 2016). Tissue microarray slides were interpreted by two pathologists (Y. Chung and K. Kim); in cases of discrepancies in microscopic findings, the two pathologists reached a consensus after review.

Statistical analysis

Data were analyzed using PASW Statistics 18 software (SPSS Inc., Chicago, IL, USA). Crosstabs, Pearson's chi-square test, and Fisher's exact test were used, as appropriate. Kaplan-Meier and Cox regression tests were employed to analyze survival and metastasis data. Differences were regarded as statistically significant at $p<0.05$.

Results

Overall clinical features of patients and p53 expression patterns

Patient median age was 62 years (range: 29-91 years). Patients were followed up over 55.8 mean months after gastric operation or endoscopic procedure. During the follow-up period, local recurrence and distant metastasis occurred in 34 (4.7%) and 41 (5.7%) patients, respectively, and 59 patients (8.1%) died from gastric cancer. On IHC for p53, heterogeneous pattern, overexpression, and absence was observed in 424 (58.5%), 209 (28.8%), and 92 (12.7%) cases, respectively, and the representative cases with each staining pattern are shown in Figure 1.

Clinical features according to p53 staining pattern

Comparing the clinical features between wild type and mutant pattern showed (Table 1), many significant differences, including greater frequency of male patients ($p=0.001$), higher pT stage ($p<0.001$), and more frequent lymph node metastasis ($p=0.007$) in mutant pattern of

p53. Most notably, local recurrence was more common among mutant pattern (p<0.001), although there was no difference in resection margin involvement between the two groups. In addition, cardia/fundus (21 out of 40 cases, 52.5%) was the most common intragastric location of the mutant pattern, compared to body (101 out of 298 cases, 33.9%) and antrum (170 out of 377 cases, 45.1%)(p=0.004). There was no significance difference in distant metastasis and presence of synchronous tumor between the two groups. In subgroup analysis divided into early and advanced gastric cancer, local recurrence remained significantly correlated with mutant pattern in both subgroups (p<0.001 and p=0.040 in the early and advanced groups, respectively; Table 2). However, male predominance (p=0.001) and higher frequency in the cardia/fundus than body and antrum (52.4% vs. 32.0% and 44.2%, p=0.011) in mutant pattern were only significant in early gastric cancers.

Histological features according to p53 staining pattern

Mutant pattern showed differentiated histology, including frequent tubular adenocarcinoma of WHO classification (p<0.001), intestinal type of Lauren classification (p<0.001), and absence of signet-ring cell components (p<0.001; Table 3). In subgroup analysis between early and advanced cases, these histologic associations remained significant for early gastric cancer only (Table 4). The histologic features indicating tumor

Table 1. Clinical differences according to p53 staining patterns.

	Total cases	Wild type	Mutant pattern	*p*-value
Sex				0.001
male	519 (71.6)	283 (66.7)	236 (78.4)	
female	206 (28.4)	141 (33.3)	65 (21.6)	
Location				0.004
cardia/fundus	40 (5.6)	19 (4.5)	21 (7.2)	
body	298 (41.7)	197 (46.6)	101 (34.6)	
antrum	377 (52.7)	207 (48.9)	170 (58.2)	
Synchronous tumor				>0.999
absent	694 (95.7)	406 (95.8)	288 (95.7)	
present	31 (4.3)	18 (4.2)	13 (4.3)	
Stage by AJCC 8th edition				<0.001
I	283 (58.5)	192 (63.8)	91 (49.7)	
II	94 (19.4)	60 (19.9)	34 (18.6)	
III/IV	107 (22.1)	48 (16.3)	58 (31.7)	
Lymph node metastasis				0.007
absent	328 (67.8)	218 (72.4)	110 (60.1)	
present	156 (32.2)	83 (27.6)	73 (39.9)	
Distant metastasis				0.141
absent	684 (94.3)	405 (95.5)	279 (92.7)	
present	41 (5.7)	19 (4.5)	22 (7.3)	
Resection margin involvement				0.212
absent	708 (97.7)	417 (98.3)	291 (96.7)	
present	17 (2.3)	7 (1.7)	10 (3.3)	
Local recurrence				<0.001
absent	691 (95.3)	416 (98.1)	275 (91.4)	
present	34 (4.7)	8 (1.9)	26 (8.6)	

Values are presented as number of cases (%).

