Clinical significance of p53 protein expression and TP53 mutation status in colorectal cancer

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Research Article

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Abstract

In human colorectal cancer (CRC), *TP53* is one of the most important driver genes. Immunohistochemistry (IHC) has been used most often to assess the mutational status of *TP53*. Recently, next-generation sequencing (NGS) of the *TP53* gene has increased. However, to our knowledge, a comparison between *TP53* status evaluated by IHC and NGS has not been studied. Therefore, the primary aim of this study was to compare the clinical effect of *TP53* status evaluated by IHC and NGS in patients with CRC. The secondary aim was to investigate the correlation between expression of p53 by IHC and mutational status of *TP53* by NGS. We performed immunohistochemical staining of p53 and sequencing of *TP53* by NGS in 204 human samples of CRC. We then analyzed the correlation between mutational status of *TP53* and p53 expression, along with their prognostic impact in CRC patients. There was significant correlation between p53 expression and *TP53* mutation ($P < 0.001$), *TP53* mutation and higher N stage ($P = 0.024$), and positive p53 expression and higher N stage ($P = 0.003$). Positive p53 expression ($P = 0.018$) was significantly associated with overall survival (OS) of CRC patients by univariate analysis and was revealed as an independent prognostic factor by multivariate analysis. Additionally, the nonsense/frameshift (p53 proportion, 0%) p53 expression pattern showed a significantly better prognosis than the wild type and missense p53 expression patterns. However, the mutational status of *TP53* was not significant in OS of CRC patients. These results suggest that IHC expression of p53 protein correlates with mutation status of *TP53* and expression of p53 protein rather than mutation status of *TP53* has more significant impact on the OS of CRC patients.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death worldwide [1]. In human CRCs, *TP53* along with *APC*, *KRAS*, and *SMAD4* are frequently mutated genes by genome-wide analysis [2, 3]. Mutations of these genes are thought to contribute to the various properties of colon cancer cells, such as stemness, proliferation, dedifferentiation, impaired genome maintenance, invasiveness, and metastatic ability [4]. Among the genes that are frequently mutated, the *TP53* gene is one of the most important drivers for development and progression of CRC [5].

The well-known tumor suppressor p53, which is the product of the *TP53* gene, induces cell-cycle arrest, senescence, or apoptosis under cellular stress, such as DNA damage, hypoxia, nutrient depletion, and oncogenic signaling [6, 7]. The p53 protein promotes these responses by regulating target molecules, including p21, Puma, Tiger, and PAI-1 [8]. *TP53* mutations can be subdivided into missense mutations and nonsense/frameshift mutations. The *TP53* loss-of-function mutation promotes tumorigenesis due to decreased p53 target induction under cellular stress [5]. Accumulation of evidence indicates that missense-type mutations at the DNA binding domain of *TP53* can induce oncogenic function [9, 10].

CRC is reported to be the most common cancer entity that harbors *TP53* mutation, with 43.28% of CRCs reported to have *TP53* mutation (IARC *TP53* database, R20; https://p53.iarc.fr/TP53SomaticMutations.aspx, accessed on 27 October 2021). Therefore, the roles of alterations in *TP53* are actively studied in CRCs. *TP53* mutations have been reported to be correlated with the poor prognosis of patients with CRC [11, 12]. In patients with advanced stage of CRC with metastasis, the rate of *TP53* mutations is reported to be as high as 80% [13]. In addition, missense-type *TP53* mutations are reported to be associated with chemoresistance in CRCs [14].

Immunohistochemical staining of p53 has long been used as a surrogate marker for mutation status of *TP53*. However, because of the high yield in genes or genomic regions that can be evaluated by next-generation sequencing (NGS) at low cost and relatively faster turnaround time, sequencing of the *TP53* gene through NGS is increasing. Recently, there has been a study on the relationship between p53 IHC and *TP53* mutation status in ovarian cancer [15]. *TP53* mutations can be divided into missense and nonsense/frameshift mutations [15]. Missense mutations disturb MDM2-induced ubiquitination and degradation of p53, which lead to aberrant p53 accumulation in the nucleus [15]. Nonsense/frameshift mutations cause premature stop codons and trigger nonsense-mediated RNA collapse, and protein translation can be disrupted by

frameshifts or aberrant splicing [15]. A nonsense/frameshift mutation in *TP53* can cause a decrease or complete absence of p53 protein expression [15]. However, the interpretation of p53 IHC varies and has not been confirmed in many cancers including CRC.

In this study, we used IHC to investigate the cutoff value of p53 expression that is highly relevant to survival of CRC patients and the optimal cutoff value reflecting *TP53* mutation and compared the clinical significance of the two values. We do not believe that the optimal cutoff values reflecting mutations must coincide with those that best reflect the pathological role of p53 expression in cancer. Therefore, we believe it is meaningful to find and compare cutoff values of p53 expression that are highly relevant to prognosis and cutoff values that reflect the status of mutations. Furthermore, we aimed to compare the prognostic effects of p53 protein expression by IHC and *TP53* mutation status by NGS in CRC.

