p53 Immunohistochemical Staining Predicts Residual Disease after Chemoradiation in Patients with High-Risk Rectal Cancer


INTRODUCTION

Rectal cancer affects approximately 38,000 patients annually (1). Because of the anatomical constraints of the pelvis, attaining adequate local control while preserving sphincter function can be a challenge to the surgeon. Local recurrence of rectal cancer after surgery occurs in as many as 25% of cases and is associated with significant morbidity (2–7). The social and psychological consequences of a permanent colostomy are obvious. Adjuvant radiation with or without 5-fluorouracil-based chemotherapy delivered before or after surgery has been demonstrated to improve local control in patients with high-risk rectal cancers (2–5), and preoperative radiotherapy has been used successfully as an adjuvant to surgery, providing good local control and potentially enhancing sphincter preservation rates (5–7). Presently, little is known about pretreatment characteristics that may predict response to adjuvant chemoradiation in patients with rectal cancer.

The p53 tumor suppressor gene is the most commonly mutated gene in human cancers, and alterations in this gene have been found in up to 70% of colorectal cancers (8–10). The function of the p53 gene is not completely understood, but its protein product can serve as a transcription factor that is known to have important roles in regulating the cell cycle and apoptosis, especially following exposure of the cell to DNA-damaging agents (11–18). Recent studies have confirmed that induction of apoptosis by the agents commonly used in cancer treatment (such as chemotherapeutic drugs and ionizing radiation) is highly dependent on normal p53 function (15–18). p53 immunohistochemical staining has been widely used in the study of p53 status in multiple cancers, including colorectal cancer. The wild-type p53 protein has a short half-life and is virtually undetectable by immunohistochemical staining. However, mutations in the p53 gene often result in stabilization of the protein, and accumulation of p53 protein has been demonstrated to correlate with p53 mutations (19).

Although p53 status has been widely studied in colorectal cancer, little is known about the correlation between p53 status and sensitivity of these tumors to chemoradiation. If a correlation were established, this information could be used to identify patients who may benefit from novel treatment strategies. To test the hypothesis that p53 status correlates with response of rectal cancer to chemoradiation, we examined the relationship between p53 protein accumulation, as measured by immunohistochemical staining, and response to chemoradiation. The data suggest that p53 immunohistochemical staining may be used as a predictor of residual disease after preoperative chemoradiation in patients with high-risk rectal cancer.

PATIENTS AND METHODS

Patients and Specimens. The study group comprised patients with rectal cancer treated with preoperative chemoradiation followed by surgical resection at The University of
There were 30 men and 22 women in the study. The median age of the patients was 63 years (range, 33–82 years). Before surgery, patients were treated with a chemoradiation regimen that consisted of 45 Gy of external-beam irradiation (1.8 Gy/day for 5 days/week) with concurrent continuous-infusion 5-fluorouracil therapy (300 mg/m²/day for 5 days/week). Six weeks after chemoradiation, the involved segments of bowel and mesorectum were resected. Additional clinical information is listed in Table 1.

Formalin-fixed, paraffin-embedded tumor blocks were obtained from the Department of Pathology at the M. D. Anderson Cancer Center. Sections of 4–6 μm in thickness were cut from the pretreatment biopsy and surgical specimen tissue blocks for immunohistochemical staining and grading of response to treatment, respectively.

**Immunohistochemical Staining for p53.** An immunoperoxidase method using an avidin-biotinylated horseradish peroxidase complex (Oncogene Science, Inc., Manhasset, NY) was used to detect p53 protein in the deparaffinized pretreatment biopsy sections. The anti-p53 monoclonal antibody DO1 (Oncogene Science), which recognizes both the human wild-type and mutant p53 molecules, was used. Specimens were irradiated in a microwave to assist in antigen retrieval. Staining was developed in 3,3′diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO), and slides were counterstained with hematoxylin (Richard Allen, Inc., Richland, MI).

Scoring of sections stained for p53 was performed by a single pathologist (K. R. C.) blinded to clinical characteristics and pathological grade of response. Ten high-powered fields or the total number of cancer cells in the biopsy specimen was evaluated. Specimens were assigned one of three staining scores: positive (more than 50% of cancer cells with positive nuclear staining); focally positive (10–50% of cancer cells with positive nuclear staining); or negative (less than 10% of cancer cells with positive nuclear staining). Photomicrographs of representative specimens from each group are presented in Fig. 1.

