p53 Nuclear Accumulation and Multiploidy Are Adverse Prognostic Factors in Surgically Resected Stage II Colorectal Cancers Independent of Fluorouracil-Based Adjuvant Therapy

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Key Words: Colorectal cancer; DNA multiploidy; p53; Prognosis

Abstract
To identify the prognostically highest risk patients, DNA content and p53 nuclear or cytoplasmic accumulation, evaluated by monoclonal antibody DO7 and polyclonal antibody CM1, were determined in 94 surgically resected stage II (Dukes B2) colorectal cancers, treated or not with adjuvant 5-fluorouracil-based chemotherapy.

Sixty-one (65%) of the tumors were aneuploid, 16 (17%) of which had a multiploid DNA content; 50 (53%) displayed DO7 nuclear p53 accumulation, and 44 (47%) showed cytoplasmic CM1 positivity. In multivariate analysis, only multiploidy and p53 nuclear positivity emerged as independent prognostic indicators of a poorer outcome. Positivity for p53 was associated with shorter survival in 5-fluorouracil–treated and untreated patients.

Therefore, in patients with Dukes B2 colorectal cancer, a biologic profile based on the combined evaluation of DNA multiploidy and p53 status can provide valuable prognostic information, identifying patients to be enrolled in alternative, more aggressive therapeutic trials.

About 40% of all colorectal cancers fall into stage II (Dukes B2), a group of tumors that penetrate the full bowel wall to the serosa without involving lymph nodes. Even if these tumors, in the majority of cases, are cured by surgical resection, they are biologically heterogeneous and often associated with uncertain clinical outcome. In fact, the failure of standard treatment ranges from 10% to 30%. Although adjuvant treatment may improve the survival of patients with nodal and extranodal disease (stage III-IV), the efficacy of conventional chemotherapy-radiotherapy in stage II patients is still controversial.

Several authors have studied a number of biologic parameters, such as DNA ploidy and p53 gene mutations, to identify patients at a higher risk of relapse. However, in this context, currently available data often are controversial, and the prognostic value of DNA ploidy and p53 need further validation as recommended by the College of American Pathologists Consensus Statement on prognostic factors in colorectal cancer. As such, the same parameters could be particularly valuable for detecting Dukes B2 stage patients with a more aggressive tumor. However, at present, only a few studies have investigated the correlation among DNA ploidy, p53 status, and clinical outcome in patients without nodal involvement.

Patients with stages I through IV colorectal cancer reportedly have an adverse clinical outcome when tumors harbor either multiploid cell populations or p53 alterations detectable even by immunohistochemical methods. In addition, some authors have shown that the impact of p53 on the clinical outcome of the disease is strictly dependent on the localization of the oncoprotein, either in the nuclei or in the cytoplasm of neoplastic cells. This raises the question of whether the prognostic value of p53 is related to its cellular compartmentalization in stage II colorectal cancers as well.
In these patients, therefore, the analysis of the relationship between these two variables, namely, multiploidy and intracellular p53 protein accumulation, may provide information of clinical interest, influencing the choice of alternative postoperative therapeutic strategies.

This study aimed at identifying the prognostically highest risk subgroup of 94 patients who underwent surgical resection of stage II colorectal cancer and were treated or not with adjuvant 5-fluorouracil (5-FU)–based chemotherapy. We wanted to determine DNA ploidy and verify the prognostic implications of multiploidy, investigate whether nuclear or cytoplasmic compartmentalization of the p53 protein could provide different prognostic information using both the DO7 monoclonal antibody and the CM1 polyclonal antiserum, and analyze the impact of the combination of multiploidy and intracellular p53 accumulation on disease-free survival (DFS) and overall survival (OS) both in 5-FU–treated and untreated patients.

