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ORIGINAL ARTICLE

Prediction of *TP53* mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer

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Background: Recently, molecular classifications of gastric cancer (GC) have been proposed that include *TP53* mutations and their functional activity. We aimed to demonstrate the correlation between p53 immunohistochemistry (IHC) and *TP53* mutations as well as their clinicopathological significance in GC. **Methods:** Deep targeted sequencing was performed using surgical or biopsy specimens from 120 patients with GC. IHC for p53 was performed and interpreted as strong, weak, or negative expression. In 18 cases (15.0%) with discrepant *TP53* mutation and p53 IHC results, p53 IHC was repeated. **Results:** Strong expression of p53 was associated with *TP53* missense mutations, negative expression with other types of mutations, and weak expression with wild-type *TP53* ($p<.001$). The sensitivity for each category was 90.9%, 79.0%, and 80.9%, and the specificity was 95.4%, 88.1%, and 92.3%, respectively. The TNM stage at initial diagnosis exhibited a significant correlation with both *TP53* mutation type ($p=.004$) and p53 expression status ($p=.029$). The Kaplan-Meier survival analysis for 109 stage II and III GC cases showed that patients with *TP53* missense mutations had worse overall survival than those in the wild-type and other mutation groups ($p=.028$). Strong expression of p53 was also associated with worse overall survival in comparison to negative and weak expression ($p=.035$). **Conclusions:** Results of IHC of the p53 protein may be used as a simple surrogate marker of *TP53* mutations. However, negative expression of p53 and other types of mutations of *TP53* should be carefully interpreted because of its lower sensitivity and different prognostic implications.

Key Words: Gastric cancer; p53; *TP53*; Next-generation sequencing; Immunohistochemistry

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TP53 is a tumor suppressor gene that encodes the protein p53, which is involved in cell cycle arrest in damaged cells that require DNA repair or in cases of damage beyond repair, triggering apoptosis. A defect in *TP53* is a crucial step in carcinogenesis. Previous studies noted that either a defect of the *TP53* gene itself or of a gene upstream or downstream of *TP53* was found in virtually all human cancers [1-3]. In gastric cancer (GC), p53 overexpression has been reported in 37.8%–54% of cases [4-6]. According to those studies, overexpression of p53 was generally associated with worse overall survival (OS) as well as well-known prognostic factors such as vascular invasion and lymph node metastasis.

In 2014, The Cancer Genome Atlas (TCGA) Research Network Group proposed a molecular classification of GC [6]. The four subgroups were Epstein-Barr virus (EBV)–positive, microsatellite instability, genomic stability, and chromosomal instability. *TP53* alteration is a characteristic of the chromosomal instability group. In the following year, the Asian Cancer Research Group (ACRG) presented a different molecular classification that considered the three factors of microsatellite instability, epithelial-mesenchymal transition, and *TP53* mutation [7]. The four groups classified by those factors exhibited different prognoses. However, one of the limitations of those two studies was that the methodology used requires high-end and high-cost technologies such as next-generation gene sequencing. Different groups have attempted to develop a more practical imple-

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mentation of the molecular classification of GC in clinical settings based on the biomarkers of TCGA and ACRG studies [8-10]. The immunohistochemistry (IHC) of p53 was used to practically predict the mutation status of *TP53*, but interpretation of p53 IHC was varied and has yet to be confirmed. Köbel et al. [11] demonstrated that optimal p53 IHC can accurately predict the mutation status of *TP53* in ovarian cancer, which can be very useful in diagnosis of high-grade serous carcinoma. This technique has yet to be validated for GC.

In this study, we aimed to measure the sensitivity, specificity, and accuracy of p53 IHC as a representation of *TP53* mutation status and to investigate the correlation between clinicopathologic features and p53 IHC or *TP53* mutations in GC. Therefore, we performed next-generation sequencing (NGS) and p53 IHC in 120 GC cases, and the *TP53* mutation statuses were compared with the p53 IHC results.

MATERIALS AND METHODS

Characterization of patients and sample acquisition

The study population was composed of 120 patients treated at Seoul National University Bundang Hospital (Seongnam, Korea) from 2009 to 2019. The median age was 60 years (range, 34 to 82 years), and 85 patients (70.8%) were men. Thirty-eight of the 120 cases (31.7%) were stage II at initial diagnosis, 71 (59.2%) cases were stage III, and 11 (9.2%) were stage IV. Among them, 109 stage II and III patients (90.8%) underwent curative radical resection (R0 resection) without preoperative chemotherapy or radiotherapy. In the 11 stage IV cases, endoscopic biopsy specimen was collected in one case, metastatectomy specimens in four cases, conversion surgery specimens after chemotherapy in five patients, and gastrectomy specimen in one case for the experiments. Analysis according to the World Health Organization (WHO) classification [12] revealed that tubular adenocarcinoma accounted for 54.2% (65 cases) of diagnoses, mucinous adenocarcinoma for 3.3% (4 cases), papillary adenocarcinoma for 3.3% (4 cases), poorly cohesive carcinoma for 30.0% (36 cases), and other minor histologic types for 9.2% (11 cases). For survival analysis, 109 patients with stage II and III GC were followed up from the date of surgery to the date of death or final follow-up. The median follow-up period was 42.2 months (range of 5.4-87.7 months).