**Grade of Pathological Response.** The response to chemoradiation in the surgical specimens was determined from both the grade of the response of the tumor and the absence of cancer in the lymph nodes. A grade of pathological response (20) was assigned to H&E-stained sections of surgical specimens by one pathologist (K. R. C.), who was blinded to the clinical characteristics and p53 immunohistochemical staining status. Specimens were assigned one of four grades based on the amount of residual viable tumor: I, more than 90% viable tumor cells; II, 10–90% viable tumor cells; III, less than 10% viable tumor cells; or IV, no viable tumor cells. Photomicrographs of representative specimens from each group are presented in Fig. 2. The pathological status of lymph nodes was reported as positive if tumor cells were present in the lymph nodes of the surgical specimens and negative if tumor cells were not present. Patients who did not have lymph nodes identified in their surgical specimens (n = 7) were excluded from the assessment of lymph node status.

**Statistical Analysis.** The strength of association between p53 immunohistochemical staining and grade of pathological response was calculated using a Spearman CC. The χ² test and Fisher's exact test were used for a 2-by-2 table for hypothesis testing of associations. The relationship between p53 immunohistochemical staining and degree of histological response was also examined in a multiple logistic regression with complete response (yes or no) as the binary outcome. In addition, we examined p53 immunohistochemical staining in relation to the degree of pathological response (1, 2, 3, or 4) in a polychotomous (ordinal) logistic regression. In both models, we computed the strength of the association (the adjusted relative odds) between p53 immunohistochemical staining and histological response while adjusting for the following covariates: tumor size, lymph nodes status, T stage (all determined by preoperative ultrasonography), histological grade, and distance of the tumor from the anal verge. A value of $P < 0.05$ was considered statistically significant. The analyses were performed with SPSS for Windows, Release 6.1 [Statistical Package for the Social Sciences (SPSS), Inc., Chicago, IL].

**RESULTS**

**Immunohistochemical Staining for p53.** Normal rectal mucosa was uniformly negative for p53. Twenty pretreatment biopsy specimens were positive for p53 on immunohistochemical staining, with cancer cells clearly demonstrating nuclear staining. Little to no nuclear staining of cancer cells was noted in 19 specimens, which were scored as negative. Three specimens were scored as focally positive, with more than 10% but...
less than 50% of the tumor cells positively stained. No correlation between p53 immunohistochemical staining pattern and patient age, tumor size, tumor location, or pretreatment endoscopic ultrasonographic nodal status was found.

Response to Chemoradiation. Grade of pathological response and lymph node status are summarized in Table 2. A grade IV or complete response was observed in 13 patients (31%). In all but 6 patients (17%), no tumor was present in the lymph nodes following chemoradiation. No correlation between pathological response to chemoradiation and patient age, tumor location, or pretreatment endoscopic ultrasonographic nodal status was found. The correlation between tumor size greater than 5 cm and a lesser response to chemoradiation approached statistical significance, with a $P$ of 0.06.

Correlation between p53 Immunohistochemical Staining and Response to Chemoradiation. The three patients with focally positive tumors on p53 immunohistochemical staining were grouped with the 20 patients with positive tumors for statistical analysis of the correlation between p53 immunohistochemical staining of pretreatment biopsy specimens and response to chemoradiation. A significant inverse relationship between p53 protein accumulation and pathological response to chemoradiation was observed ($P = 0.02$; $CC = -0.36$; Table 3). If one considers only complete pathological responses (grade IV), the results are more striking ($P = 0.005$; $CC = -0.43$; Table 3). Only 3 of the 13 complete responses occurred in patients with p53 protein accumulation in their pretreatment biopsy specimens. By multivariate analysis, p53 immunohistochemical staining was an independent predictor of grade of pathological response and complete pathological response to chemoradiation. The relationship p53 immunohistochemical staining to complete pathological response was examined using a multiple logistic regression model. When confounding covariates [tumor size, lymph nodes status, and T stage (all determined by preoperative ultrasonography); histological grade; and distance of the tumor from the anal verge] were controlled for in a logistic regression model, the adjusted odds ratio for p53 negativity in relation to a complete pathological response was 10.6 (95% confidence interval, 1.15–97.9; $P = 0.03$). An essentially equivalent result was obtained using a polychotomous (ordinal) logistic regression for degree of pathological response. Importantly, all six patients with residual cancer in the lymph nodes of their surgical specimens had aberrant p53 protein accumulation in their pretreatment biopsy specimens, and in fact, positive
immunohistochemical staining for p53 correlated with the presence of residual cancer in the lymph nodes of the surgical specimen ($P = 0.02; CC = 0.39$; Table 3).

