in vivo *21*: 113-118 (2007)

Bcl-2, Bax and p53 Expression in Rectal Adenocarcinoma. Correlation with Classic Pathologic Prognostic Factors and Patients' Outcome

ATHANASSIOS C. TSAMANDAS[1], DIMITRIOS KARDAMAKIS[2], THEODORE PETSAS[3], V. ZOLOTA[1], V. VASSILIOU[2], TH. MATATSORIS[4], HARALABOS KALOFONOS[4], CONSTANTINE E. VAGIANOS[5] and CHRISOULA D. SCOPA[1]

Departments of [1]Pathology, [2]Radiotherapy, [3]Radiology, [4]Medical Oncology and [5]Surgery, University of Patras School of Medicine, Patras, Greece

Abstract. *Background: Bcl-2 oncoprotein inhibits apoptosis, whereas bax protein promotes apoptosis by enhancing cell susceptibility to apoptotic stimuli. This study examined the bcl-2, bax and p53 expression in rectal adenocarcinomas and their relationship with tumor prognosis. Patients and Methods: Paraffin-embedded 4-μm tumor sections obtained from patients with rectal adenocarcinoma who underwent colectomy for therapeutic reasons, were analyzed with a standard streptavidin biotin peroxidase method, using polyclonal and monoclonal antibodies. Patients were followed up for 1.5-83 (mean±SD: 47.19±6.2) months. Results: Positive immunoreactivity for bcl-2, bax and p53 was detected in 21 (37%), 28 (50%) and 45 (80%) tumors, respectively. Bax was co-expressed in 17 out of 21 bcl-2(+) cases, whereas p53 was co-expressed in 18 out of 21 bcl-2(+) and 17 out of 28 bax(+) cases. Loss of bax expression was associated with advanced stage and high grade tumors (p<0.01). Local (n=6) or distant (n=5) tumor recurrence was established in 11 cases. All these cases were bax(+), bcl-2(–) and p53(+). Bax and p53 expressions were correlated with adverse outcome (p<0.05) while bcl-2 presence did not influence survival. Bcl-2(–)/bax(+)/p53(+) cases showed lower survival than bax(+)/bcl-2(+)/p53(+)cases (p<0.001). Conclusion: In rectal adenocarcinoma, bax and bcl-2 proteins co-express frequently with p53. Co-expression of bax with p53 protein is associated with poor clinical outcome, especially in cases without concomitant expression of bcl-2.*

Correspondence to: Dimitrios Kardamakis, Associate Professor, Department of Radiotherapy, University of Patras Medical School, 26500, Rion Patras, Greece. Tel: +302610999540, Fax: +302610994475, e-mail: kardim@med.upatras.gr

Key Words: Rectal adenocarcinoma, bcl-2, bax, p53, immunohistochemistry, tumor recurrence.

Colorectal cancer is one of the most common cancers, affecting men and women equally. Its prognosis is related to several clinical and pathological parameters. The rectum is one of the most frequently involved sites and accounts for 25% of primary colorectal cancers. The stage of the tumor, along with the histologic type and grade, have been used as parameters for predicting prognosis and planning appropriate therapeutic approaches (1).

Today it is accepted that tissue growth during tumor development is the result of cell proliferation or enhanced cell survival (through inhibition of apoptosis) or it results from a combination of both mechanisms (2). Apoptosis is a morphologically distinct, gene-directed form of cell death, characterized by cytoplasmic fragmentation and nuclear condensation that contributes to both physiological and pathological processes (2).

One of the oncogenes that regulate apoptosis is bcl-2. It is located at chromosome 18q21 and its expression was first described in studies regarding t(14;18) chromosomal translocation in B-cell follicular lymphoma (3). Bcl-2 encodes a 26-kD protein that blocks programmed cell death without affecting cellular proliferation (4). It has emerged as a key regulator of apoptosis, because it can protect cells from death induced by a number of injuries including radiation, chemo-therapy or growth factor deprivation. Bcl-2 expression has been detected in the long-lived, self-renewing populations of stem cells that line the basement membrane of several epithelia including skin, colon and prostate, but not in the terminally differentiated cells found at the surface of those epithelia, which are believed to die by apoptosis (5).

