Changes of Angiogenesis and Tumor Cell Apoptosis during Colorectal Carcinogenesis

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ABSTRACT
Activation of the angiogenic process occurs during tu-
morogenesis, as does disturbance of cell proliferation and
apoptosis. Seeking a potential correlation, we investigated
tumor cell apoptosis, proliferation, and angiogenesis in the
adenoma-carcinoma sequence of colorectal carcinogenesis
using an in situ apoptosis detection kit and MIB-1 and
anti-CD34 antibodies in 27 adenomas with low dysplasia, 17
adenomas with high dysplasia, and 26 carcinomas in ade-
nomas, as well as assessed p53 and bcl-2 expressions. The
results showed that the potential for apoptosis was aug-
mented, paralleling the increment of proliferation, in ade-
nomas with low dysplasia but diminished when adenomas
progressed from low dysplasia to high dysplasia and cancer.
A gradual increment of microvessel density was observed
during the progression with an increase during transition
from low dysplasia to high dysplasia and cancer. Correlation
coefficient test showed an inverse correlation between apop-
totic index and microvessel density when all of the lesions
were taken into account. No apparent impact of aberrant
p53 on angiogenesis or bcl-2 on apoptosis was observed in
this study. These results suggest that the angiogenesis ini-
tiates during transition from low dysplasia to high dysplasia
and cancer, which may, in turn, contribute to the reduction
of tumor cell apoptosis during colorectal carcinogenesis.

INTRODUCTION
Increasing evidence supports the hypothesis that tumors
result from the disturbance of the balance between cell prolif-
eration and cell death, mainly through apoptosis (1, 2). Although
cell proliferation has been well studied, less is known about the
incidence, regulation, and role of apoptosis during tumor devel-
opment. Colorectal cancer is a major cause of cancer death in
many countries and, in most cases, is believed to have de-
veloped from adenoma (3–5). As tumors of various stages from
dysplasia to malignancy are available, the well-established
adenoma-carcinoma sequence of colorectal carcinogenesis pro-
vides an ideal model for the study of the deregulated cell
proliferation and apoptosis in human cancers. Using this model,
disturbed cell proliferation and apoptosis during colorectal
carcinogenesis have been demonstrated (6–8). However, contro-
versy exists with regard to the alteration of apoptosis in the
adenoma-carcinoma sequence, and little is known about how
such alterations occur (7, 8). On the other hand, a crucial role for
angiogenesis is evident in tumorogenesis. Despite the fact that
activation of an angiogenic switch has been observed in prema-
llignant stages of several human cancers (9), few papers have
addressed the process of neovascularization in the adenoma-
carcinoma sequence (10). In vivo studies have shown that an-
ghiogenesis inhibitors can induce and sustain dormancy of ex-
perimental tumors and micrometastasis by elevating the
incidence of apoptosis in tumor cells, while the proliferation rate
remains unchanged (11, 12). A potential correlation between
tumor cell apoptosis and the initiation of angiogenesis is likely
in colorectal carcinogenesis. We, therefore, investigated tumor
cell proliferation, apoptosis, and angiogenesis in colorectal ad-
nomas with either low or high dysplasias and carcinomas in
adenoma, as well as evaluated the status of bcl-2 and p53 as
established modulators of tumor cell progression and viability.
We have found that the potential of tumor cell apoptosis was
augmented in adenomas with low dysplasia, paralleling the
increased proliferative activity, but diminished subsequently in
adenomas with high dysplasia and cancers coinciding with the
apparent increment of MVD3 in those lesions.

MATERIALS AND METHODS
Tissue Samples. Specimens used for this study were
composed of 44 adenomas and 26 carcinomas in adenoma that
were resected surgically or endoscopically at the Hospital of
Fukui Medical University from 1988 to 1992. To justify com-
parisons, lesions from patients with known familial colon cancer
syndromes and suspected de novo cancers were excluded. Di-
agnosis and histopathological classification were determined
according to the General Rules for Clinical and Pathological
Studies on Cancer of the Colon, Rectum, and Anus by the
Japanese Research Society for Cancer of the Colon and Rectum
(13) and were reviewed on histological sections with H&E
staining. The dysplasias for adenomas were reclassified into two
groups of low (27 cases) and high (17 cases) grade, according to