DISCUSSION

In model systems using thymocytes, transformed fibroblasts, and hematopoietic cells, p53 status has been demonstrated to be a crucial mediator of apoptosis in response to chemotherapeutic agents and irradiation (15–18). Cellular protein levels of p53 rise quickly in response to treatment with DNA-damaging agents. A p53-induced G1 checkpoint may then allow for DNA repair prior to entry into S phase, or, if repair is not possible, the cell may be deleted by apoptosis (14). A correlation between p53 immunohistochemical staining and a poor response to chemotherapy has been observed in patients with lung and ovarian cancers (21, 22). However, the role of p53 in determining the response of colorectal cancer to these agents has been questioned and has been studied only in patients treated with postoperative adjuvant therapy (23, 24). Some investigators have observed a correlation between p53 protein accumulation and a poor outcome for colorectal cancer patients (25, 26), and Goh et al. (24) have demonstrated a lower likelihood of benefiting from adjuvant therapy when a mutation in the highly conserved regions of the p53 gene is present.

Importantly, the role of p53 status in determining the effectiveness of adjuvant therapy may be difficult to measure in a postoperative setting. Although the survival end point used in the studies of postoperative therapy is critical, the response to chemoradiation can be determined clearly when adjuvant therapy is delivered preoperatively and response is assessed pathologically in the surgical specimen. We have examined the responsiveness of a population of rectal cancers with similar clinical features treated with a standard preoperative chemoradiation regimen. Aberrant p53 protein accumulation correlated with a poor response to chemoradiation as measured by grade of response and the presence of residual cancer in the lymph nodes of the surgical specimen.

Local-regional factors, such as positive mesorectal lymph nodes, radial extent of tumor, and adequate distal margins, play important roles in the outcome of rectal cancer, and markers that correlate with the extent of residual disease after preoperative chemoradiation may be helpful in the management of this disease. A major response to radiation or chemoradiation may increase the surgeon's ability to preserve the sphincter and attain tumor-free resection margins. Minsky et al. (7) have reported enhanced sphincter preservation rates for patients with low rectal cancers treated with preoperative radiation and chemoradiation, and Marks et al. (6) have described the feasibility of local excision in patients.
with low rectal cancers that have responded to preoperative radiotherapy. Because local excision precludes adequate lymphadenectomy in rectal cancer, biological predictors of residual disease in the mesorectal lymph nodes may be useful in selecting patients for such treatment. In our study, no patients with p53-negative tumors on immunohistochemical staining (and therefore presumed functioning p53) manifested residual disease in the resected lymph nodes. This population of patients with mostly T3 tumors would be expected to have an approximately 65% rate of positive mesorectal lymph nodes if taken directly to surgery without preoperative adjuvant therapy (27).

Little information concerning predictors of response to chemoradiation in this population is available to guide the surgeon and radiation oncologist. An analysis by Willet et al. (28) of rectal cancer patients treated with preoperative radiation therapy suggested that tumor size and immunohistochemical staining for proliferating cell nuclear antigen may be helpful predictors of response. Proliferating cell nuclear antigen status, a measurement of cellular proliferation, may provide additional information when combined with analysis of p53 status. The dynamic balance between proliferation and mechanisms of programmed cell death may provide the most accurate description of tumor biology and response to chemoradiation. Tumor size approached statistical significance as a predictor of response in our analysis, but other clinical parameters failed to predict the presence or absence of residual disease.

Immunohistochemical analysis of pretreatment biopsy specimens for accumulation of p53 is a simple and inexpensive test. Clear differences in staining pattern between the positive and negative groups were observed (Fig. 1). Correlation between p53 mutational status and accumulation of p53 protein demonstrated by immunohistochemical staining has been reported to be 67% (19). Immunohistochemical analysis may miss gene deletions, insertions, and truncations that do not result in p53 protein accumulation. The more sophisticated techniques of DNA sequencing and PCR single-strand conformation analysis are better able to detect these genetic alterations (29). However, these techniques are laborious, may require microdissection of biopsy specimens, and may not be suitable for routine diagnostic purposes. Also, these techniques may not detect abnormalities related to p53 binding with other protein complexes or dislocation of p53 between cellular compartments. Therefore, no one method of analysis will detect all p53 abnormalities in biopsy specimens from patients with rectal tumors. We were interested in a technique that would give reproducible results, could be done quickly on small pretreatment biopsy specimens, and would detect a majority of p53 abnormalities observed in rectal cancer. Nevertheless, conclusions drawn from these data are limited by the above realities.