Regarding bcl-2 expression in malignant tumors and its relation with prognosis, data are conflicting. Bcl-2 protein expression has been linked either to a favorable prognosis of malignant tumors (6) including colon carcinomas (7), or to a worse prognosis (8), whereas other studies failed to demonstrate any relation between bcl-2 expression and

prognosis, in malignant tumors and more specifically colorectal cancer (2).

Bax protein is a homologue of bcl-2 that promotes apoptosis (9). Bax may bind to bcl-2 forming bax/bcl-2 heterodimers, or may bind to itself forming bax/bax homodimers (9). The ratio of bax to bcl-2 determines the susceptibility of a cell to apoptosis. Thus, in cells with bax overexpression, bax homodimers predominate, and the susceptibility of such cells to apoptotic stimuli is enhanced. Bax/bcl-2 heterodimers predominate in cells that overexpress bcl-2, and the susceptibility of these cells to apoptosis is reduced (9, 10). Previously, we have shown that in patients with rectal adenocarcinoma, an elevated bcl-2/bax ratio in tissue specimens suggests increased resistance of the specific tumors to radiotherapy and thus it may serve as a potential molecular marker for prediction of tumor prognosis (11).

The wild-type p53 is a direct transcriptional activator of the bax gene suggesting the presence of a complexed interaction of bax, bcl-2 and p53 during cellular growth and apoptosis (12, 13). Recent studies have shown that in cases of colorectal cancer, p53 expression is related to a higher rate of distant metastases and a lower rate of local relapse (14), whereas others have demonstrated either no prognostic implication or adverse prognostic impact (15).

This study investigates bcl-2, bax and p53 expression in cases of rectal adenocarcinoma and attempts to assess a possible correlation of these markers with important clinicopathological parameters, such as tumor grade, stage and patients' survival.

Patients and Methods

Patients. This retrospective study comprised 56 consecutive surgical specimens of primary rectal adenocarcinomas, resected at Patras University Hospital, Greece, during an 9-year period (1990-1998). Archival tissues and data derived from the pathology records as well as clinical follow-up were readily available for all patients. There were 37 male and 19 female patients with a median age at the time of diagnosis of 64 years (range 27-76 yr). All patients had resectable rectal adenocarcinoma and did not receive any prior systemic chemotherapy or radiotherapy before surgery.

A blind review of the slides and pathology report for each patient was conducted in order to confirm the pathological grade and stage. All tumors included in the study were staged according to the Astler-Coller modification of Dukes classification and furthermore, the tumors were graded according to a modification in grading system proposed by WHO (low-grade and high-grade) (16). Patients were followed up for 1.5-83 (mean±SD: 47.19±6.2) months.

Before chemotherapy was initiated, all patients were assessed by physical examination, routine hematology and biochemistry analysis, chest X-ray and CT scans. All 56 patients with rectal carcinoma underwent postoperative radiotherapy using a 6-MV linear accelerator and an isocentric three- or four-field technique, in prone position with the bladder distended. Radiotherapy was initiated 2-3 weeks after the first six weekly fractions of chemotherapy. The dose was 45 Gy to the tumor bed, perirectal tissues and regional lymph nodes (1.8 Gy per fraction, five fractions per week). An additional dose of 5.4 Gy was given as a boost to the tumor bed. The total dose was prescribed at the 95% or 90% isodose curve encompassing the target volume. Chemotherapy (5-fluorouracil 450-500 mg/m^2, leucovorin 200 mg/m^2) was given postoperatively up to a total dose of 24 weekly fractions, unless the patient was voluntarily withdrawn or unacceptable toxicity occurred. Additionally, 5-fluorouracil (400 mg/m^2) was administered as a single rapid infusion on the first 3 and the last 3 days of radiotherapy as a radiosensitizer.

