High Apoptotic Activity and Low Epithelial Cell Proliferation With Underexpression of p21\textsuperscript{WAF1/CIP1} and p27\textsuperscript{Kip1} of Mucinous Carcinomas of the Colorectum

Comparison With Well-Differentiated Type

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Key Words: Colorectal neoplasm; Mucinous carcinoma; Apoptosis; Cell cycle; p21\textsuperscript{WAF1/CIP1}; p27\textsuperscript{Kip1}; Immunohistochemistry; c-Ki-ras

Abstract

We comparatively assessed 41 mucinous colorectal carcinomas (MUCs) and 620 non-MUC (well-, moderately, and poorly differentiated adenocarcinoma) cases for clinicopathologic findings; and 41 MUCs and 115 randomly selected non-MUCs also were studied for the following: (1) apoptotic activity and Ki-67 immunoreactivity; (2) immunohistochemical expression of p21\textsuperscript{WAF1/CIP1}, p27\textsuperscript{Kip1}, p53, and bcl-2; and (3) c-Ki-ras mutations. The rates for lymph node involvement and peritoneal dissemination were higher in MUCs than in non-MUCs. Multivariate analysis showed MUCs to have a worse prognosis than well-differentiated adenocarcinomas. The Ki-67 labeling for MUCs was significantly lower than that for non-MUCs, whereas the apoptotic index was significantly higher than for the well-differentiated type. The labeling for p21\textsuperscript{WAF1/CIP1} and p27\textsuperscript{Kip1} was lower in MUCs (2.7% and 35.3%, respectively) than in well-differentiated adenocarcinomas (4.2% and 48.6%, respectively). MUCs can be considered a different tumor from the well-differentiated type, with a poor prognosis owing to frequent lymph node metastasis and peritoneal dissemination, and characterized by high apoptotic and low proliferative activities associated with low p21\textsuperscript{WAF1/CIP1} and p27\textsuperscript{Kip1} expression.

The designation of mucinous carcinoma is applied to the 6% to 15% of colorectal tumors in which mucus secretion largely contributes to tumor size.\textsuperscript{1,2} Mucinous carcinomas may exhibit more aggressive behavior and a worse prognosis than their nonmucinous counterparts,\textsuperscript{1,3} although this point is controversial, and little is known about proliferative activity, oncogene expression, and genetic alteration.\textsuperscript{4,8}

The p21 \textsuperscript{WAF1/CIP1}, p27 \textsuperscript{Kip1}, p53, and bcl-2 proteins are important regulators of the cell cycle. Wild-type p53 is a critical participant in G\textsubscript{1} cell cycle arrest through induction of the p21 \textsuperscript{WAF1/CIP1} gene product that shares in partial structural homology with p27 \textsuperscript{Kip1}.\textsuperscript{9,10} This latter cyclin-dependent kinase inhibitor is a negative regulator of the cell cycle and a potential tumor suppressor gene.\textsuperscript{11-14} The bcl-2 proto-oncogene is a known inhibitor of apoptosis and may, therefore, allow accumulation of genetic alterations that become fixed by cell division and potentially contribute to neoplastic development.\textsuperscript{15,16} Like mutant p53, bcl-2 has been shown to inhibit apoptosis triggered by wild-type p53.\textsuperscript{17,18} An inverse relationship between bcl-2 and p53 has been observed in colorectal tumors,\textsuperscript{5,19} while many workers have concentrated attention on the expression of p21 \textsuperscript{WAF1/CIP1} and p27 \textsuperscript{Kip1} in colorectal adenocarcinomas.\textsuperscript{12,14,20-22}

The c-Ki-ras protein p21 belongs to the family of guanosine triphosphate–guanosine diphosphate binding proteins with guanosine triphosphatase activity, which participate in transduction of mitogenic signals from the membrane to the cell nucleus.\textsuperscript{23} Alterations of the c-Ki-ras gene may occur during early steps of tumor formation, particularly during the development of adenomatous polyps, and have been found in 50% of advanced colorectal carcinomas.\textsuperscript{24,25} Data for c-Ki-ras mutation in mucinous carcinomas, however, are limited.\textsuperscript{5,26}
The present study was conducted to compare mucinous and nonmucinous colorectal adenocarcinomas in terms of clinicopathologic features, cell turnover, immunohistochemical expression of cell cycle regulator proteins, including p21WAF1/CIP1, p27Kip1, p53, and bcl-2, and c-Ki-ras mutations.