Materials and Methods

Patients and Pathologic Data

The study group comprised a retrospective series of stage II patients surgically treated for colorectal adenocarcinoma at the Regina Elena Cancer Institute, Rome, Italy, between January 1990 and December 1995. Eligibility criteria for the study included the following: (1) no history of malignant neoplasms, (2) colonic surgical resection margins negative for tumor, (3) no preoperative treatment for the colorectal carcinoma, and (4) snap-frozen and paraffin-embedded tissues available. Moreover, to evaluate the prognostic impact of biologic parameters on a cohort of patients as homogeneous as possible, patients who died within 30 days after surgery or who died of non–cancer-related causes and those who were lost to follow-up were excluded. This selection permitted us to analyze 94 stage II colorectal cancer patients (T3 N0, 58; T4 N0, 36). The median age was 64 years (range, 56-70 years); 56 were men, and 38 were women. There were 27 right-sided tumors (ascending colon, 22; transverse colon, 5), and 67 were left-sided tumors (descending colon, 8; sigmoid colon, 27; rectum, 32). All patients underwent radical surgery intended as a resection with clear pathologic margins and regional lymphadenectomy. Nerve-sparing and complete mesorectal excision was performed in patients with rectal cancer. All operations were carried out by the same surgical staff, and no operative mortality was recorded. Multiple samples (1 in the center and at least 4 at the peripheral edges of the lesion) of fresh tissues from both tumor and normal-appearing mucosa 5 cm distant from the tumor were taken for flow cytometric analysis.

According to the World Health Organization classification, there were 11 well-differentiated (G1), 74 moderately differentiated (G2), and 9 poorly differentiated (G3) carcinomas.

Thirty-six colorectal cancer patients underwent adjuvant systemic chemotherapy with 5-FU (370 mg/m² per day) plus folinic acid (FA) (10 mg/m² per day) by rapid intravenous injection for 5 consecutive days every 4 weeks for 6 courses. Patients were selected for adjuvant treatment on the basis of adverse clinical (eg, age, performance status, intestinal subocclusion or perforation by tumor) and pathologic (eg, vascular or perineural invasion, positive peritoneal washing) factors. The 9 patients with rectal cancer also were treated with high-dose pelvic radiation.

Follow-up

No patients were lost to follow-up, and written informed consent was obtained from the patients before they were enrolled in the study. During the first 2 years, all patients underwent a physical examination and serum carcinoembryonic antigen, CA 72.4, and CA 19.9 monitoring bimonthly; a liver sonogram every 3 to 6 months; and a chest radiograph, colonoscopy, and abdominopelvic computed tomographic scan every 12 months. Thereafter, the frequency of these diagnostic procedures was halved. The median follow-up period of this series was 60 months.

Flow Cytometric Analysis

Flow cytometric analysis of the DNA content was performed starting with frozen tumor material.

A single nuclei suspension from the tumor biopsy specimens was obtained by mechanical disaggregation. Briefly, the tissue fragments were minced with scissors in cold phosphate-buffered saline. The nuclei suspensions were centrifuged at 130g for 10 minutes and fixed in 50% acetone/methanol (1:4 vol/vol) cold solution in phosphate-buffered saline and stored at 4°C until flow cytometric analysis was performed. Then, 4 × 10⁵ nuclei were incubated in a solution containing 75 KU/mL RNase (Sigma Chemical, St Louis, MO) and 50 µg/mL propidium iodide (Sigma Chemical) for 30 minutes at room temperature in the dark. The stained specimens then were filtered through an 80-µm nylon mesh and measured by a FACScan (Becton Dickinson, Sunnyvale, CA) at a flow rate of 200 cells per second. DNA-associated propidium iodide fluorescence was collected after a 620-nm-long pass filter, and 20,000 events were acquired for each sample. Normal human peripheral lymphocytes were used as internal and external standards for ploidy identification. Tumor ploidy was evaluated in terms of DNA index (DI), indicating the ratio between the tumor G₀/G₁ fluorescence peak channel and the corresponding channel of the standard reference. Tumors with a DI of more than 0.95...
but less than 1.05 were classified as diploid. The mean coefficient of variation for the G₀/G₁ diploid peak was 3.1 (range, 2.2-5) and was estimated as the width at half-maximum peak height divided by the mean channel and a factor of 2.35. Tumors were defined as aneuploid when only 1 aneuploid peak was found and as multiploid when more than 1 aneuploid peak was evidenced.