Immunohistochemical stain for the evaluation of bcl-2, bax and p53 protein expression. The detection of cells that express bcl-2, bax and p53 proteins, relied on immunohistochemistry based on a standard streptavidin biotin peroxidase method (Multilink kit, Biogenex, San Ramon, CA, USA) as described previously (2, 11). Paraffin sections (4 μm thick) were used and primary antibodies included: monoclonal antibody to bcl-2 (DAKO, USA in a dilution of 1:40), polyclonal antibody to bax (Santa Cruz, CA, USA, in a dilution of 1:1000) and mouse monoclonal antibody to p53 (Biogenex, San Ramon, CA, USA, ready-to use). All incubations were performed for 30 min at room temperature. Diaminobenzidine (Sigma Fast DAB tablets, D-4293, Sigma St Louis, MO, USA) was used as chromogen. Cytoplasmic staining for bcl-2 and bax and nuclear stain for p53 were considered as positive. For positive control purposes, paraffin sections from human tonsils were used. For negative control purposes, the same streptavidin-biotin technique was used on tissue sections in which 1% BSA in PBS substituted the primary antibody.

Morphometric analysis. A well-established method that has been described in detail elsewhere was used for blind morphometric estimations (2, 11). Briefly, cell counts were performed manually at a x400 magnification using a 10x10-microscope grid. Both the number of immunoreactive cells and the total number of tumor cells (at least 500 cells) at selected areas were tallied in five different fields per section. For each field the percentage of immunoreactive cells for bcl-2, bax and p53 was obtained. The values between fields did not differ by more than 10% per section. Cases were considered positive if at least 5% of tumor cells displayed cytoplasmic staining for bcl-2 or bax and nuclear staining for p53.

Statistical analysis. Results were expressed as median values. Correlation of pathologic parameters with staining results, were performed using one-way analysis of variants (ANOVA). Whenever the equal variance test or normality tests failed, the Kruskal-Wallis non-parametric test was applied. In order to address the problem of multiple comparisons, the ANOVA and Kruskal-Wallis tests were followed by a *post hoc* Bonferroni test. The Spearman rank correlation was used to detect the relationship between bcl-2, bax and p53. The Kaplan-Meier procedure was also used to compare the survival curves. Data were analysed using the SPSS statistical package (SPSS©, Release 10.0.1, USA). Any p-value <0.05 was considered significant.

Results

Patients. Twenty-seven tumors were of stage B_2 and 29 tumors of stage C. Forty six adenocarcinomas, which were well or moderately-differentiated, were recorded as low-grade tumors, whereas the remaining 10 poorly-differentiated adenocarcinomas were listed as high grade tumors.

Table I. *Bcl-2, bax and p53 expression in relation to tumor stage and grade.*

Pathological factors	Number of cases	Bcl-2 [No of (+) cases (median of positive cells %)]	Bax [No of (+) cases (median of positive cells %)]	p53 [No of (+) cases (median of positive cells %)]
Tumor stage				
B_2	27	10 (35)	13 (45)[a]	21 (41)
C	29	11 (32)	15 (30)[a]	24 (39)
Tumor grade				
Low (I+II)	46	20 (37)	21(58)[b]	36 (38)
High (III)	10	1 (29)	7 (36)[b]	9 (40)
Total	56	21	28	45

Differences are statistically significant at $p<0.01$ level in cases with matching letters a, b.

Table II. *Survival and bcl-2, bax, p53 expression.*

Factors	Number of cases (n=56)	Survival (No of alive patients n=44)	Survival (months median value)
Bcl-2(–)	35	24	52
Bcl-2(+)	21	20	59
Bax(–)	28	28[a]	60[b]
Bax(+)	28	16[a]	41[b]
p53(–)	11	11[c]	61[d]
p53(+)	45	33[c]	45[d]
Bcl-2(+)/Bax(+)	17	16[e]	58[f]
Bcl-2(+)/Bax(–)	4	4	59
Bcl-2(–)/Bax(+)	11	0[e]	43[f]
Bcl-2(–)/Bax(–)	24	24	56
Bcl-2(+)/p53(+)	18	17	51
Bcl-2(+)/p53(–)	3	3[g]	61[h]
Bcl-2(–)/p53(+)	27	16[g]	48[h]
Bcl-2(–)/p53(–)	8	8	57
Bax(+)/p53(+)	17	5[i]	43[j]
Bax(+)/p53(–)	11	11	51
Bax(–)/p53(+)	28	28[i]	54[j]
Bax(–)/p53(–)	--	--	--
Bcl-2(–)/Bax(+)/p53(+)	11	0[k]	44[l]
Bcl-2(+)/Bax(–)/p53(+)	4	4[k]	55[l]

Differences are statistically significant at the $p<0.05$ levels in numbers with matching letters a, b, c, d, g, h, i, j, and at the $p<0.01$ level in numbers with matching letters e, f , k, l.