Next-generation sequencing

Targeted sequencing of 170 cancer-related gene panels was performed using formalin-fixed, paraffin-embedded tissue (FFPE) samples as previously described [13]. All FFPE materials had a short cold ischemic time not exceeding 2 hours, fixation time ranging from 8 to 72 hours, and were aged between 0 and 9 years.

In brief, approximately 3 μg of genomic DNA was extracted from FFPE tumor tissues, and the sequencing library was prepared using an Agilent SureSelect Target Enrichment Kit (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's guidelines. High-throughput sequencing was performed using the HiSeq 2500 system (Illumina, San Diego, CA, USA) (Macrogen Inc., Seoul, Korea). After quality control of the FASTQ files, sequencing reads were aligned to the reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner-MEM (BWA-MEM) [14]. Single nucleotide variants and small insertions and deletions (INDELs) were detected using the MuTect2 algorithm [15]. SnpEff and SnpSift v4.3i [16] with dbNSFP v2.9.3 [17] were used for variant annotation with various databases including the OncoKB [18] and ClinVar archives [19].

IHC staining

Immunohistochemical (IHC) staining for p53 (DO7, mouse monoclonal, Dako, Agilent Technologies) was performed on 3-μm-thick slides using an automated immunostainer (BenchMark XT, Ventana Medical Systems, Tucson, AZ, USA) following the manufacturer's protocol. The p53 IHC was interpreted in three tiers: strong nuclear staining in more than 10% of the tumor cells was considered strong positivity, samples without any nuclear staining of tumor cells (complete absence) were interpreted as negativity, and cases exhibiting weak, scattered, or patchy positivity were regarded as weak positivity. Representative images for each category are shown in Fig. 1. Cut-offs of 20% and 30% nuclear positivity were additionally applied for validation of the results.

For cases where gene mutation and protein expression status did not match (18 cases or 15.0%), p53 IHC was repeatedly performed and interpreted. In most cases (17 out of 18 or 94.4%), repeated immunohistochemical assays did not alter the initial interpretation. Tumor heterogeneity accounted for the change in one case. Initially, strong nuclear expression of p53 was observed in some areas of the tumor (< 10%) but was not sufficient to be classified as strong expression. Subsequent IHC was performed on another section of the same tumor, exhibiting overall strong expression of p53.

EBV in-situ hybridization

The EBV status was tested using EBV in-situ hybridization as previously described [20]. A fluorescein-conjugated EBV en-

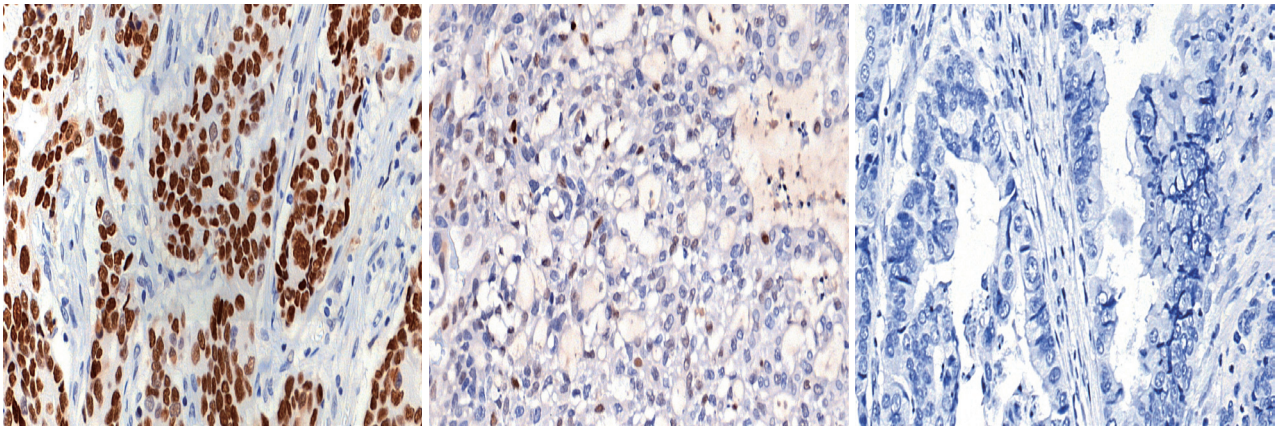

Fig. 1. Representative images of strong expression (A), weak expression (B), and loss of expression (C).

coded small RNA (EBER) oligonucleotide probe (INFORM EBVencoded RNA probe, Ventana Medical Systems) was used, and positive cases were defined as diffuse nuclear reactivity for EBER in tumor cells.