Materials and Methods

Patients and Materials

We studied a total of 661 cases of histologically confirmed single primary adenocarcinoma of the colon or rectum invading through the muscularis propria into the perimuscular connective tissue (pT3 and pT4); patients underwent curative resection and diagnosis at the Department of Pathology, Kitasato University East Hospital, Sagamihara, Japan, during the period between June 1986 and December 1999. Cancers complicating ulcerative colitis, Crohn disease, radiation colitis, familial polyposis coli, or hereditary nonpolyposis colorectal cancer syndrome were excluded. Cases with other carcinomas outside the colon and rectum also were excluded. To assess peritoneal cancer dissemination and liver metastasis, macroscopic examination at the time of the surgery was done. Lymph node metastasis was evaluated histologically using surgically resected materials. Surgical and pathology records were reviewed for sex, age, tumor site and size, lymph node metastasis, peritoneal dissemination, liver metastasis, and prognosis.

Histologic Examination

Tissue slices were fixed routinely in 10% formalin and embedded in paraffin. Four-micrometer-thick sections were cut and used for H&E staining. Histologic examination of the lesions revealed 41 of these carcinomas to be mucinous carcinomas, mucin accounting for more than 50% of the tumor mass as observed microscopically; 348 well-differentiated adenocarcinomas, mucin accounting for more than 50% of the tumor mass as observed microscopically; 202 moderately differentiated adenocarcinomas; and 70 poorly differentiated adenocarcinomas; 28

Immunohistochemical Analysis

We randomly selected 41 mucinous carcinomas and 40 well-differentiated, 40 moderately differentiated, and 35 poorly differentiated adenocarcinomas for immunostaining, performed using a streptavidin-biotin-immunoperoxidase complex method with 4-µm-thick sections, which had been deparaffinized and heated in citrated buffer (0.01-mol/L concentration, pH 6.0) solution for 15 minutes using a microwave oven to retrieve antigenic activity. This was not performed for the anti–single stranded DNA (ssDNA) antibody. The sections were incubated with primary antibodies, polyclonal anti–Ki-67 (1:100 dilution, DAKO, Glostrup, Denmark), monoclonal anti-p53 (clone DO-7, 1:500 dilution, Novocastra Laboratories, Newcastle upon Tyne, England), monoclonal anti–bcl-2 (clone 124, 1:300 dilution, DAKO), monoclonal anti–p21WAF1/CIP1 (clone Ab-1, 1:300 dilution, CALBIOCHEM, Cambridge, MA), monoclonal anti-p27Kip1 (clone 57, 1:300 dilution, Transduction Laboratories, Lexington, KY), and polyclonal anti-ssDNA (DAKO). 3,3’-Diaminobenzidine was used as the final chromogen, and nuclei were faintly counterstained with 0.3% methyl green solution to facilitate microscopic assessment.

Apoptotic Indices

Detection of apoptotic cells in H&E-stained sections was performed under high-power view, applying standard morphologic criteria. The distinctive features of apoptotic cells were marked condensation of chromatin and cytoplasm with or without nuclear fragments. Percentage counts of apoptotic bodies provided apoptotic indices (AIs) after examining at least 1,500 tumor cells for each case.

Evaluation of Each Variable

To generate Ki-67, p21WAF1/CIP1, p27Kip1, and ssDNA labeling indices (LIs), at least 1,500 tumor cells at the invasive front were evaluated, and those that were unequivocally positive for nucleic staining were counted to give percentage values. For the evaluation of these markers, the area of mucinous carcinomas where fronts are composed mainly of lakes of mucin without cancer cells were excluded. Then cellular areas were selected.

Immunoreactivity for bcl-2 was quantified according to the classification of Sinicrope et al with scoring as follows: 1, weak; 2, moderate; 3, intense. The staining intensity of infiltrating lymphocytes was set at 3. Percentages of positive tumor cells were assigned to 1 of 5 categories: 0, less than 5%; 1, 5% to less than 25%; 2, 25% to less than 50%; 3, 50% to less than 75%; 4, 75% or more, and immunoreactive scores for bcl-2 were calculated by multiplication of the values for the 2 parameters. Those for p53 were categorized into 4 groups: 0, 0%; 1, less than 1%; 2, 1% to less than 30%; 3, 30% or more.