Materials And Methods

Patients and follow-up

In total, 204 patients with CRC who underwent surgery at Jeonbuk National University Hospital between May 2018 and May 2019 were enrolled in this study. Medical records were reviewed to obtain clinicopathologic information of sex, age, histologic grade, tumor location, tumor size, carcinoembryonic antigen (CEA), T stage, N stage, and TNM stage, as summarized in Table 2. For analysis, the entire colon was divided into the right and the left. The right-side colon was defined as the segment from the cecum to the proximal two-thirds of the transverse colon, and the left-side colon was defined as the segment from the distal one-third of the transverse colon to the rectum. Histologic slides were reviewed by two pathologists according to the WHO classification of tumors of the digestive system [16]. The TNM stage of the CRC patients was classified by the 8th edition of the American Joint Committee Cancer Staging System [17]. Postoperative surveillance for CRC patients was performed every 3 months. Laboratory tests including serum tumor marker CEA were performed. Abdominal computed tomography (CT) was used to detect recurrence and metastasis. This study was approved by the Institutional Review Board of Jeonbuk National University Hospital (IRB number, CUH 2019-04-053) and was conducted according to the Declaration of Helsinki.

Table 2
Correlation of TP53 mutation with two different cut-off points for immunohistochemical expression of p53

		p53 IHC		*p*	p53 IHC		*p*
		Wild type pattern (1 ~ 79%)	Aberrant type pattern (0% or ≥ 80%)		Negative (≤ 55%)	Positive (> 55%)	
All cases		53	151		80	124	
TP53 **mutation status**							
Wild type	55	34 (61.8%)	21 (38.2%)		38 (69.1%)	17 (30.9%)	
Mutant type	149	19 (12.8%)	130 (87.2%)	< 0.001	42 (28.2%)	107 (71.8%)	< 0.001

Next-generation sequencing (NGS)

Targeted NGS was performed using formalin-fixed paraffin-embedded (FFPE) tumor tissue. Hematoxylin and eosin-stained slides were reviewed, and tumor areas with sufficient viable tumor cells were marked and used as a guide for macrodissection. Areas with greater than 50% tumor volume were used for examination. In brief, total nucleic acid was isolated from tumor tissue using a RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion, Austin, TX, USA) according

to the manufacturer's specifications. After extracting DNA and RNA from FFPE specimens, library preparation for an Oncomine Comprehensive Assay v1 (OCAv1, Thermo Fisher Scientific, Waltham, MA, USA) was performed. An IonTorrent S5 XL platform was used for sequencing following the manufacturer's instructions. The OCAv1 is an amplicon-based targeted assay and includes the entire coding sequence of exons 2–11 of *TP53*. Reads were aligned to the hg19 reference genome, and variants with allele frequencies less than 3% were excluded. Genomic data obtained by sequencing were analyzed by IonReporter Software v5.6 (Thermo Fisher Scientific). Additionally, a manual review of the variant call format file and integrated genomic viewer was conducted.

Immunohistochemistry (IHC)

Immunohistochemical staining for p53 (clone: D07, dilution: ready to use, Roche Diagnostics, Mannheim, Germany) was performed using Ventana BenchMark ULTRA (Roche). Staining was performed on the whole section of the representative slide and was evaluated by two pathologists (KMK and MJC) without knowledge of the clinical status of the patient. Tumor cells were considered positive when they showed moderate to strong nuclear staining. Stained areas were semiquantitatively recorded for the area of positive cells. We set two cut-off points for immunohistochemical expression of p53. To investigate the prognostic impact of p53 expression in CRC patients, we performed receiver operating characteristic (ROC) curve analysis for patient survival with a cut-off point at 55%. Thereafter, CRC patients with p53 expression level equal to or less than 55% were classified as the negative expression group, and patients with greater than 55% expression were classified as the positive expression group.

Next, the p53 IHC cut-off value according to mutation type was obtained. The *TP53* mutation was classified into two types (missense and nonsense/frameshift mutations), and the cutoff values of p53 IHC reflecting each were investigated. ROC curve analysis for missense mutation was performed by NGS, and the cut-off point of p53 expression was 80%. For nonsense/frameshift mutations, a 1% cut-off point for p53 expression was set after ROC curve analysis. In summary, CRC patients with p53 expression showing 1 ~ 79% were classified as the wild type expression pattern, and other patients (0% or ≥ 80%) were classified as the aberrant type expression group.

Statistical analysis

The prognosis of CRC patients was evaluated for overall survival (OS) and relapse-free survival (RFS) through March 2021. In the OS analysis, death of the patient as a consequence of CRC was treated as an event. Patient death due to other causes or alive at the last follow-up were censored. In RFS analysis, relapse of CRC or patient death by CRC were treated as an event. Death of a patient due to other causes or alive at the last follow-up without relapse were censored. Cox proportional hazards regression analysis and Kaplan-Meier survival analysis were used to evaluate the prognosis of CRC patients. Pearson's chi-square test was used to investigate the relationships between p53 expression and *TP53* mutation status with other clinicopathological factors and the correlation between p53 expression and *TP53* mutation. SPSS software (IBM, version 19.0, Armonk, NY) was used for statistical analysis. P values less than 0.05 were considered statistically significant.