Microsatellite instability analysis

Representative tumor tissues and matched normal gastric mucosal tissues were selected for microsatellite instability (MSI) testing. Five NCI markers (BAT-26, BAT-25, D5S346, D17S250, and S2S123) amplified through polymerase chain reaction were analyzed using an automated sequencer (ABI 3731 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). MSI-high was defined as two or more markers with unstable peaks, MSI-low was defined as one unstable marker, and microsatellite stable was defined as no unstable marker.

Statistical analyses

Chi-square or Fisher exact tests were used to assess significant differences in the distribution of *TP53* mutations and p53 expression. For univariate survival analysis, Kaplan-Meier survival curves were plotted in 109 patients with stage II and III GC cases. The survival differences were compared using the log-rank test. For multivariate survival analysis, the Cox regression model was used. All statistical analyses were performed using SPSS Statistics ver. 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Gene mutation and protein expression correlation

Table 1 summarizes the p53 IHC results according to *TP53* mutations. *TP53* mutations were present in 52 cases (43.3%), of which missense mutations were the most common (33 of 52 cases, 63.5%). Strong expression was observed in 34 cases (28.3%) and negative expression was observed in 27 cases (22.5%). When *TP53* mutations were compared with p53 IHC, 30 of the 33 missense mutation cases (90.9%) exhibited strong p53 expression, but negative expression of p53 was the dominant pattern (15 cases, 78.9%) among the 19 cases of other types of mutations ($p < .001$). Based on clinical significance, 37 cases (30.1%) had pathogenic or likely pathogenic *TP53* mutations, of which 22 cases (59.5%) exhibited strong expression of p53, 13 cases (36.1%) negative expression, and two cases (5.4%) weak ex-

Table 1. Comparison between *TP53* genetic mutations and p53 immunohistochemistry

TP53 mutation	p53 expression by IHC			Total	p-value
	Strong	Negative	Weak		
Mutation status					<.001
Wild-type	1 (2.9)	12 (44.4)	55 (93.2)	68 (56.7)	
Mutation present	33 (97.1)	15 (55.6)	4 (6.8)	52 (43.3)	
Variant summary					<.001
Wild-type	1 (2.9)	12 (44.4)	55 (93.2)	68 (56.7)	
Missense	30 (88.2)	0	3 (5.1)	33 (27.5)	
Other	3 (8.9)	15 (55.6)	1 (1.7)	19 (15.8)	
Stop-gained	2 (5.9)	3 (11.1)	1 (1.7)	6 (5.0)	
Splice region	0	5 (18.5)	0	5 (4.2)	
Frameshift	0	7 (25.9)	0	7 (5.8)	
In-frame deletion	1 (2.9)	0	0	1 (0.8)	
Clinical significance[a]					<.001
Wild-type	1 (2.9)	12 (44.4)	55 (93.2)	68 (56.7)	
Pathogenic or likely pathogenic	22 (64.7)	13 (48.1)	2 (3.4)	37 (30.1)	
Uncertain significance	5 (14.7)	2 (7.4)	1 (1.7)	8 (6.7)	
Conflicting interpretation	6 (17.6)	0	1 (1.7)	7 (5.8)	
Total	34	27	59	120	

Values are presented as number (%).
[a]According to the ClinVar and OncoKB databases accessed on March 18, 2020.

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pression ($p < .001$). Nevertheless, most cases of uncertain significance (62.5%) and conflicting interpretations (85.7%) also showed strong expression of p53 by IHC.

Detailed information about *TP53* mutations and p53 expression status is shown in Table 2. Two mutations were observed in three cases, of which one representative mutation was included in this table. One among seven cases with *TP53* mutations of conflicting interpretations regarding pathogenicity had weak expression of p53 (case No. 27 in Table 2). There have been reports suggestive of the "likely benign" and "uncertain significance" nature of this mutation. The mutations c.659A > G, c.742C > T, c.817C > T, c.796G > A, c.1024C > T, and c.375G > A were found in two cases, and c.818G > A mutation was found in three cases. The IHC results matched in cases with the same mutation. In 44 cases with single nucleotide polymorphism, C:G to T:A conversion was observed in 32 (72.7%), C:G to A:T in four (9.1%), C:G to G:C in two (4.5%), T:A to C:G in four (9.1%), and T:A to G:C in two (4.5%).