Analysis of c-Ki-ras Mutations

c-Ki-ras mutations were detected with the nonradioactive polymerase chain reaction single-strand conformation polymorphism approach, as described in detail previously. A total of 39 cases of mucinous carcinomas and 40 well-differentiated, 40 moderately differentiated, and 32 poorly differentiated adenocarcinomas were analyzed. For extraction of DNA, 10 slides, each 10 µm thick, of formalin-fixed,
paraffin-embedded tissue were taken from each tumor and sampled by microdissection. The oligonucleotide primers used were as follows: 5'-GACTGAAATATAACCTTGG-3' and 5'-CTATTGTGGGATCATATTGCG-3' for amplification of c-Ki-ras codons 2 to 26 (exon 1) and 5'-GATTCC-TAAGGAAGCAATG-3' and 5'-CTATAATGGGTAATCCTG-3' for amplification of codons 38 to 97 (exon 2).

**Statistical Analysis**

Results are expressed as the mean ± SD. Comparisons between groups were made by chi-square, Mann-Whitney U, or Kruskal-Wallis tests as appropriate. The association between AI and ssDNA LI was analyzed using the Spearman rank correlation coefficient. A P value less than .05 was considered to indicate statistical significance.

Survival curves were calculated by the Kaplan-Meier method, and the statistical significance between curves was tested by using the log-rank test. Univariate survival analysis was performed with the Cox proportional hazards model, and the hazard ratio (HR) and 95% confidence interval (CI) were assessed for each factor; P values less than .05 were considered statistically significant. The Cox proportional hazards model also was used for multivariate analysis, and model selection was based on an ascending stepwise procedure (adopted P value: <.15). Statview software (Abacus Concepts, Berkeley, CA) was used for all statistical analyses.

### Results

**Clinicopathologic Features**

As shown in Table II, for cases of mucinous carcinomas, sex distribution was almost equal, and the mean age was 60.0 years (range, 24-89 years); for cases of nonmucinous carcinomas (well-, moderately, and poorly differentiated types), male predominance was exhibited, and the mean age was 63.3 years (range, 24-90 years). The most frequent site of mucinous carcinomas (17/41 [41%]) was the proximal colon (cecum, ascending colon, and transverse colon), followed by the distal colon (descending and sigmoid colon, 13/41 [32%]) and the rectum (11/41 [27%]), similar to the case for poorly differentiated adenocarcinomas. Higher proportions of well- and moderately differentiated tumors arose in the rectum and distal colon followed by the proximal colon. Mucinous carcinomas were significantly larger than well- or moderately differentiated adenocarcinomas. Rates for positive lymph node involvement and peritoneal dissemination of mucinous carcinomas were 63% (26/41) and 15% (6/39), respectively, significantly higher than those for well-differentiated adenocarcinomas (44.1%, and 3.8%, respectively). The prevalence of liver metastasis was significantly lower in mucinous carcinoma than in the poorly differentiated type (P < .05).

A survival advantage was observed for patients with the following: distal colon cancer (log-rank test, P = .0158), no

### Table II

**Clinicopathologic Characteristics of the Colorectal Carcinomas Studied*\n
<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonmucinous Carcinoma</th>
<th>Well-Differentiated</th>
<th>Moderately Differentiated</th>
<th>Poorly Differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (49)</td>
<td>221 (63.9)</td>
<td>126 (62.4)</td>
<td>37 (53)</td>
</tr>
<tr>
<td>Female</td>
<td>21 (51)</td>
<td>125 (36.1)</td>
<td>76 (37.6)</td>
<td>33 (47)</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>60.0 ± 14.9</td>
<td>63.4 ± 11.4</td>
<td>62.9 ± 10.6</td>
<td>64.3 ± 14.2</td>
</tr>
<tr>
<td><strong>Tumor site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal colon†</td>
<td>17 (41)</td>
<td>87 (25.1)</td>
<td>51 (25.2)</td>
<td>32 (46)</td>
</tr>
<tr>
<td>Distal colon</td>
<td>13 (32)</td>
<td>134 (38.6)</td>
<td>68 (33.7)</td>
<td>16 (23)</td>
</tr>
<tr>
<td>Rectum</td>
<td>11 (27)</td>
<td>126 (36.3)</td>
<td>83 (41.1)</td>
<td>22 (31)</td>
</tr>
<tr>
<td><strong>Tumor size (cm)†‡</strong></td>
<td>7.2 ± 3.9</td>
<td>5.5 ± 2.0</td>
<td>5.5 ± 2.1</td>
<td>6.5 ± 2.3</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present‡§</td>
<td>26 (63)</td>
<td>153 (44.1)</td>
<td>107 (53.0)</td>
<td>48 (69)</td>
</tr>
<tr>
<td>Absent†</td>
<td>15 (37)</td>
<td>194 (55.9)</td>
<td>95 (47.0)</td>
<td>22 (31)</td>
</tr>
<tr>
<td><strong>Peritoneal dissemination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present‡</td>
<td>6 (15)</td>
<td>13 (3.8)</td>
<td>15 (7.4)</td>
<td>12 (17)</td>
</tr>
<tr>
<td>Absent</td>
<td>33 (85)</td>
<td>332 (96.2)</td>
<td>187 (92.6)</td>
<td>58 (83)</td>
</tr>
<tr>
<td><strong>Liver metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present†</td>
<td>3 (7)</td>
<td>42 (12.1)</td>
<td>34 (16.8)</td>
<td>16 (23)</td>
</tr>
<tr>
<td>Absent†</td>
<td>38 (83)</td>
<td>306 (87.9)</td>
<td>168 (83.2)</td>
<td>54 (77)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) or mean ± SD. Statistical analysis indicated significant differences between mucinous and other carcinomas, as well as between different degrees of differentiation of nonmucinous carcinomas. Data were not available for all variables for mucinous carcinoma and nonmucinous well-differentiated carcinoma.