In a follow-up period of 1.5-83 (mean±SD: 47.19±6.2) months, 44 patients (79%) of the patients are alive. In 11 cases the cause of death was tumor recurrence and in one case the patient died due to diabetic coma 25.5 months post surgery; however until that time no tumor recurrence developed.

Immunohistochemistry. Table I shows the immunohistochemical results in relation to the classic pathologic prognostic factors. Bcl-2 expression was detected in 21 (37%) of the tumors. The pattern of staining was cytoplasmic or perinuclear (Figure 1A). In the adjacent non-neoplastic colonic mucosa, bcl-2 was present within the cytoplasm of epithelial cells in the base of the crypts and in the infiltrating lymphocytes. Bcl-2 expression was higher (in terms of % positive cells) in low-grade tumors, however this difference did not reach statistical significance.

Bax expression was detected in 28 (50%) of the tumors. It displayed a granular cytoplasmic pattern of staining (Figure 1B). Bax was expressed in 17/21 bcl-2 (+) cases. Loss of bax expression with advanced stage and high grade was observed ($p<0.01$). This difference was recorded when the median values (% of positive tumor cells) were taken into account and not the number of cases.

P53 expression was recorded in 45 (80%) of the tumors. It exhibited typical nuclear staining (Figure 1C). As shown in Table II, p53 was co-expressed in 18/21 bcl-2(+) and in 17/28 bax(+) cases. Statistical analysis did not reveal any significant differences between any combination of p53/bcl-2, p53/bax or bcl-2/bax expression and stage and grade of the tumors ($p>0.05$). However, a substantial difference was seen when tumor recurrence was studied. Tumor recurrence was established in 11 cases, of which 6 were local and 5 were distal. All these cases were bax positive, bcl-2 negative and p53 positive. An inverse correlation was observed between the percentage of bax(+) cells and the time (in months) the recurrence was discovered but this was not statistically significant ($p=0.075$).

Survival analysis. A statistically significant association was observed between stages (B_2 *vs.* C : $p<0.05$) and between grades (high *vs.* low : $p<0.01$), with tumors of advanced stage and grade exhibiting shorter survival. Similar observations were made when the length of survival (in months) was compared to each of these classic prognostic factors (Table III).

Statistical correlations, between the immunohistochemical results for bcl-2, bax and p53, revealed that bax and p53 expression was correlated with adverse outcome (tumor recurrence) and shorter overall survival ($p<0.05$), while bcl-2 presence did not influence survival. The latter finding is in full accordance with a previous study done by this group (2).

Table II includes the correlations of all possible combinations between bcl-2, bax and p53 expression. Statistically significant differences were recorded between: i) bax(+)/bcl-2(–) and bax(+)/bcl-2(+) cases ($p<0.01$), ii) bcl-2(–)/p53(+) and bcl-2(+)/p53(–) cases ($p<0.05$), iii) bax(+)/p53(+) and bax(–)/p53(+) cases ($p<0.05$) and iv)