Results

TP53 **mutation analysis**

We used targeted NGS (by OCAv1) to characterize CRC for *TP53* mutations. For this method, *TP53* mutation analysis was performed on the entire exome. The *TP53* mutations were observed in 73% (149/204) and are summarized in Table 1. Of the *TP53* mutations, 108 (72.5%) were missense mutations (MS), 23 (15.4%) were nonsense mutations (NS), and 18 (12.1%) were frameshift mutations (FS). Among the functional domains of *TP53*, mutations were observed most often in the DNA binding domain (DBD), in 86.6% of patients (129/149 cases). By mutation type, 98.2% of MS mutations (106/108), 43.5% of NS mutations (10/23), and 72.2% of FS mutations (13/18) were observed in DBD. Compared to MS or FS mutations, NS mutations were more commonly observed in domains other than the DBD, 30.4% (7/23) in the

tetramerization domain and 26.1% (6/23) in the nuclear localization signaling (NLS) region. MS mutations were evenly distributed in the subregions within the DBD (L2, L3, LSH, and other). However, NS and FS mutations were observed mostly in non-zinc binding regions (excluding L2, L3, and LSH), at 91.3% (21/23) and 83.3% (15/18), respectively.

Table 1
Summary of TP53 mutations for 204 colorectal carcinoma patients

	Total n = 149	Missense n = 108	Nonsense n = 23	Frameshift n = 18
Functional domains				
Transactivation	0	0	0	0
Proline rich region	3	0 (0%)	0 (0%)	3 (16.7%)
DNA binding region	129	106 (98.1%)	10 (43.5%)	13 (72.2%)
Nuclear localization signalling	6	0 (0%)	6 (26.1%)	0 (0%)
Tetramerization	9	1 (0.9%)	7 (30.4%)	1 (5.6%)
Regulatory	2	1 (0.9%)	0 (0%)	1 (5.6%)
Sub-regions of DB domain				
L2	31	28 (25.9%)	1 (4.3%)	2 (11.1%)
L3	22	20 (18.5%)	1 (4.3%)	1 (5.6%)
LSH	31	31 (28.7%)	0 (0%)	0 (0%)
Other	65	29 (26.9%)	21 (91.3%)	15 (83.3%)
Exons				
Exon4	9	1 (0.9%)	1 (4.3%)	7 (38.9%)
Exon5	36	33 (30.6%)	1 (4.3%)	2 (11.1%)
Exon6	22	12 (11.1%)	7 (30.4%)	3 (16.7%)
Exon7	26	24 (22.2%)	0 (0%)	2 (11.1%)
Exon8	44	35 (32.4%)	7 (30.4%)	2 (11.1%)
Exon9	1	0 (0%)	1 (4.3%)	0 (0%)
Exon10	10	2 (1.9%)	6 (26.1%)	1 (5.6%)
Exon11	2	1 (0.9%)	0 (0%)	1 (5.6%)

Correlation between immunohistochemical p53 expression and TP53 mutation

Despite the increased incidence of NGS testing in CRCs, IHC is used most commonly to evaluate *TP53* status. Therefore, we investigated the correlation of immunohistochemical expression of p53 with *TP53* mutations. In this study, we classified p53 expression based on two criteria. First, p53 expression was categorized as wild type pattern (p53 proportion, 1 ~ 79%) or aberrant type pattern (0% or ≥ 80%), and this classification showed significant correlation with *TP53* mutation ($P < 0.001$) (Table 2). Sensitivity and specificity for detecting TP53 mutation using this criterion were 87.2% and 61.8%, respectively (Table 3). The other criterion of classifying p53 expression into positive (p53 proportion, > 55%) and negative

(p53 proportion, ≤ 55%) groups was also significantly correlated with *TP53* mutation ($P < 0.001$) (Table 2). Sensitivity and specificity for detecting *TP53* mutation using this criterion were 71.8% and 69.1%, respectively (Table 3).

Table 3
Sensitivity, specificity, and accuracy of p53 immunohistochemistry for detecting *TP53* mutation

Mutation type	Sensitivity	Specificity	Accuracy
Binary (IHC: wild/aberrant)	87.2%	61.8%	80.4%
Binary (IHC: positive/negative)	71.8%	69.1%	71.1%
Missense mutation	88%	84.4%	86.3%
Nonsense/frameshift mutation	70.7%	92.6%	88.2%
Wild type	61.8%	87.2%	80.4%

As mentioned above, *TP53* mutation can be further classified into missense and nonsense/frameshift types. Accordingly, we subdivided the p53 aberrant type pattern into missense (p53 proportion, ≥ 80%) and nonsense/frameshift (p53 proportion, 0%) type. This subgrouping of p53 expression showed a significant correlation with *TP53* mutation types (Table 4). The sensitivity, specificity, and accuracy for detecting *TP53* mutations are shown in Table 3.

Table 4
Correlation between p53 immunohistochemical expression pattern and TP53 mutation type

		p53 IHC			*p*
		Wild type pattern (1 ~ 79%)	Aberrant type pattern		
			Nonsense/frameshift pattern (0%)	Missense pattern (≥ 80%)	
All cases		53	41	110	
TP53 **mutation status**					
Wild type	55	34 (61.8%)	11 (20%)	10 (18.2%)	
Missense mutation	108	12 (11.1%)	1 (0.9%)	95 (88%)	
Nonsense/frameshift mutation	41	7 (17.1%)	29 (70.7%)	5 (12.2%)	< 0.001

Immunohistochemical expression of p53 and TP53 mutation in CRC patients and their correlation with clinicopathologic characteristics

Correlation between the clinicopathologic factors of *TP53* mutation and p53 expression is summarized in Table 5. In the immunohistochemical staining of p53, an aberrant expression pattern was identified in 151 (74%) patients, and 124 (60.8%) patients were classified in the positive (> 55%) expression group. The aberrant p53 expression pattern was significantly correlated with lower histologic grade, higher N stage, and TNM stage. The positive p53 expression group showed a significant correlation with left-side CRC, higher N stage, and TNM stage. The *TP53* mutation showed a significant correlation with smaller tumor size, higher N stage, and TNM stage.

