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Immunohistochemical Expression of bcl-2 in Dukes' Stage B and C Colorectal Carcinoma Patients: Correlation with p53 and ki-67 in Evaluating Prognostic Significance

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The objective of this study was to evaluate the expression of bcl-2 in Dukes' stage B and stage C (AJCC/UICC stage I and III) colorectal adenocarcinomas and to examine its association with clinicopathological features, p53, ki-67 and long term outcome. Paraffin embedded specimens from 61 patients with Dukes' stage B (AJCC/UICC stage I) and 39 patients with Dukes' stage C (AJCC/UICC stage III) colorectal adenocarcinoma who were treated with surgery were assessed. We used immunohistochemistry to determine the expression of bcl-2, p53 and ki-67 with a five-year follow-up. Positive bcl-2 expression was seen in 27 cases (27%). Expression of bcl-2 protein was related to tumor stage (p=0.0117). There was very strong evidence of an association between bcl-2 staining and ki-67 score (p<0.001). There was a trend towards increased survival in patients whose tumors expressed bcl-2 protein (p=0.001). When entered into a multivariate analysis model, which also included p53 and stage, bcl-2 staining emerged as a prognostic indicator variable. Expression of bcl-2 appears to be useful in selecting a group of colorectal cancer patients with a better prognosis.

Introduction

Colorectal cancer results from a series of genetic events, which disorder the normal mechanisms controlling cell growth. The bcl-2 proto-oncogene is an inhibitor of apoptosis and may therefore permit the accumulation of genetic alterations propagating cell division and potentially contributes to tumor development. The bcl-2 gene is located at chromosome 18q21 and its product is a 24 kD protein localized to the nuclear envelope, endoplasmic reticulum and mitochondrial membranes. The bcl-2 proto-oncogene was originally identified in the studies of t(14;18) chromosomal translocation, which occurs in most human follicular lymphomas. This translocation juxtaposes the bcl-2 gene and the immunoglobulin heavy-chain locus; consequently under the control of the immunoglobulin gene promoter, so the bcl-2 protein is over-expressed in these lymphomas. bcl-2 has been proposed as the first example of a novel carcinogenesis pathway, acting through a block of cell death without affecting cell proliferation. Increased bcl-2 expression has been also reported in epithelial malignancies, e.g., carcinoma of the lung, thyroid, breast, stomach and ovaries. In the large bowel bcl-2 protein has been localized to the epithelial cells at the base of crypts, where stem cell proliferation takes place [11, 20, 31, 35, 38]. p53 is a tumor suppressor gene that plays a key role in the control of the cell cycle. Cell proliferation is inhibited by normal or wild type p53 protein, which acts by arresting the cell cycle at the G1-S phase to allow DNA repair to take place. Loss of this activity may lead to neoplastic transformation. Alteration of this suppressor gene is a common event in colorectal carcinoma and has been associated with adverse postoperative outcome and poor survival [9, 29, 32]. ki-67 is expressed in cells actively engaged in the cell cycle and has also been used as a measure of proliferation in this patient population [1].

Sinicrope et al. [34] reported the first data concerning bcl-2 expression and apoptosis in colorectal tumorigenesis: in 17 out of 24 (71%) colonic adenomas and 14

out of 21 (67%) adenocarcinomas bcl-2 immunoreactivity could be detected. In most of the clinical studies, bcl-2 cytoplasmic expression was associated with a better prognosis [15, 21]. Conversely, in some other studies, bcl-2 association with poor survival has been reported [5]. The aim of this study was to investigate bcl-2 immunoreactivity in colorectal carcinoma and to determine its association with clinicopathological features, p53, ki-67 and with long-term outcome.