Table 2. Clinical differences according to p53 staining patterns in two groups: early and advanced gastric cancer.

	Early gastric cancer (n=522)				Advanced gastric cancer (n=203)			
	Total	Wild	Abnormal	*p*-value	Total	Wild	Abnormal	*p*-value
Sex				0.001				0.221
male	378 (72.4)	211 (67.2)	167 (80.3)		141 (69.5)	72 (65.5)	69 (74.2)	
female	144 (27.6)	103 (32.8)	41 (19.7)		62 (30.5)	38 (34.5)	24 (25.8)	
Location				0.011				0.288
cardia/fundus	21 (4.0)	10 (3.2)	11 (5.3)		19 (9.8)	9 (8.3)	10 (11.8)	
body	206 (39.5)	140 (44.6)	66 (31.9)		92 (47.4)	57 (52.3)	35 (41.2)	
antrum	294 (56.4)	164 (52.2)	130 (62.8)		83 (42.8)	43 (39.4)	40 (47.1)	
Synchronous tumor				0.658				0.512
absent	500 (95.8)	302 (96.2)	198 (95.2)		194 (95.6)	104 (94.5)	90 (96.8)	
present	22 (4.2)	12 (3.8)	10 (4.8)		9 (4.4)	6 (5.5)	3 (3.2)	
Depth of invasion				0.851				
mucosa	342 (65.5)	207 (65.9)	135 (64.9)					
submucosa	180 (34.5)	107 (34.1)	73 (35.1)					
Lymph node metastasis				0.747				0.070
absent	260 (89.0)	175 (88.8)	85 (89.5)		68 (35.4)	43 (41.3)	25 (28.4)	
present	32 (11.0)	22 (11.2)	10 (10.5)		124 (64.6)	61 (58.7)	63 (71.6)	
Distant metastasis				0.280				0.107
absent	519 (99.4)	311 (99.0)	208 (100.0)		165 (81.3)	94 (85.5)	71 (76.3)	
present	3 (0.6)	3 (1.0)	0 (0)		38 (18.7)	16 (14.5)	22 (23.7)	
Resection margin involvement				0.514				0.146
absent	513 (98.3)	309 (98.4)	204 (98.1)		195 (96.1)	108 (98.2)	87 (93.5)	
present	9 (1.7)	5 (1.6)	4 (1.9)		8 (3.9)	2 (1.8)	6 (6.5)	
Local recurrence				<0.001				0.040
absent	510 (97.7)	313 (99.7)	197 (94.7)		181 (89.2)	103 (93.6)	78 (83.9)	
present	12 (2.3)	1 (0.3)	11 (5.3)		22 (10.8)	7 (6.4)	15 (16.1)	

Values are presented as number of cases (%).

Table 3. Histological features according to p53 staining patterns.

	Total cases	Wild type	Mutant pattern	*p*-value
WHO classification				<0.001
tubular	446 (61.5)	218 (51.4)	228 (75.7)	
poorly cohesive	163 (22.5)	122 (28.8)	41 (13.6)	
mucinous	6 (0.8)	3 (0.7)	3 (1.0)	
carcinoma with lymphoid stroma	22 (3.0)	20 (4.7)	2 (0.7)	
mixed	88 (12.1)	61 (14.4)	27 (9.0)	
Lauren classification				<0.001
intestinal	432 (59.6)	208 (49.1)	224 (74.4)	
diffuse	186 (25.7)	138 (32.5)	48 (15.9)	
mixed	105 (14.5)	76 (17.9)	29 (9.6)	
unclassifiable	2 (0.3)	2 (0.5)	0 (0)	
Signet-ring cell component				<0.001
absent	496 (68.4)	247 (58.3)	249 (82.7)	
present	229 (31.6)	177 (41.7)	52 (17.3)	
Lymphovascular invasion				0.101
absent	539 (74.3)	325 (76.7)	214 (71.1)	
present	186 (25.7)	99 (23.3)	87 (28.9)	
Perineural invasion				0.599
absent	616 (85.0)	363 (85.6)	253 (84.1)	
present	109 (15.0)	61 (14.4)	48 (15.9)	
Peritumoral dysplasia				0.385
absent	651 (89.8)	377 (88.9)	274 (91.0)	
present	74 (10.2)	47 (11.1)	27 (9.0)	