† P < .01 for the following: presence of peritoneal dissemination, mucinous vs well-differentiated; tumor size, mucinous vs well-differentiated and moderately vs poorly differentiated; tumor site, mucinous vs well-differentiated and mucinous vs poorly differentiated.

‡ P < .001 for the following: tumor size, well- vs moderately differentiated and moderately vs poorly differentiated; presence of lymph node metastasis, well- vs poorly differentiated.

§ P < .05 for the following: presence of lymph node metastasis, mucinous vs well-differentiated, moderately vs poorly differentiated, and well- vs moderately differentiated; presence of peritoneal dissemination, moderately vs poorly differentiated; presence of liver metastasis, mucinous vs poorly differentiated and well- vs poorly differentiated.

¶ P < .0001 for presence of peritoneal dissemination, well- vs poorly differentiated.
evidence of lymph node metastasis, liver metastasis, or peritoneal dissemination (P < .0001), and well-differentiated adenocarcinoma (P < .001; Figure 1B). Univariate Cox proportional hazards survival analysis showed almost the same parameters to have a significant prognostic value, with the other variables not influencing survival. In this analysis, the presence of peritoneal dissemination carried the highest risk of cancer death with an HR of 9.61 (CI, 6.51-14.17), followed by the presence of the lymph node metastasis (HR, 3.42; CI, 2.45-4.75). In addition, the value with mucinous carcinomas was higher than that for well-differentiated adenocarcinomas (HR, 3.20; CI, 1.84-5.58). On multivariate analysis, peritoneal dissemination, lymph node metastasis, and tumor differentiation proved to be independent prognostic factors (Table 2).

Apoptotic Indices

The AIs for mucinous carcinomas (1.6% ± 1.0%) were significantly higher than those for well-differentiated adenocarcinomas (1.1% ± 0.8%, P < .01) (Figure 2A). A similar tendency was observed for the ssDNA LI, although the difference was not significant (data not shown).

For all tumors, a significant positive correlation between AIs and ssDNA LIs was found (Spearman rank correlation coefficient, r = 0.5; P < .0001).

Ki-67 Labeling Index

The Ki-67 LIs for mucinous carcinomas were significantly lower (30.2% ± 18.2%) than those for well- (45.3% ± 18.9%, P < .001), moderately (47.4% ± 20.1%, P < .001), and poorly differentiated (52.5% ± 18.5%, P < .0001) adenocarcinomas (Figure 2B, Image 1A, and Image 1B).

p21^WAF1/CIP1^ and p27^Kip1^ Labeling Indices

The p21^WAF1/CIP1^ and p27^Kip1^ LIs for mucinous carcinoma (2.7% ± 4.7% and 35.3% ± 24.0%, respectively) were lower than those for well-differentiated adenocarcinomas (4.2% ± 6.6% and 48.6% ± 21.9%, respectively, P < .05) (Figure 2C, Figure 2D, and Image 2). No statistically significant differences were found between mucinous carcinomas and the moderately or poorly differentiated types (data not shown).

p53 and bcl-2 Immunoreactivity

No significant differences in p53 or bcl-2 immunoreactive scores were observed among the groups studied (data not shown). Finally, no significant relationships were evident among AI, Ki-67, p21^WAF1/CIP1^, p27^Kip1^, p53, and bcl-2 immunoreactivities in mucinous or nonmucinous carcinomas.

c-Ki-ras Mutation

No significant differences of the frequency of c-Ki-ras mutations in exon 1 (mucinous carcinoma [38.5%] vs well- [45.0%], moderately [35.0%], or poorly [18.8%] differentiated type) or exon 2 (mucinous, 0%; well-differentiated, 2.5%; moderately differentiated, 0%; poorly differentiated, 3.1%) were observed among the other groups.