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3 The abbreviations used are: MVD, microvessel density; AI, apoptotic
index; LI, labeling index.
the criteria for dysplasia in colorectal adenomas of Pascal (14). Due to the complex convolutions of dysplasias of different grades, assessments in a given specimen were conducted only in the area occupied predominantly by the determined grade of dysplasia. Tumors showing definite adenomatous elements adjacent to the areas of carcinoma were diagnosed as carcinomas in adenoma (here referred to simply as carcinomas), and those involved in this study were all stage I (T1, invasion confined to mucosa or submucosa) in accordance with the tumor-node-metastasis classification (13). Tumor size was measured on resected specimens and expressed as the maximum diameter. Corresponding normal mucosa was available in 43 cases in the form of histologically normal mucosa taken either from the surgical resection margins of the original specimens (31 cases) or from adjacent tissues in the case of endoscopically excised samples (12 cases). Serial sections (4-μm-thick) were cut from paraffin blocks and prepared for the following staining procedures.

**Histochemical Detection of Apoptotic Cells and Bodies and the Determination of AI.** Apoptotic cells and bodies were visualized using the ApoTag in situ detection kit (Oncor, Gaithersburg, MD). The staining protocol was as described previously (15). Briefly, after routine treatment, tissues were digested with proteinase K (20 μg/ml; Wako, Osaka, Japan) for 20 min at room temperature and washed. Slides were then quenched for endogenous peroxidase in 3% H2O2. After sections were washed and equilibration buffer was added, terminal deoxynucleotidyltransferase enzyme was pipetted onto the sections, which were then incubated at 37°C for 1 h. The reaction was stopped by placing sections in stop/wash buffer. After washing, anti-digoxigenin-peroxidase was added to the slides. Slides were washed, stained with 3,3′-diaminobenzidine substrate (DAKO A/S, Glostrup, Denmark) and counterstained with methyl green. A specimen known to be positive for apoptotic cells was used as a positive control. Substitution of terminal deoxynucleotidyltransferase with distilled water was used as a negative control. Positively labeled cells and bodies meeting the morphological characteristics of apoptosis were identified as apoptotic cells and bodies (15), and only those located at intraepithelium were taken into account. The AI was expressed as the ratio of apoptotic cells and bodies to epithelial cells in the normal mucosa or to all tumor cells and was expressed as a percentage for each case.

**Assessment of Cell Proliferation and MVD.** Cell proliferation was assessed by immunostaining using the antibody against Ki-67 (MIB-1, diluted 1:50; Immunotech, Marseille, France). MVD was assessed by counting intraepithelial or intratumoral microvessels highlighted by endothelial cells immunostaining for CD34 antigen (QB-END/10, diluted 1:25; Novocastra Laboratories, Newcastle, United Kingdom). Microwave treatment (three times at 900 W for a total of 15 min) in 0.01 M citrate buffer (pH 6.0) was required for MIB-1 immunostaining and 0.1% trypsin in 0.1% calcium chloride solution (pH 7.8) digestion for 15 min at 37°C was required for anti-CD34 staining, respectively. The standard avidin-biotin-peroxidase complex technique was applied for color development and hematoxylin for counterstaining, as described elsewhere (15–17). Positive and negative controls were used every time.

The proliferative fraction of epithelial cells in normal mucosa and tumor tissue was expressed as the ratio of positively stained cells for Ki-67 to all epithelial cells, given as a percentage for each case as a Ki-67 LI, which was determined in those areas showing the highest density of positive staining nuclei. AI, MVD, and Ki-67 LI were evaluated in the same sections, regardless of the areas used for the assessment of each factor. If available, five areas were selected for counting under 400-fold magnification; otherwise, the whole section underwent assessment.

For MVD determination, the five most vascular areas in the normal mucosa or the tumor, if available, were selected and counted under 200-fold magnification using a point counting technique; otherwise, the whole section was taken into account. The average count was recorded and expressed as the absolute number of vessels per 0.74 mm2 (per ×200 field) for each case.