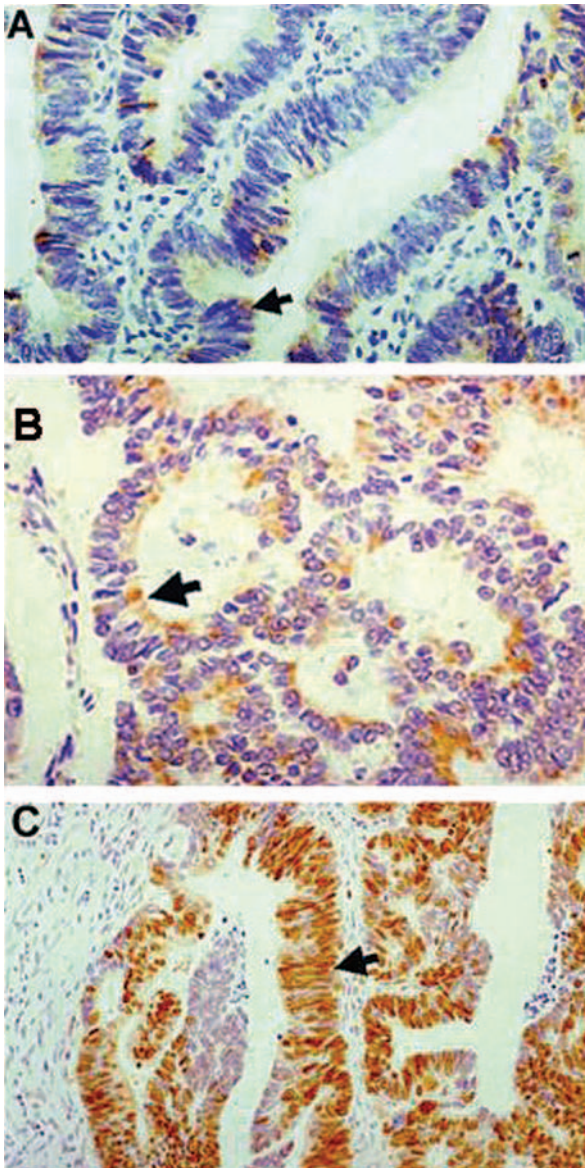

Figure 1. *A) Photomicrographs showing bcl-2 expression in a well-differentiated rectal adenocarcinoma (arrow) (streptavidin-biotin peroxidase x400). B) Photomicrographs showing bax expression in a moderately-differentiated rectal adenocarcinoma (arrow) (streptavidin-biotin peroxidase x400). C) Photomicrographs showing p53 expression in a moderately-differentiated rectal adenocarcinoma (arrow) (streptavidin-biotin peroxidase x400).*

bax(+)/bcl-2(–)/p53(+) and bax(–)/bcl-2(+)/p53(+) cases ($p<0.01$); the former cases exhibited adverse outcome and shorter overall survival, when compared to the latter cases respectively.

Figure 2 shows the bcl-2/bax/p53 expression status in relation to Kaplan-Meier survival curves. Statistically significant differences were recorded between the bcl-2(–)/ bax(+)/p53(+) and bcl-2(+)/bax(+)/p53(+) groups ($p<0.001$).

Table III. *Correlation of patient's gender, tumor stage and grade with survival.*

Factor	No of cases	Survival (No of alive patients)	Survival (months median value)
Gender			
Males	37	29	60
Females	19	15	59
Stage			
B_2	27	26[a]	61[b]
C	29	18[a]	41[b]
Grade			
Low (I+II)	46	41[c]	60[d]
High (III)	10	3[c]	10[d]
Total	56	44	

Differences are statistically significant at $p<0.05$ level in cases with matching letters a and b and at $p<0.01$ level in cases with matching letters c and d.

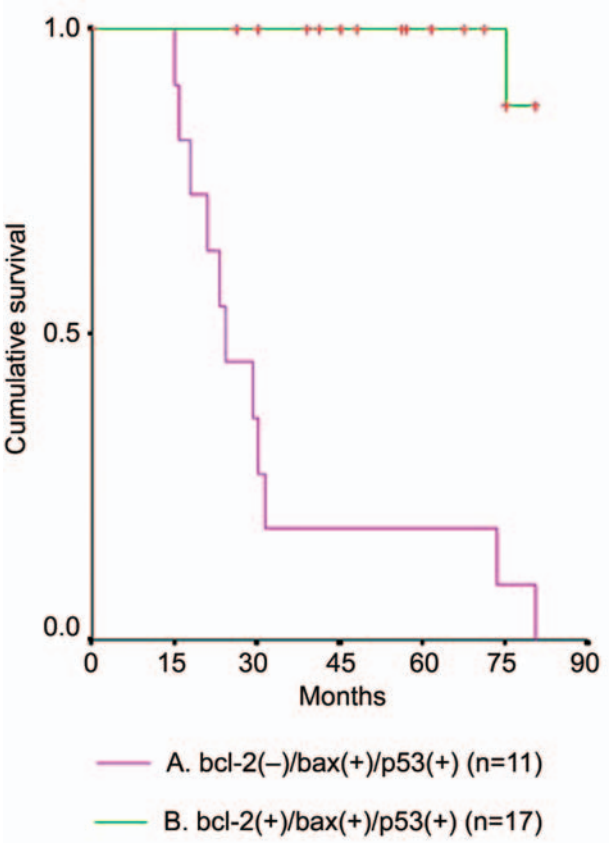

Figure 2. *Kaplan-Meier survival curves showing the relation between survival and the bcl-2/bax/p53 expression status. Statistically significant differences were recorded between the purple A. [bcl-2(–)/bax(+)/p53(+)] and the green B. [bcl-2(+)/bax(+)/p53(+)] group ($p<0.001$).*