Material and Methods

Patients

A series of 100 patients underwent surgical resection for primary colorectal adenocarcinoma at the 3rd Department of Surgery at Tzanio Hospital of Athens between 1995 and 1999. Cases of non-inherited polyposis colorectal cancer (NHPCC), familial adenomatous polyposis or ulcerative colitis and patients who died in the immediate postoperative period were excluded from the study. None of the patients had received pre/postoperative radiotherapy or chemotherapy. Each patient was regularly followed up every six months for a minimum of five years. Clinical staging was done based on Dukes' classification and was based on clinical evaluation including pre-operative chest x-ray, abdominal ultrasound or computed tomography (CT) scan and abdominal exploration during laparotomy. Tumors were histologically classified as well differentiated, moderately differentiated or poorly differentiated adenocarcinomas using the WHO criteria [39]. Survival time was calculated from the date of surgery to the date of death or last follow-up with times censored for patients dying of causes unrelated to colorectal cancer and those surviving. Median follow-up was seven years (range: 5–9 years).

Tissue specimens

Sections from the colorectal adenocarcinoma and normal mucosa at the proximal/distal resection margins were obtained at surgical resection. The slides were reviewed by two pathologists. For every case, one paraffin block with both tumor tissue and normal mucosa was selected for the detection of bcl-2, p53 and ki-67 proteins expression, using immunohistochemistry.

Immunohistochemistry

Five-micrometer thick sections were cut and mounted on glass slides coated with APS (AminoPropylmethoxy-Silane), dewaxed in xylene and rehydrated with graded alcohols. Endogenous peroxidase was blocked with 3% H_2O_2 for 15 minutes. Before application of the primary antibody, sections were immersed in 10 mM citrate buffer (pH 6.0) and rinsed in TBS (Tris 0.05 M, NaCl 0.9%, pH 7.6) and heated in a microwave oven (650–800 W) for three five-minute cycles. In order to reduce non-specific binding, they were washed with TBS buffer and the primary antibodies were applied. For bcl-2 immunostaining, we used the bcl-2 antibody (dilution 1:10 Biogenex), for p53 the DO-7 antibody (dilution 1:100, Biogenex) against both wild and mutated forms and for ki-67 the MIB-1 antibody (dilution 1:80, DAKO). Samples were subsequently incubated with the secondary antibody for 30 minutes and incubated to ABC (Avidin Biotin Complex) for 30 minutes.

Diaminobenzamine (DAB) was used as a chromogen and light hematoxylin counterstain was used. Omission of the primary antibody acted as negative control.

Immunoreactivity for bcl-2 was evaluated as a percentage of tumor cells with positive cytoplasmic staining. A cut-off of <5% tumor cells positive was used to define negative cases. Strong positive staining was seen in infiltrating lymphocytes within the tumor stroma. The infiltrating lymphocytes and the neurons were used as positive control.

Immunoreactivity for p53 was evaluated semi-quantitatively by two observers and according to the percentage of positive tumor nuclei, scored as follows: "negative" for tumors showing less than 10% of immunostained nuclei, "low" for tumors showing 10–50% of immunoreactive nuclei, and "high" for those tumors with nuclear immunoreactivity in more than 50% of tumor cells. For positive controls of p53 expression, we used a known laryngeal carcinoma case with diffuse p53 nuclear accumulation.

Immunoreactivity for ki-67 was evaluated as a percentage of positive tumor cells and scored as follows: "0" for 0% of immunostained tumor cells, "+" for 1–10% of immunostained tumor cells, "++" for 11-25%, "+++" for 26–50%, "++++" for 51–75% and "+++++" for 76–100% of immunostained tumor cells. A cut-off of <50% immunostained tumor cells positive was used to define "low" cases. As internal positive control, ki-67 positive cells of the normal colonic mucosa were used. They were confined to the middle and lower thirds of the intestinal crypts.

Statistical analysis

All analyses were performed using the statistical packages Minitab and SPlus. Categorical variables were assessed by χ^2 analysis or Fisher's exact test as appropriate. Continuous data were assessed by Wilcoxon's rank sum test. Kaplan-Meier survival curves were constructed and differences in survival between groups were compared using the log-rank test. Multivariate analyses were performed with the Cox proportional hazards model.