Table 5
Correlation between clinicopathologic factors to *TP53* mutation and p53 IHC expressions

Characteristics	Total	p53 IHC		*p*	p53 IHC		*p*	*TP53* mutation		*p*
		Wild type pattern (1 ~ 79%)	Aberrant type pattern (0% or ≥ 80%)		Negative ≤ 55%	Positive > 55%		Wild	Mutant	
All cases	204	53 (26%)	151 (74%)		80 (39.2%)	124 (60.8%)		55 (27%)	149 (73%)	
Sex										
Male	117	34 (29.1%)	83 (70.9%)		50 (42.7%)	67 (57.3%)		35 (29.9%)	82 (70.1%)	
Female	87	19 (21.8%)	68 (78.2%)	0.245	30 (34.5%)	57 (65.5%)	0.232	20 (23%)	67 (77%)	0.27
Age (years)										
< 50	12	2 (16.7%)	10 (83.3%)		5 (41.7%)	7 (58.3%)		4 (33.3%)	8 (66.7%)	
≥ 50	192	51 (26.6%)	141 (73.4%)	0.448	75 (39.1%)	117 (60.9%)	0.858	51 (26.6%)	141 (73.4%)	0.608
Histologic grade										
Well or Moderate	167	38 (22.8%)	129 (77.2%)		64 (38.3%)	103 (61.7%)		42 (25.1%)	125 (74.9%)	
Poor	37	15 (40.5%)	22 (59.5%)	0.026	16 (43.2%)	21 (56.8%)	0.579	13 (35.1%)	24 (64.9%)	0.216
Site										
Right side	69	23 (33.3%)	46 (66.7%)		34 (49.3%)	35 (50.7%)		19 (27.5%)	50 (72.5%)	
Left side	135	30 (22.2%)	105 (77.8%)	0.087	46 (34.1%)	89 (65.9%)	0.035	36 (26.7%)	99 (73.3%)	0.895
Tumor size										
< 4.5cm	114	25 (21.9%)	89 (78.1%)		43 (37.7%)	71 (62.3%)		24 (21.1%)	90 (78.9%)	
≥ 4.5cm	90	28 (31.1%)	62 (68.9%)		37 (41.1%)	53 (58.9%)	0.622	31 (34.4%)	59 (65.6%)	0.032
CEA										
< 5 ng/ml	158	40 (25.3%)	118 (74.7%)		64 (40.5%)	94 (59.5%)		38 (24.1%)	120 (75.9%)	
≥ 5 ng/ml	46	13 (28.3%)	33 (71.7%)	0.689	16 (34.8%)	30 (65.2%)	0.484	17 (37%)	29 (63%)	0.083
T stage										
T1-3	171	43 (25.1%)	128 (74.9%)		68 (39.8%)	103 (60.2%)		45 (26.3%)	126 (73.7%)	

Characteristics	Total	p53 IHC		*p*	p53 IHC		*p*	*TP53* mutation		*p*
		Wild type pattern (1 ~ 79%)	Aberrant type pattern (0% or ≥ 80%)		Negative ≤ 55%	Positive > 55%		Wild	Mutant	
T4	33	10 (30.3%)	23 (69.7%)	0.536	12 (36.4%)	21 (63.6%)	0.714	10 (30.3%)	23 (69.7%)	0.637
N stage										
N0	107	37 (34.6%)	70 (65.4%)		49 (45.8%)	58 (54.2%)		36 (33.6%)	71 (66.4%)	
N1-3	97	16 (16.5%)	81 (83.5%)	0.003	31 (32%)	66 (68%)	0.043	19 (19.6%)	78 (80.4%)	0.024
TNM stage										
Stage I, II	110	37 (33.6%)	73 (66.4%)		51 (46.4%)	59 (53.6%)		37 (33.6%)	73 (66.4%)	
Stage III, IV	94	16 (17%)	78 (83%)	0.007	29 (30.9%)	65 (69.1%)	0.024	18 (19.1%)	76 (80.9%)	0.02

Since p53 expression and *TP53* mutation showed significant correlations with N stage and TNM stage, we subdivided the p53 aberrant type pattern into missense (p53 proportion, ≥ 80%) and nonsense/frameshift (p53 proportion, 0%) types and *TP53* mutation into missense and nonsense/frameshift mutations and analyzed the correlation between N stage and TNM stage (Tables 6, 7). For p53 IHC, missense pattern and nonsense/frameshift pattern were significantly associated with higher N stage compared to wild type pattern, and missense pattern p53 expression was significantly higher in high TNM stage (Table 6). For *TP53* mutation, nonsense/frameshift mutation showed significant correlations to higher N stage and TNM stage (Table 7). Missense mutations of *TP53* were significantly related with higher TNM stage but not with N stage (Table 7).

Table 6
Correlation between p53 immunohistochemical expression pattern and lymph node stage and TNM stage.

