American Journal of Pathology, Vol. 148, No. 5, May 1996

Prognostic Significance of Bcl-2 in Clinically Localized Prostate Cancer

Lukas Bubendorf,* Guido Sauter,* Holger Moch,* Paul Jordan,† Andrea Blöchlinger,‡ Thomas C. Gasser,‡ and Michael J. Mihatsch*

From the Institute for Pathology, the University Computer Center,† the Urologic Clinics,‡ University of Basel, Basel, Switzerland*

The clinical course of prostate cancer is highly variable and cannot satisfactorily be predicted by histological criteria alone. To study the prognostic significance of Bcl-2 and p53 overexpression in prostate cancer, 137 consecutive radical prostatectomy specimens were examined by immunohistochemistry. Both Bcl-2 and p53 were associated with malignant phenotype. Bcl-2 expression was more frequent in pT3 tumors (31% positive) than in pT2 tumors (5% positive, P = 0.001). p53 overexpression (found in 8%) was associated with high Gleason score (P = 0.03) and increased tumor growth fraction (Ki67 labeling index (LI); P = 0.017). Survival analysis showed that Bcl-2 expression (P = 0.03), high Ki67 LI (P = 0.018), high grade (P = 0.0037), advanced local stage (P = 0.0005), and positive lymph nodes (P = 0.026) were predictors of progression. The combined analysis of Ki67 LI and Bcl-2 allowed the distinction of three groups with different clinical outcome. Prognosis was best in Bcl-2-negative tumors with low Ki67 LI, worst in Bcl-2-positive tumors with high Ki67 LI, and intermediate in the remaining tumors (P = 0.03). These data suggest that altered expression of both Bcl-2 and p53 play a role in prostate cancer progression. Combined analysis of factors regulating both apoptosis and cell proliferation may be relevant in prostate cancer. (Am J Pathol 1996, 148:1557–1565)

Prostate cancer is the most common malignancy among men and the second leading cause of cancer-related deaths in western countries.[1] Currently there are no reliable tools to distinguish aggressive tumors warranting immediate therapy from silent prostate cancer without influence on the patient's life expectancy. Increasing interest has been attributed to molecular pathways leading to malignant transformation and progression in prostate cancer.[2] Alterations of a variety of different oncogenes and tumor suppressor genes may ultimately result in an imbalance between proliferation and programmed cell death (apoptosis) and therefore lead to a net tumor growth and tumor progression.[3,4] Both Bcl-2 and p53 are involved in the regulation of cell cycle and/or apoptosis.

The Bcl-2 oncoprotein, localized in the mitochondrial membrane, has been shown to prolong cell survival by inhibiting apoptosis.[4,5] Dysregulation of Bcl-2 expression has been suggested to be involved in carcinogenesis in various solid organs.[6–9] A prognostic importance of Bcl-2 overexpression has been reported in breast, thyroid, and non-small-cell lung cancer.[10–12] In prostate cancer, Bcl-2 has been suggested to play a role in hormone resistance.[13–16] However, the prognostic significance of Bcl-2 overexpression in localized prostate cancer is unclear.

The p53 tumor suppressor gene plays a role in cell cycle control, DNA repair, and apoptosis.[17,18] Mutations in the p53 gene are common events in a wide variety of human malignancies.[18] The most frequent mutations are of the missense type and can result in an increased half-life of the p53 protein, making it detectable by immunohistochemistry.[19] In prostate cancer, p53 overexpression has been shown to be strongly associated with p53 mutations.[20,21] However, immunohistochemical p53 overexpression can also be caused by nonmutational mechanisms and not all mutations result in p53 overexpression.[22] Altered p53 expression has been suggested to predict adverse prognosis in different tumors,[23–25] although some of these findings have not been consistently

Accepted for publication January 31, 1996.

Address reprint requests to Dr. Lukas Bubendorf, Institute for Pathology, University of Basel, Schönbeinstrasse 40, Basel, Switzerland.

confirmed.[26,27] In prostate cancer, p53 overexpression has been found to be associated with advanced stage[20,21,28] and high risk for tumor progression.[29–32]

The purpose of this study was to investigate the prognostic significance of Bcl-2 and p53 overexpression in prostate cancer treated by radical prostatectomy and to correlate the overexpression of these proteins with tumor growth fraction (Ki67 labeling index (LI)).