Sensitivity, specificity, and accuracy of p53 IHC for predicting *TP53* mutations

In general, nonsynonymous mutations detected using NGS were related to strong p53 expression in IHC. Similarly, all other types of mutations tended to show negative expression, of p53 while cases with wild-type *TP53* exhibited weak protein expression. The sensitivity of strong expression of p53 by IHC for predicting nonsynonymous *TP53* mutations was 90.9%, sensitivity of negative expression for other types of mutations was 79.0%, and the sensitivity of weak expression for wild-type *TP53* was 80.9% (Table 3). The specificity for each category was 95.4%, 88.1%, and 92.3%, respectively. The accuracy for each category was 94.2%, 86.7%, and 85.8%, respectively. In addition, the sensitivity, specificity, and accuracy of p53 IHC at 20% and 30% cut-offs are shown in Supplementary Table S1. The sensitivity of strong expression of p53 for nonsynonymous *TP53* mutations was highest at the 10% cut-off.

Clinicopathological variables and protein expression correlations

The correlation between clinicopathological characteristics and *TP53* mutations or p53 expression status is summarized in Table 4. TNM stage at initial diagnosis was the only variable that showed significant correlation with both *TP53* mutation type and p53 expression status ($p = .004$ and $p = .029$, respectively). Of the 38 stage II gastric cancer cases, 27 (71.1%) did not exhibit any detectable mutations in the *TP53* gene, but five nonsynonymous (13.2%) and six other types of mutations (15.8%) were found. Strong p53 expression was found in seven of the 38 stage II cases (18.4%). Among the stage III cases, which accounted for 71 cases, the proportions of nonsynonymous gene mutations and strong expression of p53 mutations increased to 39.4% (28 cases) and 38.0% (27 cases), respectively. On the other hand, the proportions of wild-type *TP53* cases and weak expression cases decreased from 71.0% to 45.0% and from 55.2% to 43.6%, respectively.

TP53 mutations were more frequently observed in intestinal-type GC (25 of 45 cases, 55.6%) compared to the non-intestinal type (27 of 75 cases, 36.0%), but with borderline statistical significance ($p = .065$). Other clinicopathological variables such as sex, age, tumor location, and WHO classification were not statistically significant.

Survival analysis

One hundred nine patients with stage II and III GC at initial diagnosis were selected for survival analysis. The patients underwent curative surgery followed by adjuvant chemotherapy. Patients with any *TP53* mutations tended to have worse OS compared to those without mutations, although the difference was not statistically significant ($p = .227$). When OS was analyzed based on *TP53* mutation type, patients with nonsynonymous mutations had the worst OS, and the wild-type and other types of mutations exhibited similar OS ($p = .074$) (Fig. 2A). This trend became statistically significant when the nonsynonymous mutation group was compared to the combined wild-type and other mutation groups ($p = .028$) (Fig. 2B). The expression pattern of p53 was not significantly associated with patient OS ($p = .107$) (Fig. 2C), but it was statistically significant when strong expression of p53 was compared to the combined negative and weak expression cases ($p = .035$) (Fig. 2D). Patients with abnormal—negative and strong expression—expression did not exhibit a statistically significant survival difference compared to patients with weak expression ($p = .208$). The Kaplan-Meier survival curves of p53 expression status at 20% and 30% cut-offs were additionally plotted in Supplementary Fig. S1. The difference in survival was largest at the 30% cut-off. Multivariate Cox regression analysis showed that strong expression of p53 was associated with patient OS independent of stage with borderline significance ($p = .070$, data not shown). The presence of nonsynonymous missense mutations of *TP53* was not an independent prognostic factor in multivariate analysis ($p = .130$).

Table 2. Detailed information of *TP53* mutation and p53 expression status in gastric cancer patients with any *TP53* mutation