All details on the method of preparation of cell suspension from tissues for the flow cytometric analysis have been described.¹⁸,¹⁹

Immunohistochemical Analysis

Immunoreactivity for the p53 protein was detected using the monoclonal antibody (MAb) DO7 (Dako, Milan, Italy) and the polyclonal antibody (PAb) CM1 (BioGenex, Menarini, Firenze, Italy). The MAb DO7 recognizes an epitope located between amino acids 19 and 26 in both wild-type and mutant p53 protein. CM1 rabbit PAb detects both the wild and the mutant forms of p53 from amino acids 1 through 393.

Immunohistochemical staining was carried out on 5-µm-thick sections cut on silane-treated slides from routinely fixed, paraffin-embedded blocks (APES, Sigma). The sections were deparaffinized in xylene, rehydrated in graded ethanol, and incubated in a 0.3% hydrogen peroxide solution in methanol for 20 minutes to block the endogenous peroxidase. Heat-induced epitope retrieval was performed, pretreating the sections twice in a microwave oven at 750 W for 5 minutes in citrate buffer (pH 6.0, citric acid monohydrate, 10 mmol/L, adjusted with 2N sodium hydroxide) and allowed to cool at room temperature. Sections were incubated with primary antibodies for 30 minutes at room temperature. The DO7 antibody was used at a dilution of 1:100 and the CM1 antibody at a dilution of 1:40. The reaction was visualized using a sensitive streptavidin-biotin immunoperoxidase system (LSAB kit, Dako) and a 3 amino-9-ethyl carbazole solution (Dako) as the chromogenic substrate. Sections then were slightly counterstained with Mayer hematoxylin.

Colorectal cancers with high levels of p53 DO7 and CM1 immunoreactivity were used as positive controls. Negative controls were obtained by omission of the primary antibody. All slides were examined and scored independently by 2 investigators (S.B., M.M.) without any pathologic or clinical information concerning the cases under study. Expression of p53 was considered positive when tumor cells were stained, irrespective of the percentage of positive cells. However, we did not include faint staining cells or positive cells located on the margin of the section or in poor morphologic areas.

Statistical Methods

The correlation between variables was tested by using the Pearson chi-square test.

The DFS and OS curves were estimated by the Kaplan-Meier product-limit method. The log-rank test was used to assess differences between subgroups. Significance was defined as \( P < .05 \). The relative risk and the confidence limits were estimated for each variable using the Cox univariate model and adopting the most suitable prognostic category as referent group. A multivariate Cox proportional hazards model also was developed using stepwise regression (forward selection) with predictive variables that were significant in the univariate analyses. The enter limit and the remove limit were \( P = .10 \) and \( P = .15 \), respectively.

The BMDP statistical package (BMDP, Los Angeles, CA) was used for analysis. The BMDP P1L program was used for the Kaplan-Meier method and log-rank statistics to analyze DFS and OS. The BMDP P2L program was used for multivariate analysis for all covariates using Cox regression analysis.

Results

Flow Cytometric and Immunohistochemical Analysis

Thirty-three patients (35%) were classified as having diploid and 61 (65%) aneuploid tumors. The latter then were defined as single aneuploid when 1 aneuploid peak was found (\( n = 45 \) [48%]) or multiploid (\( n = 16 \) [17%]) when more than 1 aneuploid peak was evidenced \( \text{Figure 1}. \) Furthermore, most of the aneuploid or multiploid histograms included in this case series presented a diploid peak referred to as the peak showing the lowest DNA content.