Characteristics	Total	p53 IHC		*p*	Total	p53 IHC		*p*
		Wild type pattern (1 ~ 79%)	Missense pattern (≥ 80%)			Wild type pattern (1 ~ 79%)	Nonsense/frameshift pattern (0%)	
N stage								
N0	88	37 (42%)	51 (58%)		56	37 (66.1%)	19 (33.9%)	
N1-3	76	16 (21.1%)	60 (78.9%)	0.004	37	16 (43.2%)	21 (56.8%)	0.03
TNM stage								
Stage I, II	90	37 (41.1%)	53 (58.9%)		57	37 (64.9%)	20 (35.1%)	
Stage III, IV	74	16 (21.6%)	58 (78.4%)	0.008	36	16 (44.4%)	20 (55.6%)	0.052

Table 7
Correlation of TP53 mutation type with lymph node stage and TNM stage.

Characteristics	Total	*TP53* mutation status		*p*	Total	*TP53* mutation status		*p*
		Wild type	Missense			Wild type	Nonsense/frameshift	
N stage								
N0	90	36 (40%)	54 (60%)		53	36 (67.9%)	17 (32.1%)	
N1-3	73	19 (26%)	54 (74%)	0.061	43	19 (44.2%)	24 (55.8%)	0.019
TNM stage								
Stage I, II	92	37 (40.2%)	55 (59.8%)		55	37 (67.3%)	18 (32.7%)	
Stage III, IV	71	18 (25.4%)	53 (74.6%)	0.047	41	18 (43.9%)	23 (56.1%)	0.022

Prognostic impact of immunohistochemical expression of p53 and TP53 **mutation in CRC patients**

Table 8 shows univariate analysis for OS and RFS of CRC patients. Histologic grade, T stage, N stage, TNM stage, and positive p53 expression ($P = 0.018$) were significantly associated with OS of CRC patients. The CRC patients with positive p53 expression had a 4.35-fold [95% confidence interval (95% CI); 1.29–14.71, $P = 0.018$] increased risk of death. Tumor size, T stage, N stage, and TNM stage were significantly correlated with the RFS of CRC patients by univariate analysis. CRC patients with an aberrant p53 expression pattern showed a tendency ($P = 0.081$) for shorter RFS. The mutational status of *TP53* was not associated with OS or RFS.

Table 8
Univariate Cox proportional hazards regression analysis for overall survival and relapsefree survival in colorectal cancer patients.

Characteristics	OS		RFS	
	HR (95% CI)	p	HR (95% CI)	p
Sex, female (vs. male)	0.646 (0.263–1.588)	0.341	0.985 (0.473–2.052)	0.968
Age, y ≥ 50 (vs. < 50)	1.394 (0.187–10.369)	0.745	1.874 (0.255–13.759)	0.537
Histologic grade, Poor (vs. Well or Moderate)	3.324 (1.419–7.784)	0.006	1.211 (0.494–2.964)	0.676
Site, Left side (vs. Right side)	0.725 (0.31–1.696)	0.725	1.003 (0.469–2.144)	0.993
Tumor size, ≥ 4.5cm (vs. < 4.5cm)	1.95 (0.833–4.565)	0.124	2.109 (1.014–4.387)	0.046
T stage, T4 (vs. T1-3)	3.262 (1.367–7.779)	0.008	2.695 (1.231–5.899)	0.013
N stage, N1-3 (vs. N0)	3.129 (1.224- 8)	0.017	4.262 (1.826–9.943)	0.001
TNM Stage, III or IV (vs. TNM Stage, I or II)	3.326 (1.301–8.503)	0.012	3.775 (1.679–8.489)	0.001
CEA, < 5 ng/ml (vs. ≥ 5 ng/ml)	1.389 (0.542–3.557)	0.493	1.048 (0.425–2.584)	0.919
p53 IHC, Aberrant pattern (vs. Wild pattern)	0.96 (0.376–2.454)	0.932	3.591 (0.854–15.097)	0.081
p53 IHC, positive, > 55% (vs. negative, ≤ 55%)	4.352 (1.288–14.712)	0.018	0.919 (0.446–1.894)	0.818
TP53, **Wild type (vs. Mutant type)**	1.739 (0.588–5.142)	0.317	1.36 (0.583–3.174)	0.477

Kaplan-Meier survival analysis curves for OS and RFS of CRC patients according to *TP53* status or p53 IHC are presented in Fig. 1. For OS, the group with positive expression for p53 had significantly shorter OS than the negative expression group ($P = 0.01$). The OS of CRC patients with *TP53* mutation or aberrant p53 expression pattern did not show a significant difference from the *TP53* wild type or p53 wild type expression pattern. The mutation status of *TP53* and p53 expression showed no difference on the RFS of CRC patients.

In addition, we further divided the p53 aberrant type pattern into missense and nonsense/frameshift type and *TP53* mutation into missense and nonsense/frameshift mutation and performed Kaplan-Meier analysis for OS of CRC patients (Fig. 2). The p53 expression patterns were significantly associated with OS survival of CRC patients ($P = 0.04$). The CRC patients with nonsense/frameshift pattern of p53 expression showed significantly better prognosis compared to patients with missense or wild type pattern ($P = 0.012$, $P = 0.025$, respectively). However, there was no significant difference in OS of CRC patients according to type of TP53 mutation (Fig. 2).

