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Immunohistochemical Detection of p53 Expression in Patients with Preoperative Chemoradiation for Rectal Cancer: Association with Prognosis

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Purpose: The expression of p53 in patients with rectal cancer who underwent preoperative chemoradiationand and its potential prognostic significance were evaluated. **Materials and Methods:** p53 expression was examined using immunohistochemistry in pathologic specimens from 210 rectal cancer patients with preoperative chemoradiotherapy and radical surgery. All patients were classified into two groups according to the p53 expression: low p53 (<50% nuclear staining) and high p53 (≥50%) groups. **Results:** p53 expression was significantly associated with tumor location from the anal verge (p=0.036). In univariate analysis, p53 expression was not associated with disease-free survival (p=0.118) or local recurrence-free survival (p=0.089). Multivariate analysis showed that tumor distance from the anal verge (p=0.006), ypN category (p=0.011), and perineural invasion (p=0.048) were independent predictors of disease-free survival; tumor distance from the anal verge was the only independent predictor of local recurrence-free survival. When the p53 groups were subdivided according to ypTNM category, disease-free survival differed significantly in patients with ypN+ disease (p=0.027) only. **Conclusion:** Expression of p53 in pathologic specimens as measured by immunohistochemical methods may have a significant prognostic impact on survival in patients with ypN+ rectal cancer with preoperative chemoradiotherapy. However, it was not an independent predictor of recurrence or survival.

Key Words: p53, rectal cancer, immunohistochemistry

INTRODUCTION

Total mesorectal excision after preoperative chemoradiation remains the cornerstone of treatment for patients with potentially resectable, locally advanced rectal cancer, as it allows for increased control of local disease, greater sphincter preservation rates, and improved disease-free survival.[1-3] Pathologic variables related to

tumor responses to chemoradiation, including ypT category, ypN category, and tumor response grade, remain important prognostic indicators for long-term oncologic outcomes.[4-6] However, these measures are insufficient, as patients at the same disease stage may exhibit different clinical outcomes and different responses to adjuvant therapy. Therefore, identification of additional prognostic markers in radiated rectal cancer specimens at the molecular level is needed to supplement the standard tumor staging system.

The p53 tumor suppressor gene is the most frequently mutated tumor-associated gene in malignant human tumors, including colorectal cancer.[7] The prognostic significance of p53 expression in rectal cancer remains unclear,[8-13] although a few studies have reported associations of p53 expression with prognosis in patients with rectal cancer after preoperative chemoradiation.[9,11] Thus, this study attempted to outline the prognostic role of immunohistochemical evaluation of expression of p53 in pathologic specimens from patients with rectal cancer after preoperative chemoradiotherapy and radical surgery.

MATERIALS AND METHODS

A total of 568 patients with rectal cancer underwent surgical resection after preoperative chemoradiation at Samsung Medical Center in Korea between January 2007 and March 2011. Eligibility criteria were as follows: 1) histologically confirmed adenocarcinoma, 2) tumors located within 10 cm of the anal verge, 3) locally-advanced (cT3-4 or N-positive) tumors, 4) curative-intent treatment for rectal cancer, and 5) no evidence of distant metastatic disease. Individuals who met the following criteria were excluded: 1) history of any other malignancy associated with hereditary colon cancer syndrome, 2) history of previous chemotherapy or radiotherapy, or 3) a complete pathologic response after preoperative chemoradiation due to an inability to perform immunohistochemical staining. Ultimately, 210 patients were included in the final analysis. This study was approved by our Institutional Review Board.

All patients received preoperative chemoradiation. Details of the preoperative chemoradiation protocol followed by our institution have been published previously.[14,15] Briefly, preoperative radiotherapy was delivered to the whole pelvis region at a dose of 40.4–50.4 Gy. Preoperative chemotherapy was concurrently administered with radiotherapy based on a 5-fluorouracil or capecitabine regimen. All patients underwent radical surgery 6 to 8 weeks after preoperative chemoradiation. Of the 210 patients, 195 (92.9%) received postoperative adjuvant chemotherapy. The tumors were staged according to the 7th American Joint Committee on Cancer TNM classification. Assessment of the tumor response to chemoradiation was evaluated according to the tumor response grade (TRG), as described by Mandard, et al.[16]: TRG0 (no regression), TRG1 (minimal regression), TRG2 (moderate regression), TRG3 (near total regression), and TRG4 (complete regression). TRG3–4 scores were defined as a good TRG response in this study.