Values are presented as number of cases (%).

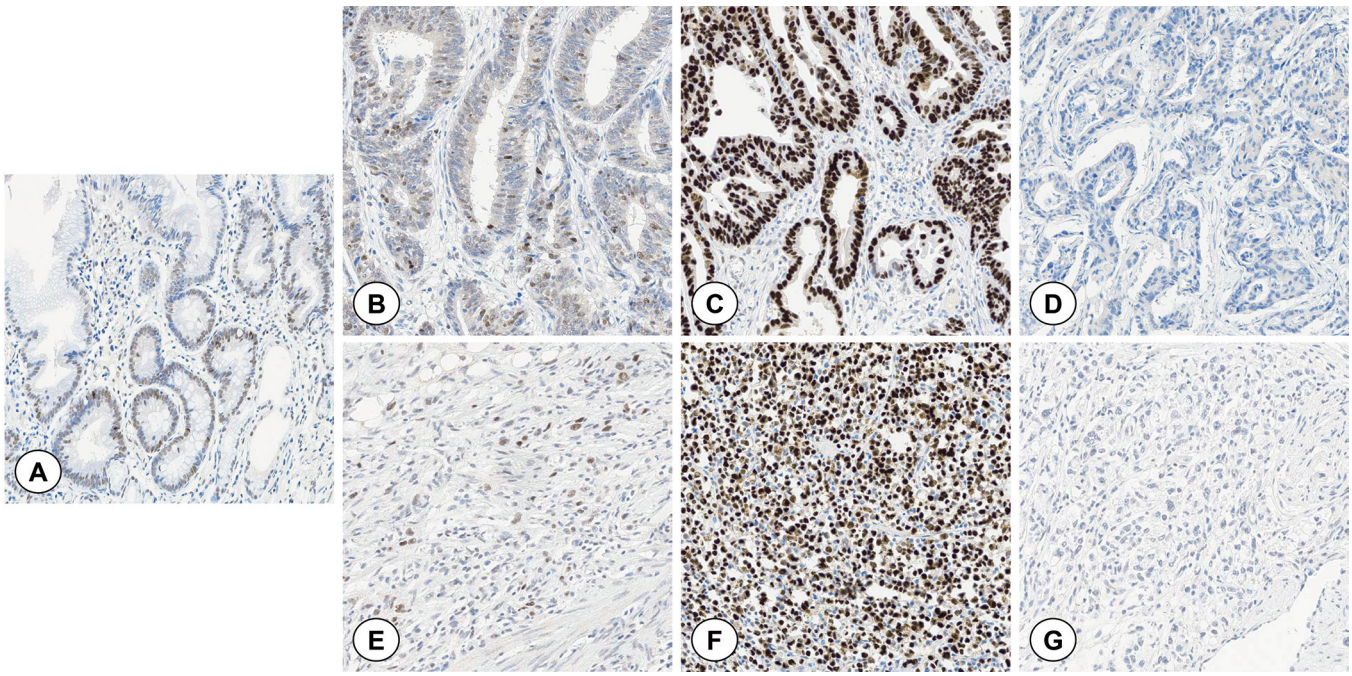

Fig. 1. Representative figures of immunohistochemistry for p53 with normal gastric mucosa **(A)**, intestinal-type gastric cancer **(B-D)**, and diffuse-type gastric cancer **(E-G)**. Normal gastric mucosa heterogeneous nuclear staining with various staining intensity **(A)**. Heterogeneous nuclear positivity with variable staining intensity is regarded as wild type **(B and E)**. Diffuse positivity more than 90% with strong nuclear staining **(C and F)** and rare stained tumor cells less than 10% **(D and G)** are regarded as overexpression and absence pattern, respectively. x 200.