Discussion

This study showed that in patients who underwent rectal resection and received adjuvant chemotherapy and radiotherapy for rectal adenocarcinoma: a) bax and bcl-2 proteins co-express frequently with p53, and b) expression of bax protein is associated with poor clinical outcome, especially in cases with co-expression of p53 and absence of bcl-2. Previous studies have shown that bcl-2 protein expression occurs in various premalignant lesions, including colorectal adenomas, suggesting that this oncogene may lead to the accumulation of long-living cells, resulting in tumor development (17, 18). Therefore, it seems that bcl-2 expression is an early event in tumorigenesis although its role during the development or progression of colorectal carcinomas has not been fully elucidated. On the other hand, the loss of bcl-2 expression in high-grade tumors observed in this and other studies (2, 11) may reflect progression of these tumors to a more deregulated state where bcl-2 is no longer required for maintenance of cell survival (19).

The relationship between bcl-2 expression and survival in cases of colorectal cancer has been studied in the past, however the results have been contradictory. Some studies have shown that bcl-2 expression is related to a favorable prognosis (18), others that it is related to poor survival (20) and a third category failed to demonstrate any relation between bcl-2 presence and outcome (2). Bcl-2 prevents apoptosis induced by either chemotherapy or irradiation and for that reason bcl-2(+) tumor cells are considered as chemotherapy and radiotherapy resistant (2). Thus, it would be expected that bcl-2(+) tumors are correlated with an adverse outcome. However, bcl-2 has also been shown to slow down cell proliferation, due to a prolongation of the G1 phase of the cell cycle and for that reason bcl-2(+) tumor cells may express a low mitotic rate (21). In neoplastic clones with a low mitotic rate, the rate of acquisition of complementary genetic defects and the growth fraction are also low, implying a less aggressive mechanism of transformation along with delayed tumor progression, and therefore bcl-2(+) tumors may be associated with an indolent clinical course. In this study, bcl-2 expression did not influence survival. However bcl-2 expression was found to correlate with favorable prognosis in the subgroup of bax(+) patients, as bax(+)/bcl-2(+) patients showed increased survival when compared to bax(+)/bcl-2(–) patients.

Regarding bax expression in colorectal carcinomas, previous studies have shown that bax expression in colorectal carcinomas is related to marginally better (21) or longer survival (22), whereas in others, it was associated with poorer survival (17). Having in mind that bax promotes apoptosis following genotoxic damage from either chemotherapy or irradiation, the presence of bax might be expected to be related to a better prognosis (12). Previous studies have shown an increased sensitivity of bax protein expressing cells, to radiotherapy and administration of chemotherapeutic agents (11, 23, 24). Bax has also been found to accelerate cell proliferation possibly through reversing the inhibitory effect of bcl-2 (21). Thus, bax(+) neoplastic clones may express a higher mitotic rate, a higher rate of acquisition of additional genetic defects and a higher growth fraction, therefore displaying a more aggressive mechanism of transformation along with a more rapid tumor progression. Consequently, bax(+) tumors seem to preserve a high malignant potential and may exhibit an aggressive clinical course. The results from this study showing an adverse prognostic role for bax may be explained in this way.

In the present study the p53 protein was expressed in 80% of the tumors. Previous studies have shown that the wild-type p53 can up-regulate bax and down-regulate bcl-2 gene expression, both *in vitro* and *in vivo* (25). In this study a coordinate immunoexpression of p53 in 18/21 bcl-2(+) and in 17/28 bax(+) tumors was recorded. Thus we are tempted to speculate that the p53(+)/bcl-2(+) and the p53(+)/bax(–) phenotypes, are caused by p53 mutants inefficient in either down-regulating bcl-2 or upregulating bax (*i.e.*, structural p53 mutations), whereas the p53(+)/bcl-2(–) and the p53(+)/bax(+) phenotypes are caused by p53 mutants which have retained either a bcl-2 suppressing or a bax up-regulating function (*i.e.*, mutations of the loop-sheet-helix motif of p53), respectively (21).