Results

bcl-2

Positive immunohistochemical staining for bcl-2 was seen in 27 out of 100 (27%) colorectal carcinomas. Strong positive staining was seen in infiltrating lymphocytes within the tumor stroma. Neurons also showed positive staining. In areas of normal colonic epithelium, bcl-2 expression was seen in the basal cells of crypts. The relationship between bcl-2 expression and a range of clinicopathological variables is summarized in Table 1. There was no significant correlation between bcl-2 staining and sex, age, tumor site or tumor grade ($p>0.05$). However, a statistically significant association was detected between bcl-2 staining and tumor stage ($p=0.0117$). No significant association was demonstrated between bcl-2 and p53 status in 66 cases in which p53 had previously been assessed (Table 2). However, there was very strong evidence of a correlation between bcl-2 staining and ki-67 score ($p<0.001$, Table 3).

TABLE 1
Clinical and pathological features of 100 patients with colorectal cancer stratified by bcl-2 status

	bcl-2 immunohistochemistry		
Variable	**Negative (n = 73) (<5%)**	**Positive (n = 27) (>5%)**	**p–value**
Sex			
Male	39	14	0.889
Female	34	13	
Age (years)			
Median	70	69	0.5
Tumor Stage			
Stage A	0	0	0.0117
Stage B	39	22	
Stage C	34	5	
Tumor Site			
Rectum	45	12	0.237
Left Colon	16	7	
Right colon	12	8	
Tumor Grade			
Well / Moderate	64	27	0.1082
Poor	9	0	

TABLE 2
bcl-2 expression and p53 status in 66 cases of colorectal cancer

	bcl-2 immunohistochemistry		
p53	**Negative (n = 50)**	**Positive (n = 16)**	**p–value**
Low staining 10–50% (27)	19	8	0.395
High staining >50% (39)	31	8	

TABLE 3
bcl-2 expression and ki-67 score in 100 cases of colorectal cancer

	bcl-2 immunohistochemistry		
ki-67 score	**Negative (n = 73)**	**Positive (n = 27)**	**p–value**
Low score (<50%) (41)	22	19	0.000
High score (>50%) (59)	51	8	

Survival analysis

The patients were followed up for five years on average. Long-term survival of the patient population was closely related ($p<0.0001$) to tumor stage (Fig. 1). Overall, there was a trend ($p<0.0001$) towards increased survival in patients whose tumors expressed bcl-2 protein (Fig. 2). There was also a statistically significant association ($p=0.0002$) between survival and p53 expression (Fig. 3). However, there was not significant evidence ($p=0.1026$) of an association between survival and ki-67 expression (Fig. 4). When entered into

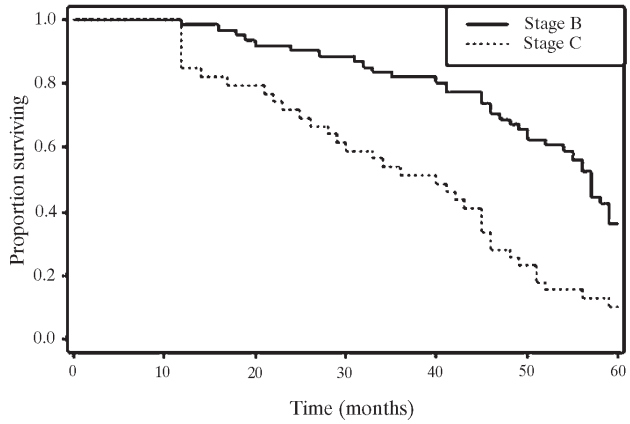

Fig. 1. Survival of 100 patients with colorectal cancer stratified by tumour stage: stage B (n=61), stage C (n=39).

