American Journal of Medicine and Medical Sciences 2016, 6(3): 87-91
DOI: 10.5923/j.ajmms.20160603.04

Immunohistochemical Detection of p53 Expression in Patients with Sphincter Saving Operations for Rectal Cancer

Nishanov D. A.

Republic Oncology Research Centre of Uzbekistan, Tashkent, Uzbekistan

Abstract **Purpose:** The expression of p53 in patients with rectal cancer who underwent sphincter saving operation 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 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), pN 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 pTNM category, disease-free survival differed significantly in patients with pN+ disease (p=0.027) only. **Conclusions:** Expression of p53 in pathologic specimens as measured by immunohistochemical methods may have a significant prognostic impact on survival in patients with pN+ rectal cancer with sphincter saving operations. However, it was not an independent predictor of recurrence or survival.

Keywords p53, Rectal cancer, Sphincter saving operations, Immunohistochemistry

1. Introduction

Total mesorectal excision 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 totumor responses to chemoradiation, including pT category, pN 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 sphincter saving operations.

* Corresponding author:
morfologia@rambler.ru (Nishanov D. A.)
Published online at http://journal.sapub.org/ajmms
Copyright © 2016 Scientific & Academic Publishing. All Rights Reserved

2. Materials and Methods

A total of 568 patients with rectal cancer underwent surgicaloperations atNational Cancer Research Centre, Uzbekistan between January 2007 and March 2012. Eligibility criteria were as follows: 1) histologically confirmed adenocarcinoma, 2) tumors located within 10 cm of the anal verge, 3) locally-advanced (T3-4 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.

All patients underwent radical surgery. 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% H2O2 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.

Table 1. Correlation between a p53 Expression and Clinicopathologicalparameters

Parameter	No.	Low p53 expression (n=83)	High p53 expression (n=127)	p value
Age, years				0.476
<53	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)	
Mucinouscarcinoma	8	5 (62.5)	3 (37.5)	
Depthofinvasion (T)				0.912
pT1-2	80	32 (40.0)	48 (60.0)	
pT3-4	130	51 (39.2)	79 (60.8)	
Lymphnode involvement (N)				0.292
pN-	138	51 (37.0)	87 (63.0)	
pN+	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)	

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

Parameter	5-yr DFS (%)	*p* value	5-yr LRFS (%)	*p* value
Age, years		0.119		0.296
<53	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	
Histology		0.680		0.428
Adenocarcinoma	82.2		91.9	
Mucinouscarcinoma	87.5		100	
Depth of invasion (T)		0.022		0.278
pT1-2	89.4		94.8	
pT3-4	78.4		90.5	
Lymphnode involvement (N)		<0.001		0.198
pN-	89.5		94.0	
pN+	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	
Perineuralinvasion		0.005		0.046
Negative	84.1		93.3	
Positive	51.8		78.9	
Tumor regression grade		0.006		0.098
Poor	78.5		90.4	
Good	95.8			
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	

3. Results

The analysis included 151 men (71.9% of the sample), and the median participant age was 56 years (range 31−79). All 210 patients 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 pT1, pT2, pT3, and pT4 cancers, respectively; 138, 55, and 17 patients had pN0, pN1, and pN2 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, pT category, pN 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), pT category (p=0.022), pN category (p<0.001), lymphatic invasion (p=0.002), perineural invasion (p=0.005), and

tumorregression 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).

4. 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 over expression 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 have suggested that the presence of p53 accumulation is associated with improved survival, independently of tumor stage or grade, in distal colorectal cancers.

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. 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, though it was not an independent predictor of survival. Confirmation of these results in larger sample sizes is needed, however.

REFERENCES

[1] Roh M.S., Colangelo L.H., O'Connell M.J., Yothers G., Deutsch M., Allegra C.J., et al. Preoperative multimodality therapy improves disease-free survival in patients with carcinoma of the rectum: NSABP R-03. J ClinOncol 2009; 27:5124-30.

[2] Sebag-Montefiore D., Stephens R.J., Steele R., Monson J., Grieve R., Khanna S., et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. Lancet 2009; 373:811-20.

[3] Abdurakhmonov O.B. Study of VEGF-A Gene and NOS-3 Polymorphism in the Patients with Nasopharyngeal Angiofibroma. // American Journal of Medicine and Medical Sciences. USA, 2015. –No.5. –Vol.3. P.126-129. DOI: 10.5923/j.ajmms.20150503.03.

[4] García-Aguilar J., Hernandez de Anda E., Sirivongs P., Lee SH., Madoff R.D., Rothenberger D.A. A pathologic complete response to preoperative chemoradiation is associated with lower local recurrence and improved survival in rectal cancer patients treated by mesorectal excision. Dis Colon Rectum 2003; 46:298-304.

[5] Huh J.W., Park Y.A., Jung E.J., Lee K.Y., Sohn S.K. Accuracy of endorectal ultrasonography and computed tomography for restaging rectal cancer after preoperative chemoradiation. J Am CollSurg 2008; 207:7-12.

[6] Habr-Gama A., Perez R.O., Nadalin W., Nahas S.C., Ribeiro

U.Jr., Silva E., Sousa A.H., et al. Long-term results of preoperative chemoradiation for distal rectal cancer correlation between final stage and survival. J GastrointestSurg 2005; 9:90-9.