We performed multivariate analysis for OS and RFS of CRC patients (Table 9). Multivariate analysis included sex, age, histologic grade, site, tumor size, T stage, N stage, and TNM stage. Along with the above-listed variables, multivariate analysis was performed and included p53 expression pattern (wild/aberrant), positive/negative p53 expression group, and *TP53* mutational status in models 1–3. For the OS of CRC patients, histologic grade, TNM stage, and positive/negative p53 expression were independent prognostic factors. The group with positive expression for p53 had a 4.1-fold (95% CI; 1.2-

14.03, *P* = 0.025) increased risk of death compared to the group with negative expression. For the RFS of CRC patients, only N stage was an independent prognostic factor.

Table 9
Multivariate Cox regression analysis for overall survival and relapsefree survival in colorectal cancer patients.

Characteristics	OS		RFS	
	HR (95% CI)	p	HR (95% CI)	p
Model 1				
Histologic grade, Poor (vs. Well or Moderate)	3.077 (1.312–7.221)	0.01	0.846 (0.336–2.131)	0.723
N stage, 1–3 (vs. N stage, 0)	0.766 (0.1-5.873)	0.798	4.262 (1.826–9.943)	0.001
TNM Stage, III or IV (vs. TNM Stage, I or II)	3.122 (1.219-8)	0.018	1.088 (0.205–5.768)	0.921
Model 2				
Histologic grade, Poor (vs. Well or Moderate)	3.375 (1.431–7.964)	0.005	0.828 (0.328–2.092)	0.69
N stage, 1–3 (vs. N stage, 0)	0.61 (0.094–3.982)	0.606	4.262 (1.826–9.943)	0.001
TNM Stage, III or IV (vs. TNM Stage, I or II)	2.543 (0.981–6.592)	0.055	1.123 (0.212–5.946)	0.892
p53 IHC, positive, > 55% (vs. negative, ≤ 55%)	4.098 (1.197–14.031)	0.025	0.77 (0.371–1.599)	0.483
Model 3				
Histologic grade, Poor (vs. Well or Moderate)	3.077 (1.312–7.221)	0.01	0.836 (0.33–2.114)	0.705
N stage, 1–3 (vs. N stage, 0)	0.722 (0.083–6.314)	0.769	4.262 (1.826–9.943)	0.001
TNM Stage, III or IV (vs. TNM Stage, I or II)	3.122 (1.219-8)	0.018	1.115 (0.211–5.878)	0.898

Discussion

In the present study, we investigated the immunohistochemical expression of p53 and the mutational status of *TP53* by NGS in CRC patients. In the 204 CRC patients, *TP53* mutations were detected in 73% of patients (149/204), with 108 (72.5%) patients harboring missense mutation and 41 (27.5%) patients with nonsense or frameshift mutation. (2) The cutoff value for p53 IHC expression reflecting missense mutations was 80%, and the cutoff value for nonsense/frameshift mutations was 0%. Subdividing p53 expression into missense (p53 proportion, ≥ 80%) and nonsense/frameshift (p53 proportion, 0%) patterns showed significant correlation with missense and nonsense/frameshift *TP53* mutations, respectively. (3) *TP53* mutation and p53 IHC expression showed correlation with poor prognostic factors such as higher N stage and TNM stage. (4) Univariate and multivariate survival analyses indicated positive p53 IHC expression (p53 proportion, > 55%) as an independent factor for poor OS in patients with CRC. (5) Nonsense/frameshift (p53 proportion,

0%) expression pattern of p53 showed a significantly better prognosis than wild type or missense p53 IHC expression pattern.

Currently, immunohistochemical staining for p53 is the tool used most often for evaluating *TP53* mutation status. However, after introduction of NGS, sequencing of the *TP53* gene in cancer has been increasing rapidly. Previous reports have demonstrated the correlation between p53 expression and *TP53* mutation detection by NGS. In a study on ovarian carcinoma, the authors classified p53 expression into wild type, overexpression, and complete absence [15]. The p53 IHC expression showed good concordance with the mutation status of *TP53*. The sensitivity of IHC for detecting gain-of-function mutations, loss-of-function mutation, and the wild type expression of p53 was 100%, 76%, and 100%, respectively [15]. The specificity of IHC for detecting gain-of-function mutations, loss-of-function mutations, and wild type expression of p53 was 95%, 100%, and 96%, respectively [15]. In gastric cancer, the IHC of p53 expression showed a significant correlation with *TP53* mutation detected by NGS. In brain glioma, the sensitivity of p53 IHC for detecting *TP53* mutation was 87% [18]. The cut-off point for p53 IHC differs according to organ studied. The cut-off point was 50% in ovarian cancer, 10% in brain glioma, and 50% in gastric cancer. In the present study, we performed ROC curve analysis to set a cut-off point for p53 IHC. The cut-off point was 80% and 1% for missense mutation and nonsense/frameshift mutation, respectively. On the other hand, there was also a report that the IHC of p53 expression cannot be used to predict *TP53* mutations [19]. However, precise validation of the cut-offs related to percent positivity of p53 IHC has been limited in CRC. To the best of our knowledge, this is the first study to report a correlation between immunohistological expression of p53 and mutational status of *TP53* gene in CRC patients. In line with previous reports, our data showed a significant correlation between IHC expression of p53 and mutational status of the *TP53* gene. Moreover, we set the cut-off point for IHC of p53 expression by analyzing the ROC curve for mutational status of *TP53*. Subclassifying p53 expression into three types (missense, nonsense/frameshift, and wild type) showed better accuracy for detecting *TP53* mutations than did subdividing p53 expression into two types, such as positive/negative or wild/aberrant type. Based on these results, if the cut-off point for p53 IHC is appropriately set, the IHC of p53 expression can predict the mutational status of *TP53* with high probability.