Survival Analysis of Mucinous Carcinomas

Univariate Cox proportional hazards analysis limited to the mucinous carcinomas demonstrated that of the 14 variables considered (sex; age; tumor site; tumor size, 6 cm or more vs <6 cm; lymph node metastasis or peritoneal dissemination...
or liver metastasis, present vs absent; AI, 1.25% or more vs <1.25%; Ki-67 LI, 27.3% or more vs <27.3%; p21WAF1/CIP1 LI, 0.17% or more vs <0.17%; p27Kip1 LI, 33.9% or more vs <33.9%; p53 score, 3 or more vs <3; bcl-2 score, 4 or more vs <4; c-Ki-ras mutation, positive vs negative), peritoneal dissemination (HR, 6.56; CI, 2.00-21.50; \(P = .0019\)) and AI (HR, 0.36; CI, 0.14-0.95; \(P = .0385\)) had significant prognostic values. On multivariate analysis, peritoneal dissemination (HR, 11.24; CI, 2.31-54.57; \(P = .0027\)), tumor site (proximal colon, HR, 0.13; CI, 0.03-0.57; \(P = .0068\); distal colon, HR, 0.08; CI, 0.01-0.46; \(P = .0049\)), and high AI (1.25% or more; HR, 0.24; CI, 0.07-0.89; \(P = .0328\)) proved to be independent prognostic factors.

**Discussion**

In the present study, mucinous carcinomas accounted for 6.2% of all deeply invasive colorectal carcinomas, a slightly lower value than previously reported.\(^2\)\(^-\)\(^5\) This discrepancy is likely to be due to the differences in diagnostic criteria for mucinous carcinoma.\(^2\)\(^-\)\(^5\) Another possibility is that racial variation exists: the prevalence ranges from 6.0% in the Japanese\(^1\) to 15% in the American population.\(^2\) Several studies have indicated a predilection for nonmucinous carcinomas to develop in the distal colon, while the prevalence is approximately equal in both distal and proximal segments.\(^1\)\(^-\)\(^3\) Most mucinous carcinomas invade beyond the serosa,\(^1\)\(^,\)\(^3\) and rates for involvement of lymph nodes or adjacent organs and peritoneum dissemination are significantly higher than those for well-differentiated adenocarcinomas,\(^1\) in line with our observations. Our data

**Figure 2** Data for the apoptotic index (A), Ki-67 labeling index (B), p21WAF1/CIP1 labeling index (C), and p27Kip1 labeling index (D). Well, well-differentiated adenocarcinoma; Mod, moderately differentiated adenocarcinoma; Poor, poorly differentiated adenocarcinoma; Muc, mucinous carcinoma. Bars indicate mean; error bars indicate SD. * \(P < .001\); † \(P < .01\); ‡ \(P < .0001\); § \(P < .05\).

**Image II** A, Mucinous carcinoma showing a low number of Ki-67+ cells (immunoperoxidase with light methyl green counterstaining, original magnification \(\times 400\)). B, Well-differentiated adenocarcinoma characterized by a high percentage of Ki-67–stained cells (immunoperoxidase with light methyl green counterstaining, original magnification \(\times 400\)).
showed the prevalence of liver metastasis in mucinous carcinoma was lowest among the group studied. The prognosis was unfavorable in most reports, and multivariate Cox proportional hazards survival analysis of our series showed a mucinous character to be an independent predictor for a poor prognosis. With mucinous carcinomas, positive peritoneal dissemination and location in the rectum also were associated with short survival.