invasiveness such as lymphovascular or perineural invasion were not related to p53 staining patterns, even in subgroup analysis between early and advanced cases (Tables 3, 4). Additionally, the presence of peritumoral dysplasia showed no correlation with p53 expression patterns (Tables 3, 4).

Survival analysis stratified by p53 expression patterns

Patients with p53 mutant pattern showed poor recurrence-free and overall survival rates compared with patients with wild type ($p<0.001$ and $p=0.006$, respectively; Fig. 2A,B). In subgroup analysis, the prognostic power of p53 mutant pattern for recurrence-free survival remained significant for both early and advanced gastric cancers ($p<0.001$ and $p=0.014$, respectively; Fig. 2C,D).

In Cox regression analysis, the p53 mutant pattern was a significant predicting factor for local recurrence (relative risk (RR)=4.882, $p<0.001$; Table 5) and overall survival (RR=2.040, $p=0.007$; Table 6). The mutant pattern remained significant in multivariate analysis for local recurrence (FF=2.934, $p=0.018$), adjusting for pT stage, and lymph node metastasis, distant metastasis, and p53 staining pattern (Table 5). For patient's survival, only pT stage and distant metastasis were significant in multivariate analysis including Lauren classification, pT stage, lymph node metastasis, distant metastasis, and p53 staining pattern (Table 6).

Discussion

In this study, we confirmed that a mutant pattern of p53 on IHC can be a prognostic factor for local recurrence and overall survival in gastric cancer patients. Additionally, gastric cancers with p53 mutant pattern