Several studies have reported that p53 does not play a substantial role in the prognosis of colorectal cancer (26), whereas others have correlated its presence with an adverse outcome (27) or even with higher rates of distant metastases together with lower rates of local relapse (14). This study showed that p53 expression was correlated with adverse outcome. This finding could be explained by the fact that mutant p53 cannot efficiently activate apoptosis following irradiation or chemotherapy and therefore p53(+) tumor cells may be considered as radio-chemoresistant (25). For that reason, postoperative residual p53(+) disease, in the form of either haematogenously pre-existing microscopic metastases or perirectally implanted tumor cells, may survive from adjuvant chemo-radiotherapy and result in distal or local relapse respectively. Furthermore, we noted that the p53(+)/bcl-2(–) subgroup of patients exhibited statistically significant worse outcome than the p53(–)/bcl-2(+) subgroup, a finding which is in accordance with a previous study (7). This may be explained by the fact that the p53(–)/bcl-2(+) phenotype is similar to the normal crypt base stem cell phenotype and therefore, a better prognosis would be likely to accompany a tumor which has retained the normal tissue phenotype (7).

In conclusion, this study demonstrates that in patients who underwent rectal resection and received adjuvant chemo-radiotherapy for rectal adenocarcinomas, the p53(+)/bax(+)/bcl-2(–) immunophenotype was associated with the highest risk of recurrences, since all the recurrent cases beared that

particular phenotype. Additionally, strong bax immunoreactivity is associated with shorter disease-free survival. Therefore, it can be suggested that patients, bearing the p53(+)/bax(+)/bcl-2(–) immunophenotype and characterized by particularly strong bax immunoreactivity, should be monitored more carefully for postoperative relapse, independently of their disease stage and tumor histologic grade. However, further investigation is warranted, with a larger series, to establish the possible usefulness of these markers in the prediction of local recurrence and distant metastasis in rectal carcinoma.

References

1 Rosai J: Large bowel. *In*: Ackerman's Surgical Pathology. 8th Edition, vol. 1, St. Louis: Mosby, pp. 729-799, 1996.

2 Scopa CD, Tsamandas AC, Zolota V, Kalofonos HP, Batistatou A and Vagianos C: The potential role of bcl-2 and Ki67 expression and apoptosis in colorectal carcinoma. A clinicopathologic study. Dig Dis Sci *48*: 1990-1997, 2003.

3 Cleary ML and Sklar J: Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci USA *82*: 7439-7443, 1985.

4 Reed JC: Bcl-2 and the regulation of programmed cell death. J Cell Biol *124*: 1-6, 1994.

5 Hockenbery DM, Zutter M, Hickey W, Nahm M and Korsmeyer SJ: Bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc Natl Acad Sci USA *88*: 6961-6965, 1991.

6 Diebold J, Baretton G, Felchner M, Meier W, Dopfer K, Schmidt M and Lohrs U: Bcl-2 expression, p53 accumulation and apoptosis in ovarian carcinomas. Am J Clin Pathol *105*: 341-349, 1996.

7 Kaklamanis L, Savage A, Whitehouse R, Doussis-Anagnostopoulou I, Biddolph S, Tsiotos P, Mortensen N, Gatter KC and Harris AL: Bcl-2 protein expression: association with p53 and prognosis in colorectal cancer. Br J Cancer *77*: 1864-1869, 1998.

8 Karamitopoulou E, Perentes E, Tolnay M and Probst A: Prognostic significance of MIB-1, p53 and bcl-2 immunoreactivity in meningiomas. Hum Pathol *29*: 140-145, 1998.

9 Oltvai Z, Milliman C and Korsmeyer SJ: Bcl-2 heterodimerizes *in vivo* with a conversed homolog, Bax, that accelerates programmed cell death. Cell *74*: 609-619, 1993.

10 Yang E and Korsmeyer S: Molecular thanatopics: a discourse on the BCL2 family and cell death. Blood *88*: 386-401, 1996.