The p53 protein, it has been established as a tumor suppressor by extensive studies [20]. Generally, tumor suppressor genes such as *BRCA1*, *RB*, and *APC* lose function through deletions or truncating mutations in cancer cells. However, unlike other tumor suppressor genes, the majority of *TP53* mutations in cancers is missense mutation [21, 22], and most of these occur in the DBD [23]. Our data supported this, showing that 98.1% of the missense mutations were located in the DBD. Many studies have confirmed that missense mutations can induce tumor progression by a gain-of-function mechanism through regulating proliferation, metastasis, genomic instability, differentiation, metabolism, and immune reactions [23]. In addition, if there is a product missense mutation of the *TP53* gene, the mutant protein product is relatively resistant to MDM2-mediated ubiquitination and accumulates in the nucleus of cancer cells, leading to overexpression of p53 [21]. There have been previous reports that p53 overexpression is related to poor survival or progression of CRC in patients [24, 25]. In our study, we investigated the prognosis of CRC patients according to the status of p53 IHC and *TP53* mutations.

As with previous reports, our data showed that the CRC patients with negative p53 expression (p53 proportion, $\leq$ 55%) have better OS than CRC patients with positive p53 expression (p53 proportion, > 55%). In addition, multivariate analysis confirmed that positive p53 IHC is an independent poor prognostic factor for CRC patients. However, no other criteria for p53 IHC (wild type pattern/aberrant type pattern) or mutational status of *TP53* affected the prognosis of CRC patients. The IHC of p53 expression reveals not only the mutational status of *TP53*, but also the post-transcriptional status of the p53 protein. Some reports emphasize the importance of post-translational modification of p53 in tumorigenesis or tumor progression [26, 27]. Our findings and previous reports suggest that the expression status of the p53 protein has a greater impact on the prognosis of CRC patients than does the *TP53* mutation itself.

Another interesting finding in our study was that CRC patients with a nonsense/frameshift pattern of p53 expression (p53 proportion, 0%) showed significantly better OS than patients with a missense pattern (p53 proportion, $\geq$ 80%) or a wild type pattern of p53 expression (p53 proportion, 1 ~ 79%). The p53 protein is actively involved in various DNA damage-response

mechanisms [28]. When cells are under stress and experience DNA damage, p53 induces cell-cycle arrest, activates DNA-repair mechanisms, and restores genomic stability [28]. In addition, various DNA-repair systems can be directly activated by the p53 protein [28]. The main adjuvant chemotherapeutic agent for advanced CRC in our institute is oxaliplatin. This agent induces DNA damage by preventing DNA replication. There are numerous reports that mutant p53 (mainly with gain-of-function missense mutations) is associated with chemoresistance via various pathways [29–31]. However, we could not find any reports about increased sensitivity to chemotherapy in cells with nonsense/frameshift TP53 mutation or absence of p53 expression. In this study, CRC patients without p53 expression had better OS than patients with p53 expression. Based on these results and the results of previous studies indicating that p53 overexpression is related to chemoresistance, we considered the possibility that the group with no p53 expression had better OS through chemosensitivity (or low chemoresistance). However, further studies are needed to determine the chemotherapy susceptibility in cancer cells lacking p53 expression.

In conclusion, our study showed that IHC of p53 expression can predict *TP53* mutation status. To predict the prognosis of CRC patients, p53 protein expression is thought to provide more information than the mutation itself. In our study, CRC patients without p53 expression had a better prognosis. Further studies are needed to establish the mechanism for differences in OS in CRC patients with or without p53 expression.

Declarations

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AUTHOR CONTRIBUTIONS

CONCEPTION: GWH, MRL, and MJC.

INTERPRETATION OR ANALYSIS OF DATA: ARA, KMK, and MJC.
PREPARATION OF THE MANUSCRIPT: MJC and KMK.
REVISION FOR IMPORTANT INTELLECTUAL CONTENT: KYJ, MJC, HSP, WSM, MJK.
SUPERVISION: MJC, and MRL.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available on “https://www.ncbi.nlm.nih.gov/sra” and the accession number is SAMN26687404

Ethics approval and consent to participate

This study was approved by the institutional review board of Jeonbuk National University Hospital (IRB number, CUH 2019-04-053) and was performed according to the Declaration of Helsinki. Each eligible participant signed an informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