Case No.	Effect	Nucleic acid alteration	Amino acid alteration	Clinical significance[a]
1	Missense_variant	c.422G>A	p.Cys141Tyr	Pathogenic or likely pathogenic
2	Missense_variant	c.422G>T	p.Cys141Phe	Pathogenic or likely pathogenic
3	Missense_variant	c.455C>T	p.Pro152Leu	Pathogenic or likely pathogenic
4	Missense_variant	c.524G>A	p.Arg175His	Pathogenic or likely pathogenic
5	Missense_variant	c.535C>G	p.His179Asp	Pathogenic or likely pathogenic
6	Missense_variant	c.542G>A	p.Arg181His	Pathogenic or likely pathogenic
7	Missense_variant	c.659A>G	p.Tyr220Cys	Pathogenic or likely pathogenic
8	Missense_variant	c.659A>G	p.Tyr220Cys	Pathogenic or likely pathogenic
9	Missense_variant	c.701A>G	p.Tyr234Cys	Pathogenic or likely pathogenic
10	Missense_variant	c.725G>A	p.Cys242Tyr	Pathogenic or likely pathogenic
11	Missense_variant	c.734G>A	p.Gly245Asp	Pathogenic or likely pathogenic
12	Missense_variant	c.742C>T	p.Arg248Trp	Pathogenic or likely pathogenic
13	Missense_variant	c.742C>T	p.Arg248Trp	Pathogenic or likely pathogenic
14	Missense_variant	c.743G>A	p.Arg248Gln	Pathogenic or likely pathogenic
15	Missense_variant	c.772G>A	p.Glu258Lys	Pathogenic or likely pathogenic
16	Missense_variant	c.817C>T	p.Arg273Cys	Pathogenic or likely pathogenic
17	Missense_variant	c.817C>T	p.Arg273Cys	Pathogenic or likely pathogenic
18	Missense_variant	c.818G>A	p.Arg273His	Pathogenic or likely pathogenic
19	Missense_variant	c.818G>A	p.Arg273His	Pathogenic or likely pathogenic
20	Missense_variant	c.818G>A	p.Arg273His	Pathogenic or likely pathogenic
21	Missense_variant	c.380C>T	p.Ser127Phe	Conflicting interpretations of pathogenicity
22	Missense_variant	c.473G>C	p.Arg158Pro	Conflicting interpretations of pathogenicity
23	Missense_variant	c.481G>A	p.Ala161Thr	Conflicting interpretations of pathogenicity
24	Missense_variant	c.613T>C	p.Tyr205His	Conflicting interpretations of pathogenicity
25	Missense_variant	c.796G>A	p.Gly266Arg	Conflicting interpretations of pathogenicity
26	Missense_variant	c.796G>A	p.Gly266Arg	Conflicting interpretations of pathogenicity
27	Missense_variant	c.1015G>A	p.Glu339Lys	Conflicting interpretations of pathogenicity
28	Missense_variant	c.329G>A	p.Arg110His	Uncertain significance
29	Missense_variant	c.380C>A	p.Ser127Tyr	Uncertain significance
30	Missense_variant	c.476C>T	p.Ala159Val	Uncertain significance
31	Missense_variant	c.797G>T	p.Gly266Val	Uncertain significance
32	Missense_variant	c.400T>G	p.Phe134Val	Uncertain significance
33	Missense_variant	c.470T>G	p.Val157Gly	Uncertain significance
34	Frameshift_variant	c.331_332insAG	p.Leu111fs	Pathogenic or likely pathogenic
35	Frameshift_variant	c.381_391delCCCTGCCCTCA	p.Pro128fs	Pathogenic or likely pathogenic
36	Frameshift_variant	c.635_669delTTCGACATAGTGTGGTG GTGCCCTATGAGCCGCCT	p.Phe212fs	Pathogenic or likely pathogenic
37	Frameshift_variant	c.660_661delTG	p.Tyr220fs	Pathogenic or likely pathogenic
38	Frameshift_variant	c.747delG	p.Arg249fs	Pathogenic or likely pathogenic
39	Frameshift_variant	c.1169delC	p.Pro390fs	Pathogenic or likely pathogenic
40	Frameshift_variant	c.778_779delTC	p.Ser260fs	Uncertain significance
41	Conservative_inframe_deletion	c.529_546delCCCCACCATGAGCGCTGC	p.Pro177_Cys182del	Pathogenic or likely pathogenic
42	Stop_gained	c.159G>A	p.Trp53*	Pathogenic or likely pathogenic
43	Stop_gained	c.437G>A	p.Trp146*	Pathogenic or likely pathogenic
44	Stop_gained	c.586C>T	p.Arg196*	Pathogenic or likely pathogenic
45	Stop_gained	c.637C>T	p.Arg213*	Pathogenic or likely pathogenic
46	Stop_gained	c.1024C>T	p.Arg342*	Pathogenic or likely pathogenic
47	Stop_gained	c.1024C>T	p.Arg342*	Pathogenic or likely pathogenic
48	Splice_region_variant&synonymous_variant	c.375G>A	p.Thr125Thr	Pathogenic or likely pathogenic
49	Splice_region_variant&synonymous_variant	c.375G>A	p.Thr125Thr	Pathogenic or likely pathogenic
50	Splice_region_variant&synonymous_variant	c.375G>C	p.Thr125Thr	Pathogenic or likely pathogenic
51	Splice_acceptor_variant&intron_variant	c.920-1G>A		Pathogenic or likely pathogenic
52	Splice_donor_variant&intron_variant	c.96+1G>A		Uncertain significance (no report)

IHC, immunohistochemistry.
[a]According to the ClinVar and OncoKB databases accessed on March 18, 2020.