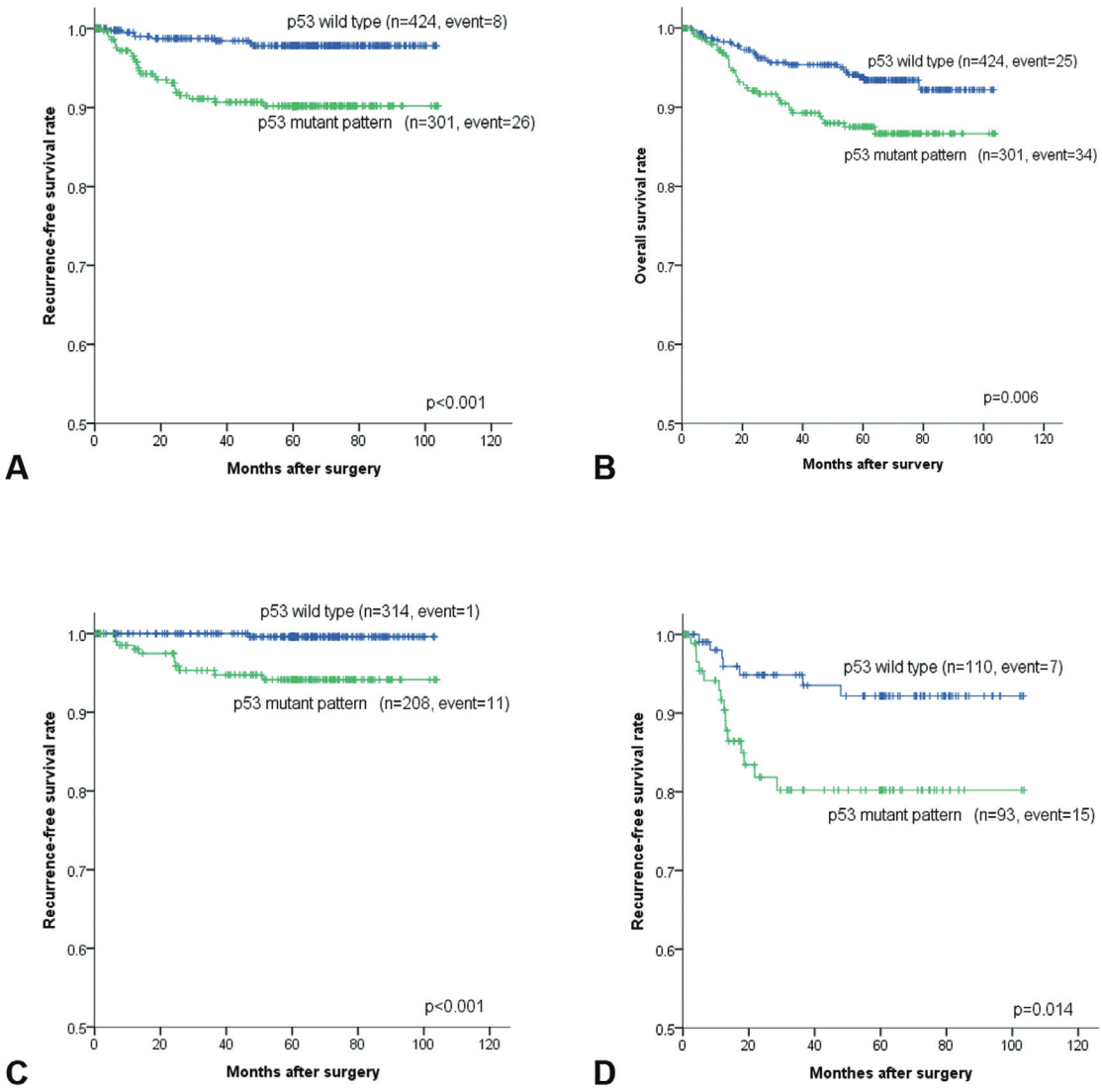

Fig. 2. Kaplan-Meier graphs for recurrence-free survival **(A, C, and D)** and overall survival **(B)** according to p53 expression pattern. Mutant pattern of p53 shows low recurrence-free **(A)** and overall survival **(B)** rates in all patients. In subgroup analysis, the recurrence-free survival rate is lower in mutant pattern than wild type in both early **(C)** and advanced **(D)** gastric cancer subgroups.

showed specific clinicopathologic features of male predominance, greater frequency in cardia/fundus, high pT stage, and more frequent lymph node metastasis. On histology, tubular subtype in WHO classification and intestinal type of Lauren classification were more frequent, while signet-ring cell was less common. Such a component tended to remain significant in early gastric cancers in subgroup analysis but not in advanced gastric cancers.

One of the main findings of our study was that p53 mutant pattern was associated with short recurrence-free survival period. And the most notable difference from previous studies was that we included not only surgical specimens, but also endoscopically resected gastric cancer tissues in this study, minimizing a selection bias that would be possible due to the limit of endoscopic resection. A recent large Korean study used multivariate analysis to show that p53 overexpression was a poor predictive factor for both overall survival in gastric cancer and recurrence in diffuse type gastric cancer (Kim et al., 2021). And in another European study, p53 overexpression was associated with both high recurrence rate and poor disease-specific survival in gastric cancer (Fondevila et al., 2004). Because these two previous reports applied a binary classifier with a 10% cut-off value to define p53 overexpression, a direct comparison with our results was not possible. However, with syntheses of results from this study and previous studies, we can suggest the mechanism of action behind TP53 mutation in gastric cancer recurrence. Our results indicate that cases with p53 mutant pattern had frequent local recurrence and shorter recurrence-free survival rates, but the presence of synchronous GC or remnant tumor in resection margins were not significantly different according to p53 expression patterns (Table 1, Fig. 2A). A previous Korean study reported that well-known risk factors for gastric cancer, such as *Helicobacter pylori* infection, atrophic gastritis, and intestinal metaplasia in gastric mucosa, showed non-significant or no correlation with p53 expression in gastric cancer (Kim et al., 2021). Therefore, it is presumed that the frequent recurrence of gastric cancer among cases with p53 mutant pattern is not due to remnant gastric cancer or precancerous lesions in the remaining gastric mucosa, but rather to the aggressive behavior of gastric cancer itself. To fully understand the contribution of *TP53* mutation to cancer recurrence, further studies with more cases and detailed molecular tests are needed.