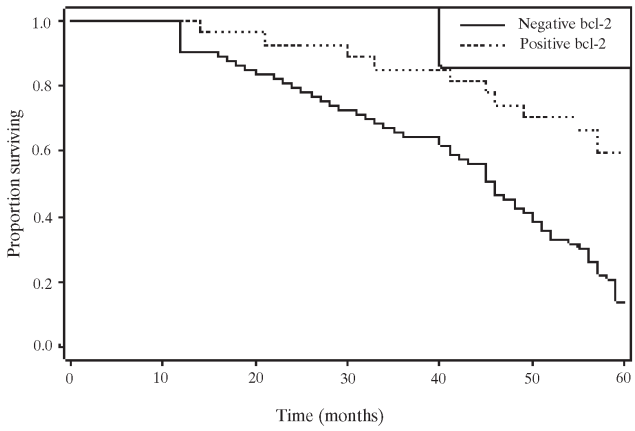

Fig. 2. Survival of 100 patients with colorectal cancer stratified by bcl-2 immunohistochemical staining: bcl-2 negative (<5%)(n=73), bcl-2 positive (>5%) (n=27).

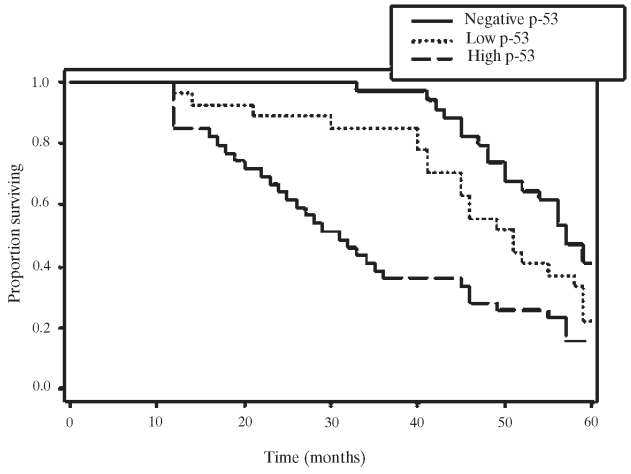

Fig. 3. Survival of 100 patients with colorectal cancer stratified by p53 expression: p53 negative (<10%) (n=34), low p53 (10-50%) (n=27), high p53 (>50%) (n=39).

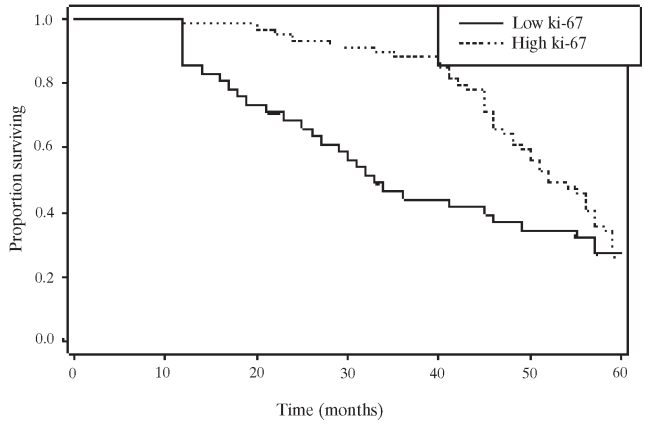

Fig. 4. Survival of 100 patients with colorectal cancer stratified by ki-67 expression: low ki-67 (<50%) (n=41), high ki-67 (>50%) (n=59).

TABLE 4
Cox regression model including bcl-2, p53 and stage; the p-value of the Cox model is p<0.001.

Variable	Coefficient	Standard error	p-value
Bcl-2	–0.985	0.349	0.00481
p53	0.744	0.267	0.00531
Stage	0.578	0.255	0.02310

a multivariate analysis model (Table 4), which also included p53 and stage, bcl-2 staining emerged as a prognostic indicator variable. From the results of Table 4 we can infer the following: there is significant evidence that the prognosis is better for patients with positive bcl-2 compared to patients with negative bcl-2 expression. There is also significant evidence that Stage B patients are associated with lower risk when compared to Stage C patients. Finally, p53 positive expression is associated with higher risk of death.