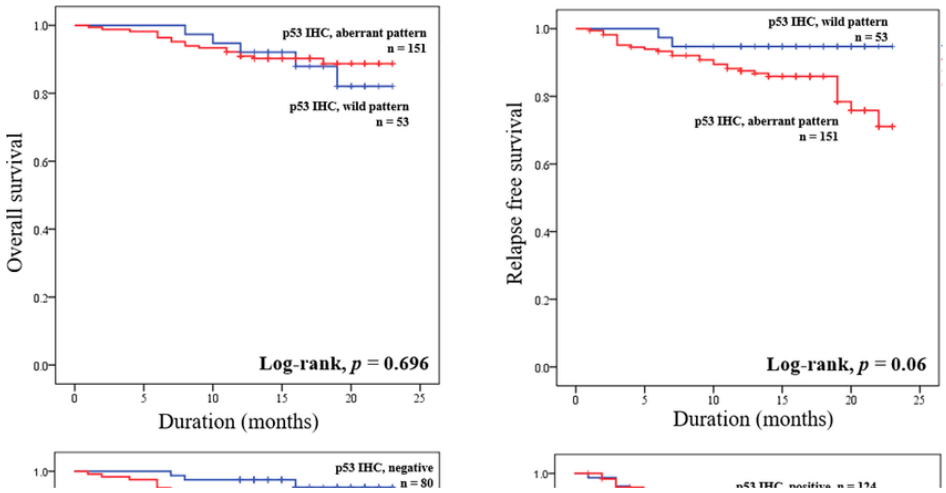

Figure 1

Survival analysis according to mutational status of *TP53* and immunohistochemical expression of p53 in colorectal carcinoma patients. Kaplan-Meier survival curves for overall survival and relapse-free survival of colorectal carcinoma patients according to the immunohistochemical expression of p53 and mutational status of *TP53*.

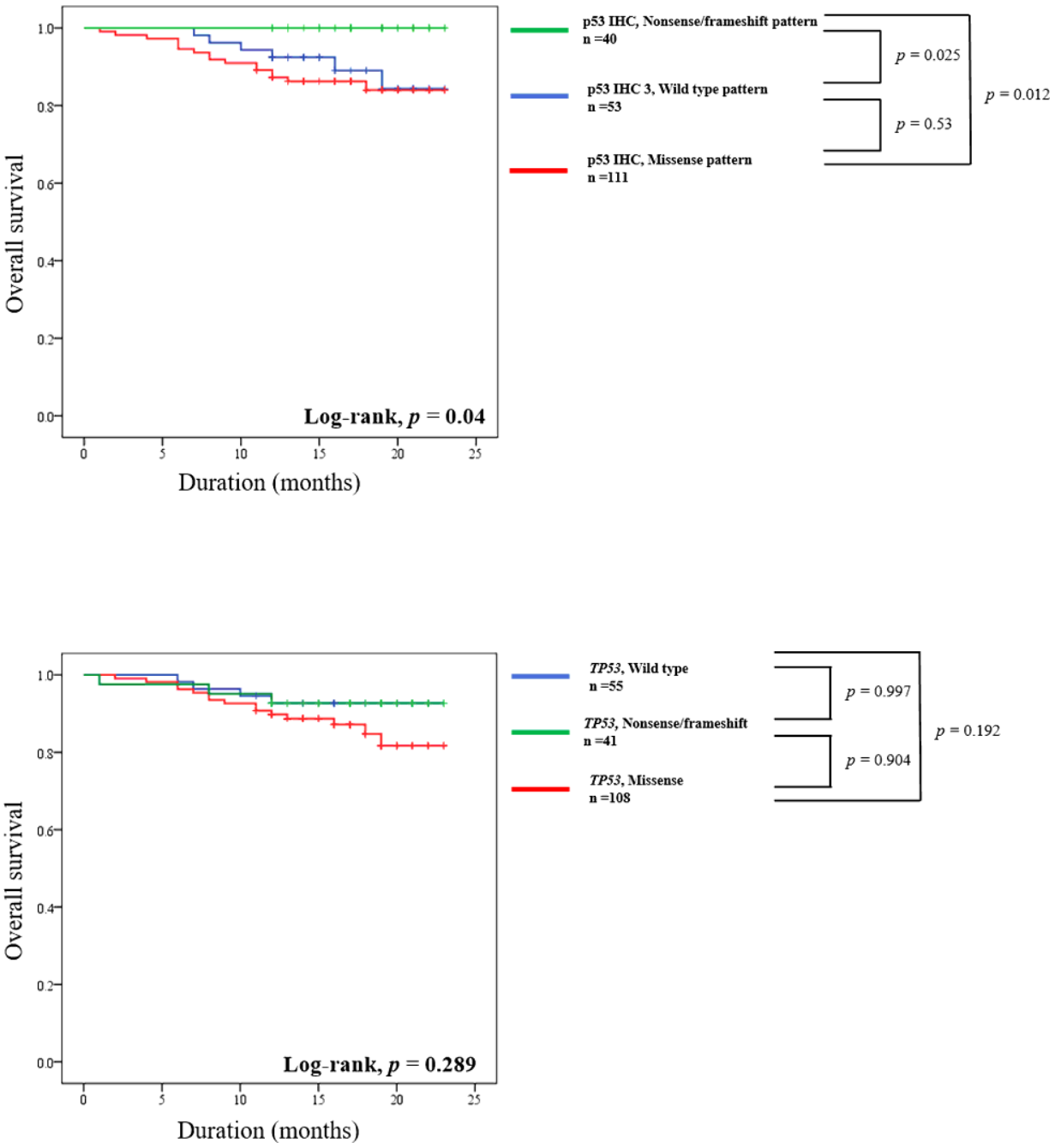

Figure 2

Survival analysis after subclassifying the *TP53* mutation and immunohistochemical expression of p53 in colorectal carcinoma patients. Kaplan-Meier survival curves for overall survival after reclassifying the *TP53* mutation into nonsense/frameshift and missense mutation and aberrant pattern of p53 expression into nonsense/frameshift and missense pattern.