There have been several recent attempts to develop a new reading method for IHC for p53 in gastric cancer to both effectively reflect *TP53* mutations and to use p53 expression as a predictive marker. With a large Central European cohort, Schoop et al. (2020) applied their own various IHC assessment algorithms to determine p53 positivity on IHC, but they did not provide an impact reading method to predict the prognosis of gastric cancer patients. In contrast, another Korean study by Hwang et

Table 4. Histologic features according to p53 staining patterns in two groups: early and advanced gastric cancer.

	Early gastric cancer (n=522)				Advanced gastric cancer (n=203)			
	Total	Wild type	Mutant	*p*-value	Total	Wild type	Mutant	*p*-value
WHO classification				<0.001				0.096
tubular	363 (69.5)	179 (57.0)	184 (88.5)		83 (40.9)	39 (35.5)	44 (47.3)	
poorly cohesive	100 (19.2)	87 (27.7)	13 (6.3)		63 (31.0)	35 (31.8)	28 (30.1)	
mucinous	1 (0.2)	0 (0)	1 (0.5)		5 (2.5)	3 (2.7)	2 (2.2)	
carcinoma with lymphoid stroma	11 (2.1)	10 (3.2)	1 (0.5)		11 (5.4)	10 (9.1)	1 (1.1)	
mixed	47 (9.0)	38 (12.1)	9 (4.3)		41 (20.2)	23 (20.9)	18 (19.4)	
Lauren classification				<0.001				0.065
intestinal	355 (68.0)	174 (55.4)	181 (87.0)		77 (37.9)	34 (30.9)	43 (46.2)	
diffuse	115 (22.0)	97 (30.9)	18 (8.7)		71 (35.0)	41 (37.3)	30 (32.3)	
mixed	50 (9.6)	41 (13.1)	9 (4.3)		55 (27.1)	35 (31.8)	20 (21.5)	
unclassifiable	2 (0.4)	2 (0.6)	0 (0)					
Signet-ring cell component				<0.001				0.147
absent	370 (70.9)	184 (58.6)	186 (89.4)		126 (62.1)	63 (57.3)	63 (67.7)	
present	152 (29.1)	130 (41.4)	22 (10.6)		77 (37.9)	47 (42.7)	30 (32.3)	
Lymphovascular invasion				0.891				0.107
absent	460 (88.1)	276 (87.9)	184 (88.5)		71 (35.0)	44 (40.0)	27 (29.0)	
present	62 (11.9)	38 (12.1)	24 (11.5)		132 (65.0)	66 (60.0)	66 (71.0)	
Perineural invasion				0.410				>0.999
absent	516 (98.9)	309 (98.4)	207 (99.5)		100 (49.3)	54 (49.1)	46 (49.5)	
present	6 (1.1)	5 (1.6)	1 (0.5)		103 (50.7)	56 (50.9)	47 (50.5)	
Peritumoral dysplasia				0.699				0.501
absent	450 (86.2)	269 (85.7)	181 (87.0)		201 (99.0)	108 (98.2)	93 (100.0)	
present	72 (13.8)	45 (14.3)	27 (13.0)		2 (1.0)	2 (1.8)	0 (0)	

Values are presented as number of cases (%).

al. (2020) divided the degree of p53 expression into three groups: strong expression (>10% of tumor cells with strong positivity), loss of expression (no nuclear staining of tumor cells), and weak expression (weak, scattered, or patchy positivity). They found that strong p53 expression was correlated with poor cumulative survival compared with patients who were p53 negative and with weak positive cases (Hwang et al., 2020). This study initially used a three-tier classifier, but the cut-off value for the strong positive group was 10%, and the negative and weak positive groups had the same prognostic significance, obtaining similar results as previous studies that used a binary classifier of a 10% cut-off value.