11 Scopa C, Vagianos C, Kardamakis D, Kourelis T, Kalofonos HP and Tsamandas AC: Bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with rectal cancer. Appl Immunohistochem Mol Morph *9*: 329-334, 2001.

12 Zhan Q, Fan S, Bae I, Guillouf C, Liebermann DA, O' Connor PM and Fornace AJ Jr: Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. Oncogene *9*: 3743-3751, 1994.

13 Miyashita T and Reed JC: Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell *80*: 293-299, 1995.

14 Giatromanolaki A, Sivridis E, Stathopoulos G, Fountzilas G, Kalofonos H.P, Tsamandas A, Vrettou E, Scopa C, Polychronidis A, Simopoulos K and Koukourakis MI: Bax protein expression in colorectal cancer: Association with p53, bcl-2 and patterns of relapse. Anticancer Res *21*: 253-260, 2001.

15 Sinricope FA, Ruan SB, Clearly KR, Stephens LC, Lee JJ and Levin B: bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res *55*: 237-241, 1995.

16 Jass JR, Sobin LH and Watanabe H: The World Health Organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. Cancer *66*: 2162-2167, 1990.

17 Saleh HA, Jackson H, Khatib G and Banerjee M: Correlation of bcl-2 oncoprotein immunohistochemical expression with proliferation index and histopathologic parameters in colorectal neoplasia. Pathol Oncol Res *5*: 273-279, 1999.

18 Bosari S, Moneghini L, Graziani D, Lee AK, Murray JJ, Coggi G and Viale G: Bcl-2 oncoprotein in colorectal hyperplastic polyps, adenomas and adenocarcinomas. Hum Pathol *26*: 534-540, 1995.

19 Krajewski S, Thor DA, Edgerton MS, Moore DH II, Krajewska M and Reed JC: Analysis of bax and bcl-2 expression in p53 immunopositive breast cancers. Clin Cancer Res *3*: 199-208, 1997.

20 Bhatavdekar JM, Patel DD, Ghosh N, Chikhlikar PR, Trivedi TI, Suthar TP, Doctor SS, Shah NG and Balar DB: Coexpression of bcl-2, c-Myc and p53 oncoproteins as prognostic discriminants in patients with colorectal carcinoma. Dis Colon Rectum *40*: 785-790, 1997.

21 Huang CL, Kohno N, Inufusa H, Kodama K, Taki T and Miyake M: Overexpression of bax associated with mutations in the loop-sheet-helix motif of p53. Am J Pathol *155*: 955-965, 1999.

22 Ogura E, Senzaki H, Yamamoto D, Yoshida R, Takada H, Hioki K and Tsubura A: Prognostic significance of Bcl-2, Bcl-xL/S, Bax and Bak expressions in colorectal carcinomas. Oncol Rep *6*: 365-369, 1999.

23 Wagener C, Bargou RC, Daniel PT, Bommert K, Mapara MY, Royer HD and Dorken B: Induction of the death-promoting gene bax-alpha sensitizes cultured breast-cancer cells to drug-induced apoptosis. Int J Cancer *67*: 138-141, 1996.

24 Olmos G, Prieto A, Herraez A, Tejedor MC, Alvarez-Mon M and Diez JC: Involvement of Bax, Bcl-2 and caspase 3 in hydroxyurea- or etoposide-induced apoptosis of mouse interleukin-3-dependent lymphoma cells. Anticancer Res *25*: 999-1008, 2005.

25 Curtis CH: Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. A review. J Natl Cancer Inst *88*: 1442-1455, 1996.

26 Tollenaar RA, van Krieken JH, van Slooten HJ, Bruinvels DJ, Nelemans KM, van den Broek LJ, Hermans J and van Dierendonck JH: Immunohistochemical detection of p53 and bcl-2 in colorectal carcinoma: no evidence of prognostic significance. Br J Cancer *77*: 1842-1847, 1998.

27 Manne U, Myers RB, Moron C, Poczatek RB, Dillard S, Weiss H, Brown D, Srivastava S and Grizzle WE: Prognostic significance of Bcl-2 expression and p53 nuclear accumulation in colorectal adenocarcinoma. Int J Cancer *74*: 346-358, 1997.

Received June 23, 2006
Revised November 22, 2006
Accepted November 27, 2006