Discussion

Tissue growth depends on both cell proliferation and the rate of cell death [19, 30]. Thus, it is conceivable that neoplastic growth may be caused or promoted by factors inhibiting cell death.

bcl-2 is a protooncogene that is involved in the regulation of cell death by inhibiting apoptosis in many cell systems in physiologic and neoplastic conditions [27, 30]. The normal biological mechanism of action of bcl-2 is not clear, although the presence of bcl-2 protein is usually associated with favorable clinicopathological features in some neoplasms [12, 15]. An increase of bcl-2 expression in mucosal regeneration following irradiation suggests that activation of bcl-2 may support stem cell survival [40]. A relationship between bcl-2 expression and transformation from normal colonic epithelium to invasive cancer is not fully understood. However, there is an evidence to suggest that bcl-2 expression is lost during the evolution of colorectal cancer [34]. There are different clinical implications of bcl-2 expression in colorectal cancer of various geographical regions. The reason remains unclear, but this controversy may be related to the characteristics of the patient population on which analyses were performed [23]. The cause of bcl-2 expression in colorectal carcinoma is unclear. One possibility is that the malignant clone is derived from a basal crypt cell and the tumor cell retains bcl-2 expression. Other possibility is translocation of the bcl-2 gene to another chromosomal site in close proximity to powerful enhancer elements in the Ig heavy chain locus, resulting in transcriptional deregulation of the bcl-2 gene and high levels of

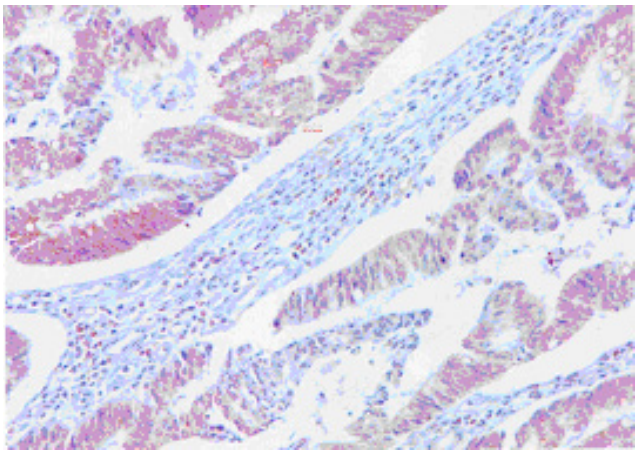

Fig. 5. Adenocarcinoma of the colon with positive immunostaining for bcl-2.

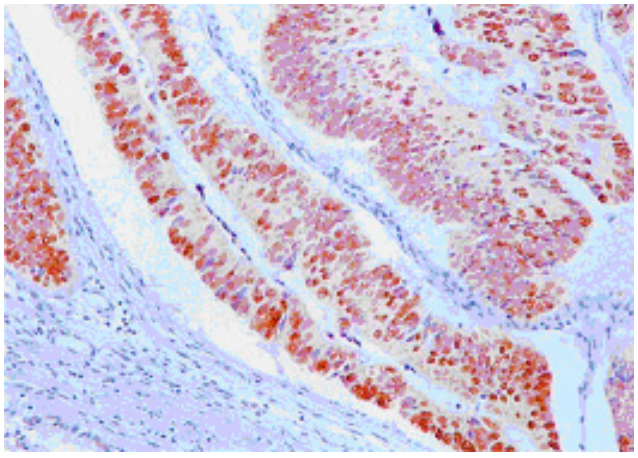

Fig. 6. Expression of p53 in colon adenocarcinoma.

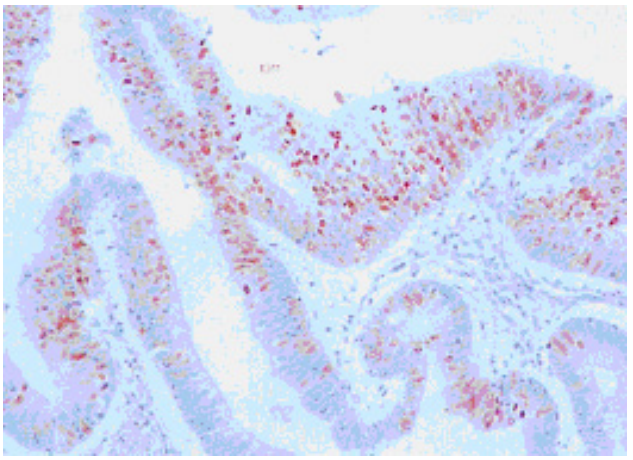

Fig. 7. Immunoreactivity for ki-67 in colon adenocarcinoma.