Immunohistochemistry

p53 expression was evaluated by immunohistochemical staining, as previously described.[13] Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue. From each paraffin-embedded block, a 2-mm punch was taken for tissue microarrays, as described by Hendriks, et al.[17] Each section of normal and tumor tissues (4 μm thick) was assessed by tissue microarray and mounted on a glass slide. The tissue microarray slides were deparaffinized, and the tissues were then rehydrated with xylene and ethanol. The slides were washed with water and phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by incubating sections in 3% H_2O_2 in PBS for 30 min, and the slides were then washed with water, after which heat-induced epitope retrieval was performed. The slides derived from normal and tumor tissues (4-μm thick) were stained with mouse monoclonal antibodies specific for p53 (clone DO-7, 1:100; Dako, Glostrup, Denmark). p53 expression was described as low if <50% nuclear staining was observed and high if ≥50% nuclear staining was observed.[18] Normal colonic epithelial tissue adjacent to tumor tissue and lymphocytes served as internal positive controls.

Statistical analysis

Statistical evaluation was carried out using the statistical package SPSS for Windows (version 14.0; SPSS Inc., Chicago, IL, USA). Association between p53 immunostaining and clinicopathological features were explored with Student's t-test and the chi-square test, as appropriate. Disease-free survival curves were calculated using the Kaplan-Meier method, and differences between curves were evaluated using the log-rank test. To identify the significant independent prognostic factors associated with disease-free survival, variables with *p*-values less than 0.2 on univariate analysis were entered into a multivariate analysis of stepwise logis-

tic regression. All values of $p<0.05$ were considered statistically significant.

RESULTS

The analysis included 151 men (71.9% of the sample), and the median participant age was 56 years (range 31–79). Of the 210 patients, 181 (86.2%) underwent sphincter-saving operations. The median number of retrieved lymph nodes from each patient was 11 (1–39). Using the 7th UICC TNM staging system, 16, 64, 125, and 5 patients had ypT1, ypT2, ypT3, and ypT4 cancers, respectively; 138, 55, and 17 patients had ypN0, ypN1, and ypN2 tumors, respectively.

The relationships between tumor p53 expression and the clinicopathological features of colorectal adenocarcinoma are summarized in Table 1. The expression of p53 in tumors was significantly associated with tumor location from the anal verge ($p=0.036$). No significant association was detected between p53 expression and age, gender, histology, tumor diameter, ypT category, ypN category, tumor regression grade, lymphatic invasion, vascular invasion, perineural invasion, or tumor budding.

During study follow-up (median of 48 months), the factors associated with lower disease-free survival in univariate analysis were tumor distance from the anal verge ($p=0.022$), ypT category ($p=0.022$), ypN category ($p<0.001$), lymphatic invasion ($p=0.002$), perineural invasion ($p=0.005$), and tumor

Table 1. Correlation between a p53 Expression and Clinicopathological Parameters

Parameter	No.	p53 expression		*p* value
		Low (n=83)	High (n=127)	
Age, yrs				0.476
<55	100	37 (37.0)	63 (63.0)	
≥55	110	46 (41.8)	64 (58.2)	
Gender				0.831
Male	151	59 (39.1)	92 (60.9)	
Female	59	24 (40.7)	35 (59.3)	
Distance from the anal verge, cm				0.036
<5	118	54 (45.8)	64 (54.2)	
≥5	92	29 (31.5)	63 (68.5)	
Histology				0.181
Adenocarcinoma	202	78 (38.6)	124 (61.4)	
Mucinous carcinoma	8	5 (62.5)	3 (37.5)	
Depth of invasion (T)				0.912
ypT1+2	80	32 (40.0)	48 (60.0)	
ypT3+4	130	51 (39.2)	79 (60.8)	
Lymph node involvement (N)				0.292
ypN-	138	51 (37.0)	87 (63.0)	
ypN+	72	32 (44.4)	40 (55.6)	
Tumor regression grade				0.903
Poor	161	64 (39.8)	97 (60.2)	
Good	49	19 (38.8)	30 (61.2)	
Lymphatic invasion				0.756
Negative	184	72 (39.1)	112 (60.9)	
Positive	26	11 (42.3)	15 (57.7)	
Vascular invasion				0.189
Negative	187	71 (38.0)	116 (62.0)	
Positive	23	12 (52.2)	11 (47.8)	
Perineural invasion				0.365
Negative	193	78 (40.4)	115 (59.6)	
Positive	17	5 (29.4)	12 (70.6)	
Tumor budding				0.336
Negative	164	62 (37.8)	102 (62.2)	
Positive	46	21 (45.7)	25 (54.3)	

regression grade (p=0.006) (Table 2). The factors associated with lower local recurrence-free survival in univariate analysis were tumor distance from the anal verge (p=0.011), lymphatic invasion (p=0.049), and perineural invasion (p=0.046) (Table 2). No significant association was observed between both disease-free survival and local recurrence-free survival and p53 expression (Table 2). A multivariate analysis showed that tumor distance from the anal verge (p=0.006), ypN cat-