Furthermore, we analyzed p53 protein accumulation by using both MAb DO7 and PAb CM1 \( \text{Image 1}. \) When MAb DO7 was used, 49 (52%) of 94 adenocarcinomas displayed nuclear p53 accumulation, while cytoplasmic expression was observed in only 1 case. Immunostaining with PAb CM1 involved complete staining of the nucleus and/or the entire cytoplasm of neoplastic cells in 44 (47%) of 94 cases, of which 27 showed cytoplasmic positivity and 17 nuclear p53 expression. As shown in \( \text{Table 1}. \), nuclear immunoreactivity was detected with both antibodies in 17 cases. In addition, 17 tumors showed positive nuclear expression with DO7 but were negative with CM1, 11 cases were positive in the cytoplasm with CM1 but negative with DO7, and 15 adenocarcinomas showed nuclear staining with DO7 and cytoplasmic staining with CM1. Only 1 case displayed cytoplasmic reactivity with both DO7 and CM1 antibodies.

Relationship Among Biologic and Clinicopathologic Parameters

\( \text{Table 2} \) summarizes the relationship between the p53 expression and the biopathologic parameters. The p53 nuclear accumulation detected with MAb DO7 was significantly
related to younger age and left-sided and aneuploid tumors. A higher frequency of p53 (DO7)-positive cases was observed in multiploid neoplasias. In contrast, p53 expression detected with PAb CM1 was correlated only with right-sided tumors. DNA aneuploidy and multiploidy were not correlated significantly with any clinicopathologic variables. There was a trend for left-sided tumors to be aneuploid compared with right-sided cancers (data not shown).

Relapse and Survival

Seventeen patients (18%) developed a relapse: 12 had abdominal-pelvic relapse and 5 had single or multiple lung metastases. Nineteen patients (20%) died of cancer-related causes. As shown in Table 3 in the multivariate analyses (Cox model), sex, tumor site, multiploidy, and DO7 p53 nuclear accumulation emerged as significant predictors of both DFS and OS. In contrast, in our series of stage II patients (Dukes B2), cytoplasmic CM1 p53 expression and DNA aneuploidy did not show any significant relation to survival when analyzed by univariate and multivariate analyses. Kaplan-Meier curves, stratified respectively for multiploidy and DO7 p53 nuclear positivity for all 94 patients, showed that significantly longer DFS and OS can be observed in patients with nonmultiploid Figure 2A and Figure 2B and
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Table 1
Cellular Compartmentalization of p53 Proprotein Detected With Monoclonal Antibody DO7 and Polyclonal Antibody CM1*

<table>
<thead>
<tr>
<th>CM1</th>
<th>DO7</th>
<th>CM1</th>
<th>DO7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>33 (66)</td>
<td>17 (34)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive nucleus</td>
<td>0 (0)</td>
<td>17 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive cytoplasm</td>
<td>11 (41)</td>
<td>15 (56)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>49</td>
<td>1</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the percentage based on CM1 totals.

Table 2
Accumulation of p53 by Antibody Type and Its Relationship With Biopathologic Variables *