The use of p53 IHC for a surrogate marker for a molecular test for *TP53* mutation has been well validated in gynecological cancers. Currently, endometrial cancer is classified into four molecular subtypes, one of which is p53-mutant endometrial cancer (Herrington, 2020; Alexa et al., 2021). IHC for p53 is used as a diagnostic test, where the tumor is classified into a p53-mutant subtype when it shows "mutant-like staining" pattern (Talhouk et al., 2015; Herrington, 2020). The term "mutant-like staining" refers to diffuse strong nuclear expression, complete absence of nuclear staining, and cytoplasmic staining (Herrington, 2020). A review article reported that both "overexpression" and "overexpression or complete absence" on IHC for p53 showed high diagnostic accuracy (AUC=0.9088 and 0.9030, respectively) (Raffone et al., 2020). Also, in

Table 5. Cox regression analysis for local recurrence.

Variables	Univariate				Multivariate			
	p-value	Relative risk	95.0% CI		*p*-value	Relative risk	95.0% CI	
			upper	lower			upper	lower
Sex (female vs. male)	0.723	0.871	0.407	1.866				
Tumor location (antrum vs. non-antrum)	0.247	0.655	0.320	1.341				
Lauren classification	0.278							
diffuse vs. intestinal	0.973	1.014	0.441	2.333				
mixed vs. intestinal	0.125	1.919	0.834	4.413				
pT stage	<0.001				<0.001			
2 vs. 1	0.938	0.922	0.120	7.089	0.291	3.646	0.331	40.206
3 vs. 1	<0.001	5.522	2.435	12.523	<0.001	17.275	3.818	78.175
4 vs. 1	<0.001	13.367	5.728	31.198	<0.001	34.736	7.479	161.323
Lymph node metastasis (present vs. absent)	<0.001	9.759	3.639	26.175				
Distant metastasis (present vs. absent)	0.004	4.042	1.555	10.510				
P53 staining pattern (mutated vs. wild type)	<0.001	4.882	2.210	10.786	0.018	2.934	1.203	7.158

CI, confidence interval.

Table 6. Cox regression analysis for overall survival.

Variables	Univariate				Multivariate			
	p-value	Relative risk	95.0% CI		*p*-value	Relative risk	95.0% CI	
			upper	lower			upper	lower
Sex (male vs. female)	0.183	1.431	0.844	2.426				
Tumor location (antrum vs. non-antrum)	0.757	1.088	0.638	1.855				
Lauren classification	0.001							
diffuse vs. intestinal	<0.001	2.965	1.655	5.312				
mixed vs. intestinal	0.004	2.770	1.378	5.569				
pT stage	<0.001				<0.001			
2 vs. 1	0.002	16.760	2.800	100.308	0.066	6.288	0.884	44.728
3 vs. 1	<0.001	70.068	16.515	297.275	<0.001	20.124	4.583	88.361
4 vs. 1	<0.001	287.283	68.486	1205.095	<0.001	68.220	15.438	301.467
Lymph node metastasis (present vs. absent)	<0.001	16.871	7.991	35.619				
Distant metastasis (present vs. absent)	<0.001	51.277	29.829	88.146	<0.001	9.355	5.225	16.750
P53 staining pattern (mutated vs. wild type)	0.007	2.040	1.217	3.420				

CI, confidence interval.

ovarian cancer, p53 expression pattern on IHC has been a powerful method to predict the presence of *TP53* mutations (Yemelyanova et al., 2011; Cole et al., 2016; Kobel et al., 2016). The combination of diffuse strong positivity and complete absence patterns were well correlated with *TP53* mutation (94.4%) in molecular analysis (Yemelyanova et al., 2011).