**Immunohistochemical Staining for bcl-2 and p53.** A monoclonal mouse antibody against bcl-2 oncoprotein diluted at 1:20 (clone 124; DAKO, Copenhagen, Denmark) and a mouse anti-human p53 antibody diluted at 1:50 (DO-7; DAKO) were used as primary antibodies for bcl-2 and p53 immunostaining. Before addition of the primary antibody, sections were heated in a microwave three times at 900 W for a total of 15 min. The other staining procedures were as for Ki-67 antigen. A positive control for p53 staining was obtained from a previous study (15), and the reactivity of infiltrating lymphocytes with bcl-2 monoclonal antibody served as an internal positive control for bcl-2 staining. Negative controls were prepared in the absence of primary antibody, as described above. The immunohistochemical results for bcl-2 were recorded as positive if >10% of cells in a tumor showed unequivocally cytoplasmic staining; otherwise, tumors were identified as negative. For p53 expression, cases with >10% tumor nuclei staining were defined as positive; the rest were defined as negative.

**Statistical Analysis.** We used Student’s t test to analyze continuous variables and the χ2 test for categorized data. Correlations between AI, Ki-67 LI, and MVD were checked by the Pearson’s rank correlation coefficient on a per case basis. SPSS software (SPSS Inc., Chicago, IL) was used for all statistical analyses. P < 0.05 was considered statistically significant.

**RESULTS**

The clinical background of patients in the three groups was shown in Table 1. Relative to the patients with adenomas with low dysplasia, the patients with carcinomas were significantly older, and their tumors were significantly larger (P = 0.005 and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinicopathological background of lesionsa</th>
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<tr>
<td>Lesions</td>
<td>Total</td>
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<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Adenomas with low dysplasia</td>
<td>27</td>
</tr>
<tr>
<td>Adenomas with high dysplasia</td>
<td>17</td>
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<tr>
<td>Carcinomas</td>
<td>26</td>
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a Statistics: carcinomas vs. adenomas with low dysplasia, P = 0.005 for age and P = 0.002 for tumor size; no significant differences were observed for each of the other two groups.
No significant differences were observed for any other two groups.

Cell Proliferation, Apoptosis, and Vasculature in Normal Mucosa of Large Bowel. In normal mucosa, MIB-1 staining was confined to the lower one-third of the crypts, corresponding to the proliferative zone. The mean value of Ki-67 LI obtained by counting MIB-1 positive nuclei was 6.0% (SD, 3.8%). In contrast, apoptotic cells and bodies identified by a brown staining and morphological features of apoptosis were observed more frequently at or near the mucosa surface. Apoptotic cells or bodies extruded into the lumen of bowel or detached from the mucosa were also seen. After excluding the positively stained cells and bodies in the lumen from counting, a mean value of normal intraepithelial AI of 0.26% (SD, 0.18%) was obtained. Anti-CD34 immunostaining showed a uniform spatial distribution of microvessels between crypts with regular lumens of vessels of ~10 μm at the most. The mean value of MVD/0.74 mm² was 74 (SD, 15).

Alterations of Ki-67 LI, AI, and MVD in Adenoma-Carcinoma Sequence. An increase of proliferative activity was observed in the adenoma-carcinoma sequence, as reflected by the increased mean value of Ki-67 LI of 16.7% (SD, 6.5%) in adenomas with low dysplasia, 25.8% (SD, 5.7%) in adenomas with high dysplasia, and 24.6% (SD, 8.8%) in carcinomas (P < 0.001 for every paired comparison, except a P of 0.61 for adenomas with high dysplasia and carcinomas; Fig. 1A). The pattern of normal spatial distribution of MIB-1 staining disappeared in adenomas and carcinomas with most of the positive cells located near the lumen or surface (Fig. 2A). The potential for cell apoptosis changed markedly during the progression from normal mucosa to malignant colorectal epithelia. The spatial differences in AI were noted as follows: the rates of apoptosis were increased in basal versus superficial regions of adenomas, which is inverted relative to normal epithelia, as pointed out previously (7, 18). The AI reached a mean value of 2.3% (SD, 2.0%; Fig. 1B) in adenomas with low dysplasia (versus normal mucosa, P < 0.001) but declined to 0.74% (SD, 0.74%) in adenomas with high dysplasia and 0.90% (SD, 0.58%) in carcinomas. Although the SDs in AI were large, reflecting considerable intertumor variability, the latter two values were higher than in normal mucosa (P < 0.001) but lower than in adenomas with low dysplasia (P = 0.004 and P = 0.001, respectively; Fig. 1B). A gradual increment of MVD was noted in the adenoma-carcinoma sequence. The MVD/0.74 mm² increased to a mean value of 82 (SD, 15) in adenomas with low dysplasia, 100 (SD, 29) in adenomas with high dysplasia, and 115 (SD, 18) in carcinomas. Although all of the increments of MVD were significant, the change from normal to adenoma with low dysplasia (P = 0.04) and that from adenoma with high dysplasia to carcinoma (P = 0.05) was moderate (Fig. 1C). The morphological changes of microvessels were not apparent in adenomas with low dysplasia but displayed a great aberrance in adenomas with high dysplasia and carcinomas as characterized by tortuous extensions, considerable diversity in the lumen, and irregular spatial distribution with highest density at the epithelial margin (Fig. 3).