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Cases</th>
<th>DO7 Positive (%)</th>
<th>P†</th>
<th>CM1 Positive (%)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;64</td>
<td>45</td>
<td>30 (67)</td>
<td>.012</td>
<td>24 (53)</td>
<td>.224</td>
</tr>
<tr>
<td>64 or older</td>
<td>49</td>
<td>20 (41)</td>
<td></td>
<td>20 (41)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>32 (57)</td>
<td>.351</td>
<td>29 (52)</td>
<td>.240</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>18 (47)</td>
<td></td>
<td>15 (39)</td>
<td></td>
</tr>
<tr>
<td>Right-sided tumors</td>
<td>27</td>
<td>9 (33)</td>
<td>.014</td>
<td>17 (63)</td>
<td>.046</td>
</tr>
<tr>
<td>Left-sided tumors</td>
<td>67</td>
<td>41 (61)</td>
<td></td>
<td>27 (40)</td>
<td></td>
</tr>
<tr>
<td>G1-G2</td>
<td>95</td>
<td>45 (53)</td>
<td>.811</td>
<td>38 (45)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>9</td>
<td>5 (56)</td>
<td></td>
<td>6 (67)</td>
<td>.209</td>
</tr>
<tr>
<td>Diploidy</td>
<td>33</td>
<td>13 (39)</td>
<td></td>
<td>15 (45)</td>
<td></td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>61</td>
<td>37 (61)</td>
<td>.048</td>
<td>29 (48)</td>
<td>.846</td>
</tr>
<tr>
<td>Nonmultiploidy</td>
<td>78</td>
<td>39 (49)</td>
<td></td>
<td>38 (49)</td>
<td></td>
</tr>
<tr>
<td>Multiploidy</td>
<td>16</td>
<td>12 (75)</td>
<td>.050</td>
<td>6 (38)</td>
<td>.412</td>
</tr>
</tbody>
</table>

* Data for DO7 and CM1 positivity are given as number (percentage).
† Pearson chi-square.

Table 3
Multivariate Analyses of Prognostic Factors for Disease-Free Survival (DFS) and Overall Survival (OS)

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Multiivariate (DFS)† RR (95% CI)†</th>
<th>P</th>
<th>Multiivariate (OS)† RR (95% CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male vs female)</td>
<td>3.13 (0.99-9.86)</td>
<td>.051</td>
<td>3.51 (1.12-11.03)</td>
<td>.031</td>
</tr>
<tr>
<td>Site (left-sided vs right-sided tumors)</td>
<td>6.20 (0.80-47.90)</td>
<td>.080</td>
<td>4.13 (0.90-18.90)</td>
<td>.067</td>
</tr>
<tr>
<td>Multiploidy (present vs absent)</td>
<td>3.39 (1.22-9.43)</td>
<td>.019</td>
<td>4.48 (1.71-11.73)</td>
<td>.002</td>
</tr>
<tr>
<td>p53 DO7 (negative vs positive)</td>
<td>5.52 (1.21-23.38)</td>
<td>.027</td>
<td>5.49 (1.26-23.89)</td>
<td>.023</td>
</tr>
</tbody>
</table>

CI, confidence interval.
† Final results of Cox regression analysis using a forward stepwise procedure (enter limit = 0.10; remove limit = 0.15).
‡ Relative risk (RR) determined by Cox regression model.

p53-negative tumors Figure 2C and Figure 2D. These findings allowed us to evaluate the impact of the combination of multiploidy and p53 nuclear expression on survival, analyzing the 3 groups of patients: with nonmultiploid/p53-negative, with nonmultiploid/p53-positive, or with multiploid/p53-positive tumors. The group of patients with multiploid/p53-negative tumors was not included in this statistical analysis since it was composed of only 4 cases. As shown in Figure 3, the results obtained provide statistically significant evidence that the presence of multiploidy and the concomitant p53 nuclear accumulation identified a group of patients with a higher probability for relapse and death. Otherwise none of the patients with nonmultiploid/p53-negative tumors experienced relapse or died of disease within 5 years of surgery.

When we stratified patients according to 5-FU plus FA-based therapy, as shown in Figure 4, p53 nuclear accumulation was associated with a shorter DFS and OS both in the group of 36 5-FU–treated patients (P = .05 for DFS; P = .1 for OS) and in 58 untreated patients (P = .0009 for DFS; P = .001 for OS). We could not perform the same analysis, according to multiploidy, owing to the limited number of cases.

Discussion

The purpose of this study was to test the hypothesis that the evaluation of ploidy status, generally associated with genetic instability, and p53 cellular compartmentalization in
colorectal cancers without nodal involvement (stage II/Dukes B2) can be helpful for identifying patients with poorer prognosis.