While diffuse strong positive and complete negative patterns are highly correlated with *TP53* mutation status, as mentioned above, some uncertainty remains about the "p53 complete absence" pattern. In gastric cancers, strong and weak positivity on IHC were well-correlated with presence and absence of *TP53* mutation, respectively, but negative staining was representative of a mixed-population with only 55.6% *TP53* mutation (Hwang et al., 2020). In another gastric cancer study, 80% of p53 negative cases showed wild-type *TP53* (Ando et al., 2015). Poor staining quality and reading errors for IHC were likely causes of the high discrepancy between loss/absence of p53 on IHC and actual *TP53* mutation status, and careful optimization of p53 IHC and use of an internal control during reading were recommended (Cole et al., 2016; Raffone et al., 2020). Therefore, when setting p53 IHC to predict *TP53* mutation status, it is recommended to conduct molecular verification at least for the "absence pattern" group on IHC, and to standardize reading criteria by using an internal control to distinguish the absence pattern from weak positivity when reading IHC.

Additionally, we performed EBV in situ hybridization and mismatch repair (MMR) test with tissue microarray to classify molecular subtypes. Among 725 cases of gastric cancer, 37 (5.1%) were EBV-associated, although EBV status was unknown in 5 cases. Among 683 non-EBV cases, 93 (13.6) were MSI-high, and 590 were non-EBV/non-MSI. The clinicopathologic significance of p53 mutant pattern in non-EBV/non-MSI gastric cancers was shown in Supplementary Tables 1 and 2, and the results were similar to those of Tables 1 and 3 for all 725 cases. Therefore, one simple p53 IHC might make significant predictions, regardless of molecular subtype.

However, our study has several limitations. First, this study was conducted using only IHC for p53. This technique can be easily performed in a pathology clinic and can be a meaningful marker for gastric cancer. However, correlation with molecular tests is essential to support our results and apply them to clinical practice. Although previous reports supported good concordance between p53 expression pattern on IHC and *TP53* mutation status, a pattern of absence has shown unclear results (Yemelyanova et al., 2011; Ando et al., 2015; Kobel et al., 2016; Hwang et al., 2020). Therefore, additional molecular studies are needed to support our findings. Second, we used TMA blocks for IHC. Schoop et al. (2020) reported that p53 was stained heterogeneously in only 11.3% of their gastric cancer cohort, suggesting that using TMA blocks was sufficient to represent all tumor characteristics. However, multicenter and large-scale studies using whole block GC tissue would be needed to validate our results and apply the prognostic role of p53 as a recurrence predictive marker in clinical pathology fields. Third, we used a semi-quantitative ternary classifier to measure p53 expression with slightly different cut-off values from previous reports. Our values were determined through consensus of two pathologists who participated in microscopic measurement of p53 expression. We believe that this method can be used to achieve global consensus and expect that the measurement of p53 expression will be further improved using computer-associated automatic measurement.

In conclusion, mutant pattern of p53 on IHC, either overexpression or absence, showed significantly distinguishable clinicopathologic characteristics from wild type cases: male predominance, high frequency in the cardia/fundus and of intestinal type histology, and higher stage tumor. Particularly, p53 mutant pattern showed a high local recurrence rate and short recurrence-free survival rate, suggesting p53 expression on IHC as a useful predictor of local recurrence after curative resection of gastric cancer.

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Conflict of interest statement. The authors have no conflicts of interest to declare.

Ethics approval. This study protocol was approved by the Institutional Review Board of Kangbuk Samsung Medical Center (IRB number:2020-11-033) and was performed according to the ethical standards of the Declaration of Helsinki, as revised in 2008. The review conducted by our Institutional Review Board confirmed that informed consent was not necessary for this study.

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Authors' contributions. KK and HWL conceived and designed this study. YC, HWL, and KK participated in pathology review. BHS and CHY reviewed medical records. YC, HWL, and KK analyzed and interpreted the results and prepared the manuscript. KK oversaw the entire project and approved the submitted draft. All coauthors read and approved the submitted manuscript.

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