Correlation between Ki-67 LI, AI, and MVD. Because the biological properties of adenomas with high dysplasia, as reflected by Ki-67 LI, AI, and MVD, resemble those of carcinomas, these two groups were counted as one, and the correlation between Ki-67 LI, AI, and MVD was then analyzed in this group and the group of adenomas with low dysplasia or as a whole by Pearson’s correlation coefficients test on a per-case basis. The augmentation of apoptosis was revealed to parallel the increment of proliferative activity in adenomas with low dysplasia (r = 0.52, P = 0.006) but not in adenomas with high dysplasia and carcinomas (r = 0.12, P = 0.46; Fig. 4A). No appreciable correlation could be found when all of the lesions were taken into account. A tendency toward diminished AI accompanying increased MVD was noted in both adenomas with low dysplasia and adenomas with high dysplasia and carcinomas, although it had not reached a significant level. However, an inverse correlation between AI and MVD appeared when all of the lesions were taken into account (r = −0.39, P = 0.001; Fig. 4B), revealing that a significant impact of increased MVD on apoptosis manifested in the transition from low dysplasia to high dysplasia and carcinoma during colorectal carcinogenesis.

Aberrant Expressions of p53 and bcl-2 in Relation to Tumor Cell Proliferation and Apoptosis. Nuclear staining for p53 was detected in 2 of 27 adenomas with low dysplasia (4%), in 11 of 17 adenomas with high dysplasia (65%) and in 15 of 26 carcinomas (58%). Differences in the rate of p53 expression were shown to be significant only for adenomas with low dysplasia in comparison with adenomas with high dysplasia and carcinomas (P < 0.001). Normal colorectal epithelium was completely negative for p53 immunostaining. bcl-2 immunostaining was detected in the base of most crypts in normal mucosa with reduced intensity up to the surface. Aberrant expression of bcl-2 characterized by a patchy distribution or diffuse homogeneous staining was observed in 11 of 27 adenomas with low dysplasia, 6 of 17 adenomas with high dysplasia, and 12 of 26 carcinomas, with no significant differences between any two groups (Table 2). The impacts of the aberrant expressions of p53 and bcl-2 on proliferation, apoptosis, and angiogenesis were evaluated. Excepting adenomas with low dysplasia (n = 27) in which the Ki-67 LI and AI in p53-positive tumors were higher than in p53-negative tumors (n = 19; P = 0.015 and P = 0.002, respectively), no significant differences could be observed for AI and Ki-67 LI. When all of the lesions were taken into account, p53-positive tumors (n = 34) showed a higher proliferative activity than p53-negative tumors (n = 36; Ki-67 LI = 25.6 (SD, 8.4) versus 18.4 (SD, 6.5); P < 0.001). A lower Ki-67 LI for bcl-2-positive tumors (n = 11) was observed in adenomas with low dysplasia [n = 16; Ki-67 LI = 13.6 (SD, 3.9) versus 18.9 (SD, 7.1); P = 0.04], and the AI of bcl-2-positive tumors was usually lower than that of bcl-2-negative tumors in each of the groups, but the difference was not significant. When all of the lesions were taken into account, bcl-2-positive tumors showed a lower mean value of AI than bcl-2-negative tumors, which was borderline significant (P = 0.07). No significant impacts on tumor angiogenesis from either aberrant p53 accumulation or bcl-2 expression were observed in any of the groups.