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Table 3. The sensitivity, specificity, and accuracy of p53 immunohistochemistry for predicting *TP53* mutation, cut-off 10%

TP53 mutation	Sensitivity (%)	Specificity (%)	Accuracy (%)
Nonsynonymous mutation by p53 strong expression	90.9	95.4	94.2
Other type mutation by negative expression of p53	79.0	88.1	86.7
Wild-type by weak expression of p53	80.9	92.3	85.8

Table 4. Clinicopathologic characteristics according to *TP53* mutation and p53 expression status

Characteristic	Total	*TP53* mutation				p53 expression			
		NS	Other	Wild	p-value	Strong	Negative	Weak	p-value
No.	120	33	19	68		34	27	59	
Age (yr)					0.248				0.470
<65	69 (57.5)	15 (45.5)	11 (57.9)	43 (63.2)		17 (50.0)	15 (55.6)	37 (62.7)	
≥65	51 (42.5)	18 (54.5)	8 (42.1)	25 (36.8)		17 (50.0)	12 (44.4)	22 (37.3)	
Sex					0.117				0.285
Male	85 (70.8)	27 (81.8)	15 (78.9)	43 (63.2)		27 (79.4)	20 (74.1)	38 (64.4)	
Female	35 (29.2)	6 (18.2)	4 (21.1)	25 (36.8)		7 (20.6)	7 (25.9)	21 (35.6)	
Location of tumor center					0.940				0.856
Lower third	53 (44.2)	15 (45.5)	10 (52.6)	28 (41.2)		16 (47.1)	11 (40.7)	26 (44.1)	
Middle third	33 (27.5)	9 (27.3)	4 (21.1)	20 (29.4)		7 (20.6)	8 (29.6)	18 (30.5)	
Upper third	34 (28.3)	9 (27.3)	5 (26.3)	20 (29.4)		11 (32.4)	8 (29.6)	15 (25.4)	
TNM at initial diagnosis					0.004				0.029
II	38 (31.7)	5 (15.2)	6 (31.6)	27 (39.7)		7 (20.6)	10 (37.0)	21 (35.6)	
III	71 (59.2)	28 (84.8)	11 (57.9)	32 (47.1)		27 (79.4)	13 (48.1)	31 (52.5)	
IV	11 (9.2)	0	2 (10.5)	9 (13.2)		0	4 (14.8)	7 (11.9)	
WHO classification					0.733				0.596
Papillary	4 (3.3)	1 (3.0)	1 (5.3)	2 (2.9)		1 (2.9)	2 (7.4)	1 (1.7)	
Tubular WD/MD	28 (23.3)	10 (30.3)	6 (31.6)	12 (17.6)		10 (29.4)	6 (22.2)	12 (20.3)	
Tubular PD	37 (30.8)	9 (27.3)	7 (36.8)	21 (30.9)		10 (29.4)	8 (29.6)	19 (32.2)	
PCC	36 (30.0)	8 (24.2)	3 (15.8)	25 (36.8)		7 (20.6)	7 (25.6)	22 (37.3)	
Mucinous	4 (3.3)	2 (6.1)	0	2 (2.9)		2 (5.9)	1 (3.7)	1 (1.7)	
Others	11 (9.2)	3 (9.1)	2 (10.5)	6 (8.8)		4 (11.7)	3 (11.1)	4 (6.8)	
Lauren classification					0.065				0.587
Intestinal	45 (37.5)	14 (42.4)	11 (57.9)	20 (29.4)		15 (44.1)	10 (37.0)	20 (33.9)	
Non-intestinal	75 (62.5)	19 (57.6)	8 (42.1)	48 (70.6)		19 (55.9)	17 (63.0)	39 (66.1)	
EBV					0.215				0.036
Negative	105 (87.5)	31 (93.9)	18 (94.7)	56 (82.4)		30 (88.2)	27 (100)	48 (81.4)	
Positive	15 (12.5)	2 (6.1)	1 (5.3)	12 (17.6)		4 (11.8)	0	11 (18.6)	
MSI					0.258				0.010
MSS/MSI-L	112 (93.3)	32 (97.0)	19 (100)	61 (89.7)		34 (100)	27 (100)	51 (86.4)	
MSI-H	8 (6.7)	1 (3.0)	0	7 (10.3)		0	0	8 (13.6)	

Values are presented as number (%).
NS, nonsynonymous; Other, other type mutation; wild, wild-type; WHO, World Health Organization; WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated; PCC, poorly cohesive carcinoma; EBV, Epstein-Barr virus; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, microsatellite instability-low; MSI-H, microsatellite instability-high.