As reported, patients with stages I through IV colorectal cancer who have multiploid tumors or p53 nuclear accumulation have an adverse clinical outcome. In this context, however, limited information is available in the early stages of the disease. To the best of our knowledge, this is the first study that investigated the prognostic implications of DNA multiploidy, accurately evaluated on multiple tumor specimens, together with the p53 accumulation in a selected series of patients with Dukes B2 colorectal cancer.

Our results demonstrated that although DNA ploidy status itself (intended as diploidy vs aneuploidy) did not prognostically stratify this series of 94 colorectal stage II patients, the probability of recurrence and death was significantly higher for patients with multiploid tumors. These findings are further reinforced when multiploid tumors concomitantly displayed p53 alterations as detected by immunohistochemical analysis.

Despite the relatively low incidence of multiploidy in colorectal cancer (17% in this study), this variable deserves greater attention because of its unfavorable independent prognostic role. Previously conflicting data have been
reported on this point. For this reason, in 1993 a consensus review of the clinical use of DNA flow cytometry in colorectal cancer emphasized the need for standardized criteria, such as the use of fresh or frozen material only, sampling of multiple tissues because of tumor heterogeneity, and strict quality control of the study method. Therefore, in the present study, DNA ploidy was determined following the methodologic approach indicated by the consensus review.

Mutations in the \textit{p53} gene frequently are associated with stable intracellular expression of the altered form of the \textit{p53} protein, leading to the stabilization of the wild-type p53 protein and suppression of its \textit{cell cycle} regulatory function. This results in an accumulation of p53-positive cells in the tumor and a decrease in the disease-free survival rate of patients with \textit{p53} DO7+ tumors compared to those with \textit{p53} DO7– tumors. This finding is consistent with the results of other studies, which have shown that \textit{p53} mutations are associated with a poor prognosis in colorectal cancer.

\textbf{Figure 3} Disease-free (A) and overall survival (B) curves for patients with colorectal adenocarcinoma categorized according to the combinations of multiploidy and \textit{p53} nuclear accumulation in patients with Dukes B2 stage colorectal adenocarcinoma. A, \textit{P} = .0005. B, \textit{P} = .0001.

\textbf{Figure 4} Disease-free (A) and overall survival (B) curves according to \textit{p53} status in 5-fluorouracil (5-FU)–treated and untreated patients with Dukes B2 colorectal adenocarcinoma. A, For patients treated with 5-FU, \textit{P} = .05 for \textit{p53}–negative vs \textit{p53}–positive patients; for patients not treated with 5-FU, \textit{P} = .009 for \textit{p53}–negative vs \textit{p53}–positive patients. B, For patients treated with 5-FU, \textit{P} = .1 for \textit{p53}–negative vs \textit{p53}–positive patients; for patients not treated with 5-FU, \textit{P} = .001 for \textit{p53}–negative vs \textit{p53}–positive patients.
In colorectal cancers, the p53 gene is frequently mutated and the protein is often overexpressed. Since some authors have shown that in colorectal cancer the prognosis is directly related only to the cytoplasmic compartmentalization of the p53 protein, in our study we correlated conventional clinicopathologic variables with p53 positivity using both the MAb DO7, which visualizes p53 nuclear accumulation, and the PAb CM1, which prevalently identifies the cytoplasmic expression of the oncoprotein. These patterns of immunoreactivity represent differential activation modalities of the protein: the first due to gene mutation and the second to the nuclear exclusion of the protein.