bcl-2 protein. Other possible mechanisms are mutation of the bcl-2 promoter causing deregulated protein expression and mutation of bcl-2 itself, thereby increasing its half-life. Other possibility is the loss of wild-type functional p53, which could lead to deregulated expression of bcl-2 protein [24]. It has been also suggested that the loss of bcl-2 expression with colorectal tumor progression may be due to wild-type p53 and some p53 mutations down-regulate bcl-2 by binding to a transcriptional silencer element within the bcl-2 promoter [24].

In our study, bcl-2 immunoreactivity was seen only in the moderately and well differentiated tumors, which is in agreement with previous reports [10, 37]. We found bcl-2 expression in 27% of our cases. Our results are somewhat lower than in other colorectal cancer studies, in which 28–35% of tumors have been positive for bcl-2 [10, 17, 18, 37]. Higher levels of bcl-2 positive cases, ranging from 50% to 67% have also been reported [7, 25, 34]. A statistically significant association was detected between bcl-2 staining and tumor stage (p=0.0117). Expression of bcl-2 has been also associated with a more favorable clinical outcome in non-small cell carcinoma of the lung and thyroid follicular carcinomas [28]. bcl-2 expression as reported in breast cancer [33] was associated with increased survival [22, 26]. In breast cancer, bcl-2 may be more appropriate as an indicator of therapeutic response than a prognostic factor [3]. However, investigations of bcl-2 in colorectal carcinoma have yielded conflicting results in survival. Basari et al. and Tollenaar et al. did not find any prognostic significance of bcl-2 expression [7, 36]. In contrast, other studies found that bcl-2 expression was associated with a favorable clinical outcome [4, 14, 26]. Many factors should be taken into consideration as an explanation for these conflicting survival results. A difficulty in the interpretation of literature data is the use of different staging systems or small groups of different stages. Furthermore, the details of patient therapy are often inadequate in prognostic marker studies. Several studies include patients with all stages of Dukes' classification. Such studies obviate the need for sub-stratification and, therefore, more appropriate assessment of the prognostic utility of a marker. The choice of the patient group is also determined by the questions asked, as markers would have little potential use in stage A comparison to stage B or C disease [8]. Our study, with a five-year follow-up, suggests that bcl-2 expression is associated with better clinical course. It seems paradoxical that bcl-2, which inhibits apoptosis, should be associated with good prognosis. However, bcl-2 has also been shown to slow down cell growth in colorectal cancer lines [13]. Loss of bcl-2 may be indicative of a more aggressive phenotype allowing further tumor progression under influences such as p53 changes [5].

Mutation of p53 is one of the most frequently encountered genetic alterations in solid tumors. p53 mutations play a central role in colorectal tumor progression and are present in >50% of sporadic colorectal carcinomas [16]. It has been also shown that there is an inverse relationship between bcl-2 and p53 expression in cells of both colorectal adeno-

mas and carcinomas [37]. In our study, no significant association was demonstrated between bcl-2 and p53 status in the 66 cases in which p53 had previously been assessed. However, there was very strong evidence of an association between bcl-2 staining and ki-67 score. There was also a statistically significant association between survival and p53 expression and as it is consistent with previous reports, we found no association between ki-67 scores and clinical outcome [2].

Apoptosis is a well organized process controlled by genes with positive or negative regulatory functions [13]. Its role in tumor progression is complex and incompletely understood. Multiple proto-oncogenes, regulatory factors and tumor suppressor genes appear to have a dominant role in the pathogenesis of colorectal cancer. The gene mutations produce metabolically more stable proteins that may be used as prognostic indicators in patients with colorectal cancer [5]. Our study suggests that bcl-2 expression is associated with better outcome following curative surgery, independently of Dukes' stage. Immunohistochemical evaluation of bcl-2 in colorectal cancer may be of clinical value. It may be useful as an adjunctive test in routine histopathological practice.