DISCUSSION

TP53 is the most well-known tumor suppressor gene, and p53 IHC is a method used in daily practice as a surrogate marker in various cancer patients. In this study, we performed targeted deep sequencing for detecting various *TP53* mutations and IHC for p53 using a commercially available and validated primary antibody with an automatic immunostainer. Strong expression of p53 could predict nonsynonymous missense mutations of *TP53* with a sensitivity of 90.9%, specificity of 95.4%, and accuracy of 94.2%. However, weak expression of p53 was less specific (80.9%) for predicting wild-type *TP53*, and negative expression was less sensitive (79.0%) for predicting other mutations of *TP53*. These results suggest that p53 IHC can be used as a surrogate marker in predicting *TP53* mutations, especially for strong expression, to predict nonsynonymous mutations. There

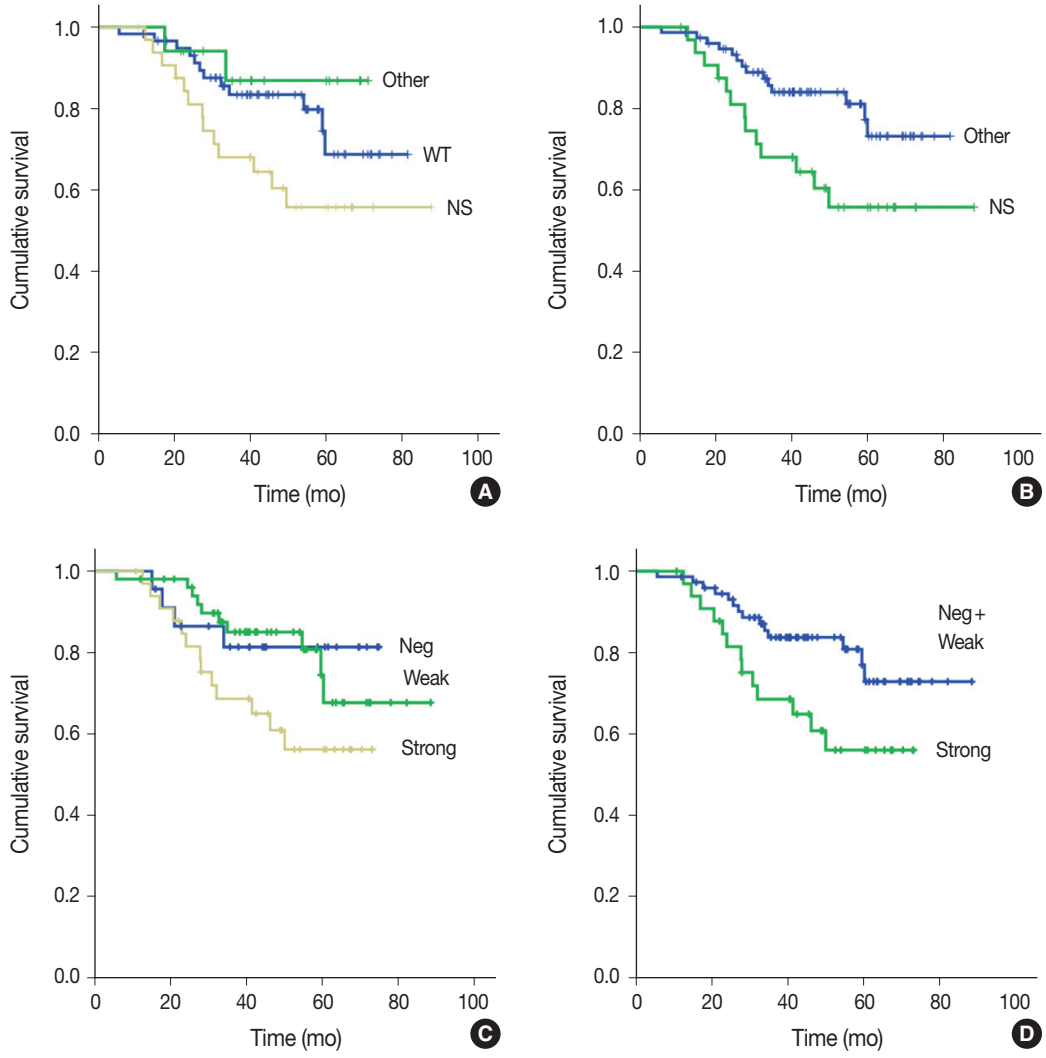

Fig. 2 . Kaplan-Meier survival curves of cumulative survival rate versus follow-up months after surgery according to mutation status (A, B) and immunohistochemistry results (C, D). (A) Nonsynonymous mutations (NS, beige) versus wild-type (WT, green) versus other types of mutations (other, green) (p = .074). (B) Nonsynonymous mutations (NS, green) versus combined wild-type and other types of mutations (other, blue) (p = .028). (C) Strong (beige) versus weak (green) versus negative expression (Neg) (p = .107). (D) Strong (green) versus combined weak and negative expression (Neg + Weak, blue) (p = .035).

have been recent attempts to use IHC for molecular classifications, and their clinicopathological significance has been increasingly important in GC [8,10]. Our results will be helpful for these new molecular classifications although negative expression should be cautiously interpreted.