In contrast with the other studies, our results suggest that only the group of colon cancers harboring p53 nuclear accumulation, as assessed by the DO7 anti-p53 antibody, represents a subset of tumors with a more aggressive clinical behavior. In fact, in our series, only nuclear staining was related to a poorer prognosis, whereas cytoplasmic p53 expression, as detected by PAb CM1, failed to correlate with survival. On the other hand, the nuclear exclusion of the p53 protein is more frequent in the advanced stages of the disease than it is in the earlier stages. In fact, in our selected cohort of stage II colorectal cancer patients, the percentage of p53 nuclear accumulation was higher than the cytoplasmic expression, in agreement with data reported by Flamini et al.

Furthermore, the MAb DO7 and the PAb CM1 seem to recognize different subpopulations of abnormal p53 proteins that, in turn, confer different biologic and clinical behavior on the neoplasms. This observation was supported by our data, which showed a close relationship between p53 intracellular localization and the tumor anatomic site. Left-sided tumors, known to be more aggressive, showed prevalently DO7 nuclear positivity, whereas we evidenced a prevailing CM1 cytoplasmic immunostaining in the more indolent right-sided tumors. In fact, the etiologic factors having a role in colorectal tumorigenesis may vary in the right and left sides of the colon, giving a different spectrum of p53 gene aberrations.

In particular, tumors of the left side of the colon and the rectum are characterized by a higher incidence of allelic losses, especially allelic deletions of 17p and 18q, than tumors arising in the proximal colon. A relationship between the molecular genetic alterations and the DNA ploidy pattern also has been shown. In fact, 17p and 18q allelic deletions and p53 gene mutations were demonstrated to be more frequent in DNA aneuploid than in diploid colorectal tumors. In the present series p53 nuclear accumulation detected by MAb DO7 was associated significantly with DNA aneuploidy and, consequently, with multiploidy according to data that reported that in colorectal carcinomas, mutant p53 can destabilize the genome and facilitate aneuploid clonal divergence.

In our series, 36 colorectal cancer patients with adverse clinical and pathologic prognostic factors were treated with 5-FU plus FA-based adjuvant therapy. The 9 patients with rectal cancer also underwent radiotherapy. To establish whether the abnormal accumulation of p53 is associated with the response to conventional regimens of adjuvant therapy, we analyzed the role of the oncoprotein separately in 5-FU–treated and in untreated patients. Although available data on p53 immunohistochemical expression and clinical outcome are numerous, a few studies, which analyzed only advanced colorectal cancer cases, compared the relationship between p53 status and outcome after 5-FU–based therapy and showed no significant correlation between response and p53 immunoreactivity. In our study, we focused on stage II patients in whom the efficacy of conventional adjuvant therapy is still controversial. Results obtained demonstrated that, even in stage II colorectal cancer, the p53 status does not influence the response to adjuvant therapy either in terms of DFS or OS. In fact, p53-positive patients, whether or not they received 5-FU plus FA adjuvant treatment, had a poor prognosis. However, owing to the small number of patient subsets included in this statistical analysis, these findings should be interpreted with caution. If these observations are successfully confirmed in a larger series of stage II patients, the evaluation of the molecular phenotype may contribute significantly to an improved selection of patients for enrollment into more aggressive and innovative therapeutic trials, ie, adjuvant therapy with high-dose leucovorin/5-FU or chronomodulated infusion of 5-FU and FA without or with oxaliplatin.

Flow cytometric analysis of DNA multiploidy on fresh or frozen multiple biopsy specimens from surgical specimens and the immunohistochemical evaluation of p53 nuclear accumulation led us to the identification of a subgroup of Dukes B2 colorectal cancer patients at higher risk of progression and death, independent of 5-FU–based treatment. Furthermore, DNA multiploidy and p53 nuclear accumulation together proved to be a better independent prognostic indicator than either of the biomarkers on their own, which may provide a valuable tool to plan alternative, more aggressive adjuvant therapy.

However, we are aware that although this study, according to the Tumor Marker Utility Grading System, provides evidences of potential usefulness of these markers in better defining prognosis of stage II colorectal cancer patients, larger prospective and controlled studies will be necessary to improve the levels of evidence for their clinical usefulness.

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