The Ki-67 LI has been reported to provide a reliable and reproducible assessment of proliferative activity without relation to prognosis in colorectal cancer. One previous report demonstrated a higher level of Ki-67 reactivity in mucinous carcinomas than in nonmucinous adenocarcinomas counting randomly selected fields, but no significant difference was found between the 2 types of tumor assessed by proliferating cell nuclear antigen immunostaining. In our study, Ki-67 LIs for mucinous carcinoma at the invasive front were significantly lower than for nonmucinous tumors. On the other hand, the apoptotic activity was higher in mucinous carcinomas than in the well-differentiated type of tumors, in contrast with the report of Zhang et al. One possible explanation for this discrepancy is regional heterogeneity of proliferative or apoptotic activity in mucinous carcinomas. Interestingly, mucinous carcinomas with relatively low apoptotic activity (AI, <1.25%) showed a poorer prognosis than those with a high value in the present study. With distal colon cancers, a low AI may predict poor survival. An association of reduced apoptosis with adverse outcome also was reported in patients younger than 45 years at the time of colorectal cancer diagnosis.

In the present study, we chose not to use the terminal deoxynucleotidyl transferase–mediated dUTP (deoxyuridine triphosphate)-biotin nick end-labeling method (ie, the TUNEL method) because it detects not only apoptotic but
also necrotic cells.\textsuperscript{35} It has been reported that morphologic criteria for identification of apoptotic cells are more reliable.\textsuperscript{23} We also comparatively studied apoptotic activity by immunohistochemical analysis for ssDNA that detects early-stage apoptosis,\textsuperscript{36} in addition to conventional morphologic assessment, and found a significant positive correlation between the methods.

In colorectal carcinomas, an association between absence or down-regulation of \textsuperscript{p21 WAF1/CIP1} and poor tumor differentiation or an unfavorable prognosis has been described.\textsuperscript{21,22} In our series, \textsuperscript{p21 WAF1/CIP1} expression was lower in mucinous carcinomas that showed poor prognosis than in the well-differentiated type, but this was without statistical significance. In addition, no correlation was found between \textsuperscript{p21 WAF1/CIP1} and \textsuperscript{p53} immunoreactivity, in line with an earlier study,\textsuperscript{20} suggesting that \textsuperscript{p21 WAF1/CIP1} expression may be regulated by \textsuperscript{p53}-independent mechanisms. The reported significant correlation between \textsuperscript{p27Kip1} expression and tumor grade, poorly differentiated carcinomas having lower levels,\textsuperscript{12} is in agreement with our findings. Lack of \textsuperscript{p27Kip1} was also associated with a poor prognosis, this being an independent prognostic marker in the multivariate analysis of another series.\textsuperscript{14} In the present study, the \textsuperscript{p27Kip1} expression, as well as \textsuperscript{p21 WAF1/CIP1}, was lower in mucinous carcinomas than in well-differentiated adenocarcinomas, but without any significant link to prognosis. On the basis of experimental evidence,\textsuperscript{37} the hypothesis has been raised that tumor growth suppression induced by \textsuperscript{p27Kip1} may be caused by an increase in the apoptosis rate. In our study, however, no correlation was found between \textsuperscript{p27Kip1} and proliferative or apoptotic activity, not lending support to this experimental conclusion.

\textsuperscript{bcl-2} and mutant \textsuperscript{p53} may enhance genetic instability by inhibiting apoptosis in colorectal neoplasms.\textsuperscript{19} The frequency of \textsuperscript{p53} gene mutations or \textsuperscript{p53} protein expression has been found to be lower in mucinous than in nonmucinous tumors with no significant difference in \textsuperscript{bcl-2} expression.\textsuperscript{5-8} In 1 report, \textsuperscript{bcl-2} and \textsuperscript{p53} staining were inversely correlated in adenomas but not in carcinomas of the colon.\textsuperscript{19} In our series, the expression of \textsuperscript{p53} and \textsuperscript{bcl-2} was not different between mucinous and nonmucinous carcinomas, and there was no inverse relationship. While the prevalence of \textsuperscript{c-Ki-ras} mutations may be higher in mucinous than in nonmucinous carcinomas,\textsuperscript{5,26} there was no significant difference in our cases. Of interest, mucinous ovarian tumors the prevalence of \textsuperscript{c-Ki-ras} mutations is higher than in nonmucinous neoplasms.\textsuperscript{38} Whether \textsuperscript{c-Ki-ras} might influence mucus production or degradation, leading to the development of mucinous-type carcinomas, remains to be determined.

Mucinous carcinomas manifesting with a poor prognosis accompanied by frequent lymph node metastasis and peritoneal dissemination are characterized by relatively low proliferative and high apoptotic activity associated with low \textsuperscript{p21 WAF1/CIP1} and \textsuperscript{p27Kip1} expression, as compared with the well-differentiated type; however, apoptotic activity seems to be an independent predictor of patient survival rates with these tumors.

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