[7] Nigro J.M., Baker S.J., Preisinger A.C., Jessup J.M., Hostetter R., Cleary K., et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989; 342:705-8.

[8] Watanabe T., Wu T.T., Catalano P.J., Ueki T., Satriano R., Haller D.G., et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med 2001; 344:1196-206.

[9] Spitz F.R., Giacco G.G., Hess K., Larry L., Rich T.A., Janjan N., et al. p53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer. Clin Cancer Res 1997; 3:1685-90.

[10] Schwandner O., Schiedeck T.H., Bruch H.P., Duchrow M., Windhoevel U., Broll R. p53 and Bcl-2 as significant predictors of recurrence and survival in rectal cancer. Eur J Cancer 2000; 36:348-56.

[11] Jakob C., Liersch T., Meyer W., Becker H., Baretton G.B., Aust D.E. Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. World J Gastroenterol 2008; 14:1060-6.

[12] Saw R.P., Morgan M., Koorey D., Painter D., Findlay M., Stevens G., et al. p53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy. Dis Colon Rectum 2003; 46:192-202.

[13] Kim H.R., Kim H.C., Yun H.R., Kim S.H., Park C.K., Cho Y.B., et al. An alternative pathway in colorectal carcinogenesis based on the mismatch repair system and p53 expression in Korean patients with sporadic colorectal cancer. Ann SurgOncol 2013; 20:4031-40.

[14] Park C.H., Kim H.C., Cho Y.B., Yun S.H., Lee W.Y., Park Y.S. et al. Predicting tumor response after preoperative chemoradiation using clinical parameters in rectal cancer. World J Gastroenterol 2011; 17:5310-6.

[15] Lee H., Park H.C., Park W., Choi D.H., Kim Y.I., Park Y.S., et al. Negative impact of pretreatment anemia on local control after neoadjuvantchemoradiotherapy and surgery for rectal cancer. RadiatOncol J 2012; 30:117-23.

[16] Mandard A.M., Dalibard F., Mandard J.C., Marnay J., Henry-Amar M., Petiot J.F., et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer 1994; 73:2680-6.

[17] Hendriks Y., Franken P., Dierssen J.W., De Leeuw W., Wijnen J., Dreef E., et al. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. Am J Pathol 2003;162:469-77.

[18] Rödel C, Liersch T, Becker H, Fietkau R, Hohenberger W, Hothorn T, Graeven U, Arnold D, Lang-Welzenbach M, Raab HR, et al. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. Lancet Oncol. 2012;13:679–687. [PubMed]

[19] Huh J.W., Lee J.H., Kim H.R. Expression of p16, p53, and Ki-67 in colorectal adenocarcinoma: a study of 356 surgically resected cases. Hepatogastroenterology 2010;57:734-40.

[20] Bouzourene H., Gervaz P., Cerottini J.P., Benhattar J., Chaubert P., Saraga E., et al. p53 and Ki-ras as prognostic factors for Dukes' stage B colorectal cancer. Eur J Cancer 2000;36:1008-15.

[21] Zeng Z.S., Sarkis A.S., Zhang ZF, Klimstra D.S., Charytonowicz E., Guillem J.G., et al. p53 nuclear overexpression: an independent predictor of survival in lymph node--positive colorectal cancer patients. J ClinOncol 1994;12:2043-50.

[22] André T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, Bonetti A, Clingan P, Bridgewater J, Rivera F, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009;27:3109–3116. [PubMed]

[23] Soong R., Grieu F., Robbins P., Dix B., Chen D., Parsons R., et al. p53 alterations are associated with improved prognosis in distal colonic carcinomas. Clin Cancer Res 1997;3:1405-11.

[24] Morgan M., Koorey D., Painter D., Findlay M., Newland R., Chapuis P., et al. p53 and DCC immunohistochemistry in curative rectal cancer surgery. Int J Colorectal Dis 2003;18:188-95.

[25] Birkenkamp-Demtroder K., Olesen S.H., Sørensen F.B., Laurberg S., Laiho P., Aaltonen L.A., et al. Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid. Gut 2005;54:374-84.

[26] Kapiteijn E., Liefers G.J., Los L.C., Kranenbarg E.K., Hermans J., Tollenaar R.A., et al. Mechanisms of oncogenesis in colon versus rectal cancer. J Pathol 2001;195:171-8.

[27] Hilska M., Collan Y.U., O Laine V.J., Kössi J, Hirsimäki .P, Laato M., et al. The significance of tumor markers for proliferation and apoptosis in predicting survival in colorectal cancer. Dis Colon Rectum 2005;48:2197-208.

[28] Leahy D.T., Salman R., Mulcahy H., Sheahan K., O'Donoghue D.P., Parfrey N.A. Prognostic significance of p53 abnormalities in colorectal carcinoma detected by PCR-SSCP and immunohistochemical analysis. J Pathol 1996;180:364-70.

[29] Watson A.J., Merritt A.J., Jones L.S., Askew J.N., Anderson E., Becciolini A., et al. Evidence of reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br J Cancer 1996;73:889-95.