Table 2. Univariate Analysis of the Prognostic Factors for 5-Year Disease-Free Survival (DFS) and Local Recurrence-Free Survival (LRFS)

	5-yr DFS (%)	*p* value	5-yr LRFS (%)	*p* value
Age, yrs		0.119		0.296
<55	79.0		90.3	
≥55	85.7		94.1	
Gender		0.821		0.638
Male	83.1		92.8	
Female	81.1		91.0	
Distance from the anal verge, cm		0.022		0.011
<5	76.5		87.9	
≥5	90.1		97.6	
Tumor diameter, cm		0.138		0.802
<2.5	84.1		91.6	
≥2.5	79.1		93.0	
Histology		0.680		0.428
Adenocarcinoma	82.2		91.9	
Mucinous carcinoma	87.5		100	
Circumferential resection margin		0.449		0.386
Negative	83.0		92.7	
Positive	76.5		87.5	
Depth of invasion (T)		0.022		0.278
ypT1+2	89.4		94.8	
ypT3+4	78.4		90.5	
Lymph node involvement (N)		<0.001		0.198
ypN-	89.5		94.0	
ypN+	69.1		88.4	
Lymphatic invasion		0.002		0.049
Negative	85.0		93.6	
Positive	56.0		82.4	
Vascular invasion		0.959		0.790
Negative	82.5		92.5	
Positive	82.6		90.7	
Perineural invasion		0.005		0.046
Negative	84.1		93.3	
Positive	51.8		78.9	
Tumor budding		0.914		0.452
Negative	82.5		91.5	
Positive	82.6		95.0	
Tumor regression grade		0.006		0.098
Poor	78.5		90.4	
Good	95.8		97.9	
Postoperative chemotherapy		0.610		0.251
No	79.0		84.6	
Yes	81.4		92.8	
p53 expression		0.118		0.089
Low	76.9		87.9	
High	86.2		94.9	

egory (p=0.011), and perineural invasion (p=0.048) were independent predictors of disease-free survival; tumor distance from the anal verge was the only independent predictor of local recurrence-free survival (Table 3).

When the low and high p53 groups were further subdivided according to their ypTNM category (Fig. 1), disease-free survival significantly differed in relation to p53 expression for ypTanyN+ disease (p=0.027), but not for ypT1-2N0 and ypT3-4N0 diseases (p=0.122 and p=0.676, respectively).

DISCUSSION

The product of the p53 tumor suppressor gene is a nuclear phosphoprotein that indirectly regulates cell growth and inhibits cells with mutagenic damage from entering S-phase by arresting the cell cycle in G1.[7] The clinical significance of p53 in the progression and metastasis of colorectal cancer is controversial.[8,13,19-23] A number of studies have reported worse clinical outcomes in patients with p53 overexpression or mutations; however, this association has not been found to be universally true, as several investigations, including a large series of 541 patients with colorectal cancers with a long follow-up period by Soong, et al.,[23] have suggested that the presence of p53 accumulation is associated with improved survival, independently of tumor stage or grade, in distal colorectal cancers. Moreover, Watanabe, et al.[8] failed to demonstrate a significant association between p53 overexpression and clinical outcomes in patients with locally advanced colon cancer. In a previous publication, we reported that p53 expression, as well as the mismatch repair system, had no prognostic impact in patients with non-radi-

Table 3. Multivariate Analysis of the Prognostic Factors for 5-Year Disease-Free Survival (DFS) and Local Recurrence-Free Survival (LRFS)

	Hazard ratio (CI)	p value
DFS		
Age	0.572 (0.293–1.120)	0.103
Distance from the anal verge	0.342 (0.160–0.731)	0.006
Tumor diameter	1.200 (0.577–2.493)	0.625
Depth of invasion	1.232 (0.503–3.014)	0.648
Lymph node involvement	2.635 (1.243–5.583)	0.011
Lymphatic invasion	1.300 (0.581–2.911)	0.523
Perineural invasion	2.454 (1.008–5.970)	0.048
p53 expression	0.666 (0.343–1.291)	0.228
LRFS		
Distance from the anal verge	0.145 (0.031–0.672)	0.014
Lymph node involvement	1.426 (0.443–4.587)	0.551
Lymphatic invasion	2.034 (0.544–7.603)	0.291
Perineural invasion	3.231 (0.876–11.911)	0.078
Tumor regression grade	0.277 (0.035–2.224)	0.227
p53 expression	0.474 (0.166–1.351)	0.162

CI, confidence interval.