Materials and Methods

Patients

A total of 137 consecutive, previously untreated patients with clinically localized prostate cancer who underwent radical prostatectomy and pelvic lymphadenectomy at the University Hospital in Basel between 1978 and 1993 were studied. The average patient age was 65.3 years (range, 45 to 82 years). All carcinomas were staged according to the TNM classification system.[33] The specimens were fixed in 4% phosphate-buffered formalin. The entire prostate was serially blocked at 3- to 4-mm intervals from apex to base in transverse sections perpendicular to the urethra. Whole-mount paraffin sections were cut at 5 μm and stained with hematoxylin and eosin. Tumor grade was determined by one pathologist (H. Moch) according to Gleason.[34] Disease progression was monitored at regular follow-up visits every 3 months during the first year, every 6 months during the second year, and then at yearly intervals or when clinically indicated. Progression of disease was defined as follows: biopsy-proven local recurrence, distant metastases on chest x-rays and bone scans, and elevation of prostate-specific antigen (PSA), prostatic acid phosphatase, or alkaline phosphatase above the normal range or increasing values of PSA or prostatic acid phosphatase even within the normal ranges of up to 12 ng/ml and up to 3.1 ng/ml, respectively. Follow-up data were available from 115 patients with a mean follow-up of 5.2 years (range, 0.5 to 15 years), and 54 of these patients received adjuvant local radiotherapy. Orchiectomy was performed in 6 patients at the time of prostatectomy. Disease progression was documented in 37 patients. The diagnosis of tumor progression was based on a PSA increase in 29 (78%), biopsy in 3 (8%), and a positive bone scan in 5 (14%) patients. Neither postoperative radiotherapy nor orchiectomy were associated with tumor progression (data not shown). An analysis of tumor-adjusted overall survival was not performed due to the low number of patients dying of disease (n = 8).

Immunohistochemistry

All immunohistochemical examinations were performed using the avidin-biotin-enhanced immunoperoxidase technique. The mouse monoclonal antibody Bcl-2 124 (1:400; Dako, Glostrup, Denmark) was used for detection of Bcl-2 overexpression. Bcl-2 staining of basal cells in benign prostatic glands, lymphocytes, and peripheral nerves was used as internal positive control. Staining for Bcl-2 was subjectively estimated as low (scattered positive tumor cells), intermediate (10 to 50% positive tumor cells), and high (>50% positive tumor cells). p53 protein was detected by the mouse monoclonal antibody DO-7 (1:500, Dako, Glostrup, Denmark), which reacts with wild-type and mutant p53 protein. Positive controls for p53 consisted of breast cancer with known positivity. Only nuclear p53 overexpression was considered. The fraction of cells showing a nuclear p53 positivity was estimated in all cases.

To minimize the risk of false positivity, a cutoff value of 10% positive cells was used to define p53 and Bcl-2 positivity on a tumor basis. Because of the lack of generally accepted standards for quantitation of Bcl-2 or p53 immunostaining, the selection of this cutoff was based on previous reports from the literature dealing with Bcl-2[10,11,35,36] or p53 immunohistochemistry,[20,22,30,37] considering the presence of just occasional Bcl-2- or p53-positive tumor cells as an overall negative staining result. For detection of Ki67 protein the monoclonal antibody MIB 1 (1:800; Dianova, Hamburg, Germany) was applied after a microwave pretreatment as described.[38] Tonsillary tissue served as positive control for Ki67. Negative controls were carried out by replacing the primary antibody by phosphate-buffered saline. For each section, the areas with the highest density of Ki67-positive cells was defined. Ki67 LI was then assessed by counting at least 500 adjacent cells in the selected areas.

Statistics

Contingency table analysis was used to study the relationship between Bcl-2, p53, and histopathological findings. Student *t*-tests were applied to analyze the association between Ki67 LI (after log transformation), pTN stage, grade, and immunohistochemical findings. Estimation of disease-free survival was performed by Kaplan Meier curves together with a log rank test. Proportional hazard model with step-

wise selection of the covariates was used to determine the parameters with greatest influence on progression. Seventy-eight surviving patients were censored at the time of their last clinical control.