The three complete responses observed in patients with abnormal p53 protein accumulation contrasts with the remainder of the data. This may be related to inaccuracies in the immunohistochemical technique used. However, these findings may also be due to alternative biological pathways for response or resistance to radiation and tumor cell death. The false-negative rate of immunohistochemical staining in the detection of p53 alterations has been reported to be as high as 30% (19). It is therefore possible that we are underestimating the value of p53 mutation status in predicting response of rectal tumors to preoperative chemoradiation.

The results presented suggest that p53 immunohistochemical staining of pretreatment biopsy specimens can predict residual disease in patients with rectal cancer. This was made evident by pathological assessment of both the status of the lymph nodes and the response of the tumor. All patients whose specimens were negative for p53 protein accumulation were found to have no tumor in their resected lymph nodes, and 10 of them (53%) experienced a pathological complete response of the primary tumor. In contrast, among patients with pretreatment accumulation of p53 protein (and presumed altered p53 protein function), six (26%) had residual tumor in their resected lymph nodes, and only three (13%) experienced a pathological complete response.

This pretreatment information may prove useful in designing therapeutic strategies. Reported pathological complete response rates for patients with rectal cancer treated with preoperative radiation with or without 5-fluorouracil-based chemotherapy range from 0 to 57% (5, 7); in the present study, 31% of patients experienced a pathological complete response. Attemps to improve the efficacy of preoperative chemoradiation by intensifying therapy would likely increase the toxicity of treatment, and those patients with responsive tumors would garner no further benefit. Our results indicate that p53 immunohistochemical staining can predict response to chemoradiation in

### Table 2

<table>
<thead>
<tr>
<th>p53 Immunohistochemical Staining</th>
<th>No. of Patients (%)</th>
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<tbody>
<tr>
<td>Positive</td>
<td>20 (48)</td>
</tr>
<tr>
<td>Focally Positive</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Negative</td>
<td>19 (45)</td>
</tr>
<tr>
<td>Grade of pathological response</td>
<td></td>
</tr>
<tr>
<td>I (&gt;90% viable tumor cells)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>II (10-90% viable tumor cells)</td>
<td>11 (26)</td>
</tr>
<tr>
<td>III (&lt;10% viable tumor cells)</td>
<td>13 (31)</td>
</tr>
<tr>
<td>IV (No viable tumor cells)</td>
<td>13 (31)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>6 (17)</td>
</tr>
<tr>
<td>Absent</td>
<td>29 (83)</td>
</tr>
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### Table 3

<table>
<thead>
<tr>
<th>p53 Immunohistochemical Staining and Response to Chemoradiation</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade of pathological response</td>
<td>p53 negative</td>
</tr>
<tr>
<td>I (&gt;90% viable tumor cells)</td>
<td>3</td>
</tr>
<tr>
<td>II (10-90% viable tumor cells)</td>
<td>1</td>
</tr>
<tr>
<td>III (&lt;10% viable tumor cells)</td>
<td>5</td>
</tr>
<tr>
<td>IV (No viable tumor cells)</td>
<td>10</td>
</tr>
<tr>
<td>Completeness of pathological response</td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>10</td>
</tr>
<tr>
<td>Incomplete</td>
<td>9</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>Absent</td>
<td>15</td>
</tr>
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\(p = 0.02; CC = -0.36, p = 0.005; CC = -0.43, p = 0.02; CC = 0.39\).
this population of high-risk rectal cancer patients with patients with abnormal accumulation of p53 protein having a lower probability of responding to treatment. If this information is supported by follow-up studies, we believe this pretreatment information would be useful in designing trials and treatment strategies. p53 immunohistochemical staining could be used to stratify patients in randomized trials, or patients with abnormal accumulation of p53 protein and therefore lower probability of response to conventional therapy would be candidates for protocols that involve additional or novel therapy. Therapy could be intensified by increasing radiation dose, using alternative radiosensitizing regimens, or even restoring wild-type p53 function through a gene therapy approach (30).

Our results from this retrospective analysis of the predictive value of p53 immunohistochemical staining in a relatively small population are promising. These results and those of others (21, 22, 24, 28) may lead to the use of molecular markers of tumor biology in multidisciplinary treatment planning for rectal cancer patients. Confirmation of these observations in a larger population, preferably by prospective analysis, is warranted.

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REFERENCES


p53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer.


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