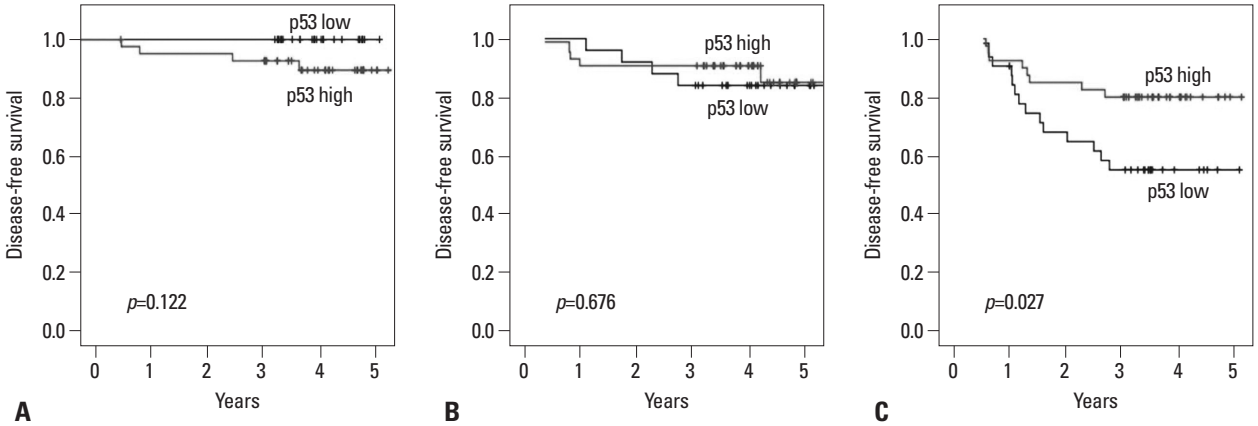

Fig. 1. Disease-free survival according to the p53 expression and ypTNM category. (A) ypT1-2N0, (B) ypT3-4N0, and (C) ypTanyN+.

ated colorectal cancer.[13] In this study, we found that p53 expression in radiated specimens was not an independent predictor of survival, although it had a significant prognostic impact on survival in patients with ypTanyN+ rectal cancer.

The prognostic value of p53 expression for the outcomes of treatment for rectal cancer is still unknown.[9-13,24] Moreover, few publications have examined the prognostic impact of p53 expression in patients with radiated rectal cancer. Schwandner, et al.[10] suggested that immunohistochemical assessment of both p53 and Bcl-2 status was a significant predictor of prognosis after curative surgery for rectal cancer and that p53 was a stronger predictor of prognosis than Bcl-2. In contrast, Morgan, et al.[24] reported that the immunohistochemical p53 status of rectal cancer was not associated with clinicopathological variables, nor predictive of oncologic outcomes. Saw, et al.[12] found no correlation between p53 expression and survival in 60 patients with rectal cancer treated with preoperative chemotherapy or radiotherapy. They also concluded that predicting the prognosis of patients with locally advanced low rectal cancer who have received preoperative therapies remains a challenge. We concur with this, as, in our study of a relatively large consecutive cohort of patients with radiated rectal cancer, p53 expression was not shown to be an independent predictor of survival.

The prognostic role of p53 expression according to the tumor location remains unclear. In colon cancer, proximal colon tumors, when compared with distal tumors, are more often found in older patients and benefit from adjuvant chemotherapy.[25,26] In this study, although p53 expression was not independent predictor for survival, distance from the anal verge had a significant prognostic impact in rectal cancer patients with preoperative chemoradiation. We found that patients with proximal rectal cancer showed better survival and greater p53 expression than patients with distal rectal cancer. This finding is consistent with the study by Hilska, et al.,[27] in which p53 overexpression showed better prognosis in rectal cancer than in colon cancer.

The differences in the results of previous studies may have been related to subjectivity in scoring, the absence of a uniform cut-off value for the definition of positive tumors, and differences in antibodies used, immunohistochemical methods, patient materials, and durations of follow-up. For nuclear markers such as p53, arbitrary values ranging from 5% to 75% have been used. Sometimes the cut-off point is based on a mean or a median staining index,[28] and sometimes the cut-off is set somewhere between the indisputable negatives and all other samples.[29] Occasionally, a cut-off is chosen on the basis of earlier studies.

We acknowledge that this study was not a proper trial with a large population sample size, and thus, there is a possibility for Type II error. Although our findings are insufficient to draw definite conclusions, they may still be of value. Further investigations with larger sample sizes are necessary to assess the prognostic role of p53 expression in patients with rectal cancer and to validate their possible value as novel therapeutic targets. In conclusion, p53 expression in resected specimens had a significant prognostic impact on survival in patients with stage III rectal cancer after preoperative chemoradiation, though it was not an independent predictor of survival. Confirmation of these results in larger sample sizes is needed, however.

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