References

1. *Allegra CJ, Paik S, Colangelo LH et al:* Prognostic value of thymidylate synthase, ki-67, and p53 in patients with Dukes' B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative study. J Clin Oncol 2003, 21(2), 241–250.
2. *Allegra CJ, Parr AL, Wold LE et al:* Investigation of the prognostic and predictive value of thymidylate synthase, p53 and ki-67 in patients with locally advanced colon cancer. J Clin Oncol 2002, 20(7), 1735–1743.
3. *Barbareschi M, Caffo O, Veronese S et al:* bcl-2 and p53 expression in node-negative breast carcinoma: a study with long term follow-up. Human Pathol 1996, 27, 1149–1155.
4. *Baretton GB, Dielbold J, Christoforis G et al:* Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Cancer 1996, 77, 255–264.
5. *Bhatavdekar JM, Patel DD, Ghosh N et al:* Coexpression of bcl-2, c-myc and p53 oncoproteins as prognostics discriminants in patients with colorectal carcinoma. Dis Colon Rectum 1997, 40, 785–790.
6. *Bhatavdekar JM, Patel DD, Chikhlikar PR et al:* Molecular markers are predictors of recurrence and survival in patients with Dukes' B and Dukes' C colorectal adenocarcinoma. Dis Colon Rectum 2001, 44(4), 523–533.
7. *Bosari S, Nonegnini L, Graziani D et al:* bcl-2 oncoprotein in colorectal hyperplastic polyps, adenomas and adenocarcinomas. Hum Pathol 1995, 26(5), 534–540.
8. *Bouzourene H, Gervaz P, Cerottini JP et al:* p53 and ki-ras as prognostic factors for Dukes' stage B colorectal cancer. Eur J Cancer 2000, 36, 1008–1015.
9. *Diez M, Enriquez JM, Camunas J et al:* Prediction of recurrence in B-C stages of colorectal cancer by p53 protein nuclear expression in comparison with standard pathological features. Eur J Surg Oncol 1995, 21, 635–639.
10. *Flohill CC, Janssen PA, Bosman FT:* Expression of bcl-2 protein in hyperplastic polyps, adenomas and carcinomas of the colon. J Pathol 1996, 178, 393–397.
11. *Garrity MM, Burgart LJ, Mahoney MR et al:* Prognostic value of proliferation, apoptosis, defective DNA mismatch repair, and p53 overexpression in patients with resected Dukes' B2 or C colon cancer: a North Central Cancer Treatment Group Study. J Clin Oncol 2004, 22(9), 1572–1582.
12. *Gee JMW, Robertson JFR, Ellis IO et al:* Immunohistocytochemical localization of bcl-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. Int J Cancer 1994, 59, 619–628.
13. *Giatromanolaki A, Sivridis E, Stathopoulos GP et al:* Bax protein expression in colorectal cancer: association with p53, bcl-2 and patterns of relapse. Anticancer Research 2001, 21, 253–260.
14. *Ilyas M, Hao XP, Wilkinson K et al:* Loss of bcl-2 expression correlates with tumor recurrence in colorectal cancer. Gut 1998, 43, 383–387.
15. *Joensuu H, Pylkkanen I, Toikkanen S:* bcl-2 protein and long-term survival in breast cancer. Am J Pathol 1994, 145, 1191–1198.
16. *Kahlenberg MS, Stoler DL, Rodriguez-Bigas MA et al:* p53 tumor suppressor gene mutations predict decreased survival of patients with sporadic colorectal carcinoma. Cancer 2000, 88(8), 1814–1819.
17. *Kaklamanis L, Savage A, Mortensen N et al:* Early expression of bcl-2 protein in the adenoma-carcinoma sequence of colorectal neoplasia. J Pathol 1996, 179, 10–14.
18. *Kaklamanis L, Savage A, Whitehouse R et al:* bcl-2 protein expression: association with p53 and prognosis in colorectal cancer. J Pathol 1998, 77, 1864–1867.
19. *Kerr JFR, Winterford CM, Harmon BV:* Apoptosis: its significance in cancer and cancer therapy. Cancer 1994, 73, 2013–2036.
20. *Kouraklis G, Kakisis J, Theoharis S et al:* Prognostic significance and correlation with survival of bcl-2 and TGF-beta RII in colon cancer. Dig Dis Sci 2003, 48(12), 2284–2289.
21. *Leek RD, Kaklamanis I, Pezzella F et al:* bcl-2 in normal human breast and carcinoma, association with oestrogen receptor-positive, epidermal growth factor receptor negative tumors and in situ cancer. Br J Cancer 1994, 69, 135–139.
22. *Manne U, Myers RB, Moron C et al:* Prognostic significance of bcl-2 expression and p53 nuclear accumulation in colorectal adenocarcinoma. Int J Cancer 1997, 74, 346–358.
23. *Manne U, Weiss HL, Grizzle WE:* Bcl-2 expression is associated with improved prognosis in patients with distal colorectal adenocarcinomas. Int J Cancer (Pred Oncol) 2000, 89, 423–430.
24. *Miyasita T, Harigal M, Hanada M et al:* Identification of a p53 dependent negative response element in the bcl-2 gene. Cancer Res 1994, 54, 3714–3717.
25. *Mueller J, Mueller E, Hoepner I et al:* Expression of bcl-2 and p53 in de novo and ex-adenoma colon carcinoma: a comparative immunohistochemical study. J Pathol 1996, 180, 259–265.
26. *Ofner D, Riehemann K, Maier H et al:* Immunohistochemically detectable bcl-2 expression in colorectal carcinoma: correlation with tumor stage and patient survival. Brit J Cancer 1995, 72, 981–985.
27. *Oren M:* The involvement of oncogenes and tumor suppressor genes in the control of apoptosis. Cancer Metastasis Rev 1992, 11, 141–148.
28. *Pilotti S, Collini P, Rilke F et al:* bcl-2 protein expression in carcinomas originating from the follicular epithelium of the thyroid gland. J Pathol 1994, 172, 337–342.
29. *Pricolo VE, Finkelstein SD, Hansen K et al:* Mutated p53 gene is an independent adverse predictor of survival on colon carcinoma. Arch Surg 1997, 132, 371–375.