DISCUSSION

The results of this study with respect to the alterations of cell proliferation and apoptosis during adenoma-carcinoma pro-
gression are consistent with reports by Sinicrope et al. (7) and Kikuchi et al. (8), although they did not show a difference between low dysplasias and high dysplasias. The AIs obtained from our study are lower than those from their reports, which may be ascribed to our strict criteria for identifying apoptotic cells and bodies, and the exclusion of detached apoptotic cells and bodies from counting. The fact that the increased Ki-67 LI was accompanied by an elevated level of apoptosis in adenomas with low dysplasia indicates that adenomas arise through increased proliferation of epithelial cells, of which most would be deleted through increased apoptosis. It was expected that the increased proliferative activity might, in turn, activate the program of cell death (apoptosis) due to lack of nutrients, competition for growth factors, or oxygen starvation resulting from the deregulated proliferation, provided that the underlying mechanisms for apoptosis had not been compromised. This may also explain why the growth of early adenomas takes decades despite the high proliferative activity indicated in most reports (3, 5–7).

In alteration, the synergetic effect of increased proliferation and decreased apoptosis may contribute to the relative rapid growth of late adenomas and carcinomas because perturbations presented not only in the control of cell proliferation but in the control of apoptosis as well in those lesions. Recent advances in molecular analysis suggest that the accumulation of genetic changes as well as their order with respect to one another, for example, mutations of ras gene and adenomatous polyposis coli (APC) gene, underlie the development of colorectal carcinomas (3–5). A further understanding of the function of such genes and their changes in the adenoma-carcinoma sequence may uncover the molecular basis for the reduction of apoptosis during this process.

The crucial role of angiogenesis in tumor growth is well documented, and the increment of MVD in colorectal adenomas and cancers has been reported (9–12, 17). Transgenic mouse models have revealed an angiogenic switch that becomes activated during the early stages of tumor development (9). In line with these studies, we found a gradual increment of MVD in the adenoma-carcinoma sequence. To our knowledge, it is the first time that a progression of angiogenesis in colorectal carcinogenesis has been studied in the established adenoma-carcinoma sequence. Given the fact that the development of adenomas usually takes years to decades and the vasculature in adenomas with low dysplasia observed in this study appears normal, the moderate increment of MVD from normal mucosa to adenomas with low dysplasia may arise from the passive extending of normal vessels rather than the initiation of tumor angiogenesis induced by the deregulated angiogenic factors. As a discrete, rate-limiting step, tumor angiogenesis seems to be initiated during the transition of adenomas from low dysplasias to high dysplasias and carcinomas as reflected by the significant rising of MVD and aberrant morphological changes of vessels as well as dysplasia and to 0.90% (SD, 0.58%) in carcinomas (normal mucosa versus each lesion, P < 0.001; a, P = 0.004; b, P = 0.001; c, P = 0.44).

Fig. 1 Alterations of Ki-67 LI, AI, and MVD in the adenoma-carcinoma sequence. Box plots display median values and interquartile ranges. Extreme values, defined as more than three box lengths, and outliers, defined as being between 1.5 and 3 box lengths, were omitted. A, Ki-67 LI increased from a mean value of 6.0% (SD, 3.8%) in normal mucosa to 16.7% (SD, 6.5%) in adenomas with low dysplasia, 25.8% (SD, 5.7%) in adenomas with high dysplasia, and 24.6% (SD, 8.8%) in carcinomas (a, P = 0.61; all other two groups, P < 0.001). B, AI reached a mean value of 2.3% in adenomas with low dysplasia (SD, 2.0%) but declined to 0.74% (SD, 0.74%) in adenomas with high dysplasia and to 0.90% (SD, 0.58%) in carcinomas (normal mucosa versus each lesion, P < 0.001; a, P = 0.004; b, P = 0.001; c, P = 0.44). C, MVD/0.74 mm² increased from a mean value of 74 (SD, 15) in normal mucosa to 82 (SD, 15) in adenomas with low dysplasia, 100 (SD, 29) in adenomas with high dysplasia, and 115 (SD, 18) in carcinomas (a, P = 0.04; b, P = 0.008; c, P = 0.05).
As one of the most significant aspects of this study, the significant increase of MVD in adenomas with high dysplasia and carcinomas coincided, temporally and spatially, with the decline of AI, and the AIs were shown to be inversely correlated with MVDs when all of the lesions were taken into account, suggesting that the initiation of tumor angiogenesis plays an additional role in inhibiting apoptosis. We have demonstrated previously an inverse correlation between tumor cell apoptosis and MVD in gastric cancer (15). A "paracrine" effect in tumor growth has been proposed to explain the rapid growth of tumors after the angiogenic switch during tumorigenesis (11, 12). The paracrine effect results from growth factors produced by capillary endothelial cells (such as fibroblast growth factor, heparin-binding epithelial growth factor, and insulin-like growth factor) or from macrophages and host cells delivered by neo-vascularization to the tumor (19). The sustaining effect of such growth factors on tumor cells may contribute to the mechanism by which the inhibition of apoptosis results from the initiated angiogenesis in colorectal carcinogenesis.