Based on the Kaplan-Meier survival analysis, strong expression of p53 was significantly associated with worse OS compared to weak and negative expression of p53 in this study. Previous studies that investigated any relationship between p53 overexpression and survival reported p53 overexpression as a poor prognostic factor [21-23], similar to the findings from our study. Some studies did not reveal the prognostic significance of p53 overexpression in GC, but a meta-analysis demonstrated that it is a poor prognostic factor [24]. In those studies, the median cut-off value was 10% [24]. Therefore, we applied a cut-off value of 10% for defining strong expression of p53. In addition to the 10% cut-off, we applied 20% and 30% cut-offs in this study. Although the survival difference was largest at the 30% cut-off, the sensitivity of strong expression of p53 for predicting nonsynonymous *TP53* mutation was highest at the 10% cut-off. Therefore, further studies are needed to validate various cut-offs.

For interpretation of p53 IHC, Köbel et al. [11,25] proposed a three-tiered scoring system, including overexpression, complete absence, and normal or wild-type pattern in ovarian cancer. The

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scoring system exhibited good correlation with *TP53* mutation status: overexpression with nonsynonymous mutation; complete absence with stop gain, frameshift, and splicing mutations; and a normal pattern with the wild-type *TP53* gene [11]. Shin et al. [26] investigated the prognostic roles of p53 expression status in patients with GC. They defined group 0 as complete absence, group 1 as weak staining in < 50%, group 2 as strong staining in 50%–90%, and group 3 as strong staining in > 90%. When the Kaplan-Meier survival analysis was performed, group 1 was associated with better survival than groups 0, 2, and 3, but with borderline statistical significance. Our results showed a similar relationship between p53 IHC and *TP53* mutation status to those of previous studies. If weak expression in this study was defined as a normal or wild-type pattern, the Kaplan-Meier survival curves did not show a significant difference between normal and abnormal expression patterns. Furthermore, patients with GC having nonsynonymous *TP53* mutations had significantly worse prognosis compared to patients with other types of mutations and the wild-type *TP53* group. Similarly, strong expression of p53, which was related to nonsynonymous *TP53* mutations, was shown to be a poor prognostic factor. The complete absence of p53 expression or other types of *TP53* mutations might not be significant for predicting prognosis.

There were 17 cases (14.2%) with discrepant results between p53 IHC and *TP53* mutations. Most discrepant cases had negative expression of p53 and wild-type *TP53*. Weak expression of p53 was observed in four missense mutation cases and one stop gain mutation case. These findings might be due to tumor heterogeneity or tissue quality issues, such as specimen ischemic time or archival age. In addition, there was one case with weak p53 expression and a missense mutation of conflicting pathogenic interpretation. Considering this case was conflicting between uncertain significance and likely benign significance, one of the possible reasons for the discrepancy is a non-pathogenic mutation. To discriminate functional and nonfunctional p53, Nenutil et al. [27] performed IHC for p53, Ki67, MDM2, and p21 in human cancers, and overexpressed p53 without increased MDM2 indicated inactivating mutations in their study. p21, a transcriptional target of p53, was considered to reflect p53 activity and could decrease false-positive results of p53 expression [28]. The ACRG considered a *TP53*-activity signature using p21 and MDM2 genes [7]. Therefore, in addition to p53 IHC, IHC for p21 and MDM2 would be helpful for evaluating the functional status of p53. The need for these additional analyses reflects a potential limitation of this study, necessitating further research.

In a total of 120 gastric cancer cases, 52 (43.3%) had *TP53* mutations, including nonsynonymous missense, frameshift, stop gain, in-frame deletion, and splice region mutations. Hot spot mutations within the central core (R175, G245, R248, R273, and R282) were observed in a minority of cases (10 out of 120 or 8.3%). In accordance with our results, a previous study reported hot spot mutations in 6.2% of gastric cancer cases [29]. Therefore, sequencing (Sanger or NGS) is suggested as a suitable method for detecting *TP53* mutations in gastric cancer.

In summary, we investigated the relationship between p53 expression and *TP53* mutation status to predict *TP53* mutations by p53 IHC and reveal their prognostic significance. *TP53* mutations were observed in 43.3% of cases. Strong p53 expression could predict nonsynonymous missense mutations with high sensitivity and specificity, but only half of the p53 negative cases (55.6%) exhibited other types of *TP53* mutations. Protein overexpression and nonsynonymous genetic mutations of *TP53* significantly predicted worse OS. p53 IHC could be regarded as a simple surrogate marker of *TP53* mutations, but negative expression of p53 and other types of *TP53* mutations should be cautiously considered in daily practice or scientific research. Overall, our study results will be informative for simple molecular classification of patients with GC.

Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2020.06.01.

Ethics Statement

This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB number: B-2001/591-105). Written informed consent was waived by the IRB.

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Conflicts of Interest

W.H.K. and H.S.L., contributing editors of the *Journal of Pathology and Translational Medicine*, were not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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