30. *Reed JC:* Bcl-2 and the regulation of program cell death. J Cell Biol 1994, 124, 1–6.
31. *Reerink O, Karrenbeld A, Plukker JT et al:* Molecular prognostic factors in locally irresectable rectal cancer treated preoperatively by chemo-radiotherapy. Anticancer Res 2004, 24(2c), 1217–1221.
32. *Remvikos Y, Tominaga O, Hammel P et al:* Increased p53 protein content of colorectal tumors correlates with poor survival. Br J Cancer 1992, 66, 758–764.
33. *Silvestrini R, Veneroni S, Daidone M et al:* The bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. J Nat Cancer Inst 1994, 86, 499–504.
34. *Sinicrope FA, Ruan SB, Cleary KR et al:* bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 1995, 55, 237–241.
35. *Sun XF, Bartik Z, Zhang H:* Bcl-2 expression is a prognostic factor in the subgroups of patients with colorectal cancer. Int J Oncol 2003, 23(5), 1439–1493.
36. *Tollenaar RA, van Krieken JH, van Slooten HJ et al:* Immunohistochemical detection of p53 and bcl-2 in colorectal carcinoma: no evidence for prognostic significance. Brit J Cancer 1998, 77, 1842–1847.
37. *Watson AJM, Merrit AJ, Jones LS et al:* Evidence for reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br J Cancer 1996, 73, 889–895.
38. *Wilson JW, Nostro MC, Balzi M et al:* Bcl-w expression in colorectal cancer. Br J Cancer 2000, 82(1), 178–185.
39. World Health Organization. International Histological Classifications of Tumors. No 15. Histological Typing of Intestinal Tumors. Geneva. WHO 1976.
40. *Yank HB, Chow NH, Shey BS et al:* The role of bcl-2 in the progression of the colorectal adenoma-carcinoma sequence. Anticancer Research 1999, 19, 727–730.

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