An important question that has arisen is how the initiation of angiogenesis is controlled at the genetic level, for which there is no answer, despite the fact that the underlying mechanisms for the control of vasculogenesis are emerging and a number of angiogenic inducers and inhibitors for angiogenesis have been identified (9, 20). Although experimental evidence suggests that p53 modulates angiogenesis (9, 10), most studies into human
Fig. 3 A panel of tissue sections illustrating the process of angiogenesis from adenoma to carcinoma. A, moderate increase of MVD in an adenoma with low dysplasia characterized by tall epithelial cells with elongated, hyperchromic nuclei keeps the spatial distribution pattern of normal mucosa and regular lumens of ~10 μm in most of the vessels (arrows). A few of normal crypts remain in the background. B, accompanying the increased MVD, the aberrance of vasculature featured by tortuous extensions, considerable diversity in the lumen, and irregular spatial distribution (arrows) become evident in an adenoma with high dysplasia characterized by stratified cells with marked nuclear pleomorphism. C, a carcinoma showing an apparent aberrance of microvessels with highest density at the epithelial margin of tumor (arrows). Original magnification, ×100.
cancer including this one have been unable to confirm a correlation between p53 gene status and MVD (10). The results in regard to the process of angiogenesis in this study suggest, in addition, that the genetic changes underlying the transition from low dysplasia to high dysplasia or cancer during colorectal carcinogenesis may, at the same time, be responsible directly or indirectly for the initiation of angiogenesis. Because cell lines representing different stages of the adenoma-carcinoma sequence are available for study (4), further investigations into the genetic basis of the angiogenic progression in the adenoma-carcinoma sequence using known genetic models both in vivo and in vitro may provide an insight into the underlying mechanisms.

The status of p53 and bcl-2 has been well studied in the adenoma-carcinoma sequence (5–7, 21, 22). In line with previous reports, p53 accumulation was observed more frequently in late adenomas and cancers, suggesting an increased incidence of mutation of p53 in the progression of colorectal carcinogenesis. The Ki-67 LI was usually higher in p53-positive tumors than in p53 negative tumors. No apparent impact of p53 on tumor cell apoptosis could be found despite a higher AI in p53 positive tumors of adenomas with low dysplasia, which may be due to the increased proliferative activity in these tumors rather than the status of p53 itself. The aberrant expression of bcl-2 was observed throughout the adenoma-carcinoma sequence. The expression of bcl-2 did not significantly differ between each stage of tumor development in this study. Nevertheless, bcl-2 has a general role in extending cell viability: the AIs in bcl-2-positive tumors only were lower than in bcl-2 negative tumors, with a borderline significance (P<0.07). These results, together with observations from other reports (8, 22), suggest that the status of bcl-2 is unlikely to be the main reason for the reduction of apoptosis in the adenoma-carcinoma sequence. A novel apoptosis inhibition gene, survivin, has been found to be expressed relatively frequently in common human tumors (23, 24). Investigations on the aberrant expression of survivin and a potential correlation between survivin expression and apoptosis inhibition during colorectal carcinogenesis are currently underway.

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REFERENCES

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Table 2  Aberrant expressions of p53 and bcl-2 in the adenoma-carcinoma sequence

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<th>Total no.</th>
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<th>bcl-2 expression</th>
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<td>16 +</td>
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<tr>
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<td>2 –</td>
<td>11 –</td>
</tr>
<tr>
<td>Adenomas with</td>
<td>17</td>
<td>6 +</td>
<td>11 +</td>
</tr>
<tr>
<td>high dysplasia</td>
<td></td>
<td>11 –</td>
<td>6 –</td>
</tr>
<tr>
<td>Carcinomas</td>
<td>26</td>
<td>11 +</td>
<td>14 +</td>
</tr>
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*Statistics: adenomas with low dysplasia vs. adenomas with high dysplasia or carcinomas for p53 expression, P<0.001 and P<0.001, respectively; no significant differences were observed between each of the other two groups for either p53 or bcl-2 expression.
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