Results

Histology

Staging of 137 total prostatectomy specimens revealed 4 stage pT1, 40 stage pT2, and 93 stage pT3 tumors. Pelvic lymph node metastases were found in 34 of 137 cases (25%). A total of 68 tumors (50%) were classified as low grade (Gleason score 2 to 6) and 69 tumors (50%) as high grade (Gleason score 7 to 10). A high Gleason score was associated with advanced pTN stage. A high tumor grade was more frequent in pT3 tumors (59%) than in pT1/2 tumors (32%, $P = 0.0028$). Lymph node metastases were present in 36% of high grade tumors but in only 13% of low grade tumors ($P = 0.0018$). Lymph node metastases were found in 36% of pT3 tumors as compared with 13% in pT1/2 tumors ($P = 0.0372$). High grade ($P = 0.0037$), advanced local stage ($P = 0.0005$), and positive lymph nodes ($P = 0.026$) were all significantly associated with tumor progression in univariate analysis.

Immunostaining

The mean Ki67 LI was 7.5% ± 5.6% (range, 0 to 33%) in the areas with the highest density of positive cells. There was no significant association of Ki67 LI with pTN stage or histological grade. A high Ki67 LI was associated with tumor progression when the mean Ki67 LI (7.5%) was used as a cutoff to define tumors with low and high growth fraction ($P = 0.018$). This result was expected as there was a large overlap between the patients in this series and the patient set of a previous report in which we described the prognostic significance of tumor growth fraction in prostate cancer.[39]

In normal tissue, positive staining of Bcl-2 was always present in basal cells of prostate glands, in lymphocytes, and peripheral neural tissue (Figure 1). Positive staining was also recorded in prostatic ducts and in seminal vesicles. A clear cytoplasmic staining for Bcl-2 in more than 10% of cells was seen in 33 of 137 tumors (24%), and 19 of these tumors (14%) showed Bcl-2 staining in 10 to 50% of tumor cells and in more than 50% of tumor cells in 14 tumors (10%). Bcl-2 staining of a representative case is shown in Figure 1c. In addition, Bcl-2 staining was limited to a few scattered cells (<10%) in 30 tumors. These tumors were considered Bcl-2 negative to avoid false positive results. The relationship between Bcl-2 and histopathology is shown in Table 1. There was a strong correlation between Bcl-2 and pT stage. Bcl-2 overexpression was seen in 31% of pT3 tumors but in only 5% of pT2 tumors ($P = 0.001$). Bcl-2 was not significantly associated with lymph node stage or Gleason score. There was no significant difference in Ki67 LI between Bcl-2-positive (9.0 ± 7.7%) and Bcl-2-negative tumors (7.0 ± 4.8%, $P = 0.083$). Comparison of Bcl-2 overexpression with tumor progression revealed a significantly better prognosis for Bcl-2-negative than for Bcl-2-positive tumors ($P = 0.03$, Figure 2). Interestingly, the combined analysis of Bcl-2 overexpression and Ki67 LI showed that prognosis was best in Bcl-2-negative tumors with low Ki67 LI and worst in Bcl-2-positive tumors with high Ki67 LI ($P = 0.03$ for Bcl-2-negative and low Ki67 LI *versus* Bcl-2-positive and high Ki67 LI), whereas the remaining tumors behaved intermediately (Figure 3). Nuclear p53 overexpression was found in more than 10% of cells in 11 tumors (8%). These tumors were considered p53 positive, and 5 of these tumors showed p53 staining in 10 to 20% of tumor cells, 2 in 20 to 50%, and 4 in more than 50% of tumor cells. A representative case is shown in Figure 4. A few scattered p53-positive cells (always less than 10%) were seen in an additional 30 tumors. These tumors were considered p53 negative. There was no staining of benign epithelium. p53 overexpression was associated with aggressive histopathological phenotype (Table 1). All p53-positive cases except one were staged pT3 ($P = 0.113$ for pT3 *versus* pT2). Of 11 p53-positive tumors, 9 were classified as high grade and only 2 were low grade ($P = 0.03$). p53 overexpression was also associated with rapid tumor cell proliferation. Mean Ki67 LI was higher in p53-positive tumors (11.3 ± 5.4%) than in p53-negative tumors (7.1 ± 5.5%, $P = 0.017$). There was a tendency toward a more frequent p53 overexpression in Bcl-2-positive tumors than in Bcl-2-negative tumors. A nuclear p53 overexpression was seen in 5 of 33 Bcl-2-positive (15.2%) but in only 6 of 98 (5.8%) Bcl-2-negative tumors ($P = 0.084$). Comparison of the clinical outcome between p53-positive and p53-negative tumors showed a tendency toward earlier progression in p53-positive tumors ($P = 0.15$, Figure 5).

Multiparameter Analysis

Cox's stepwise regression analysis including pTN stage, histological grade (Gleason score), Ki67 LI, and Bcl-2 showed that only pT3 stage and a high

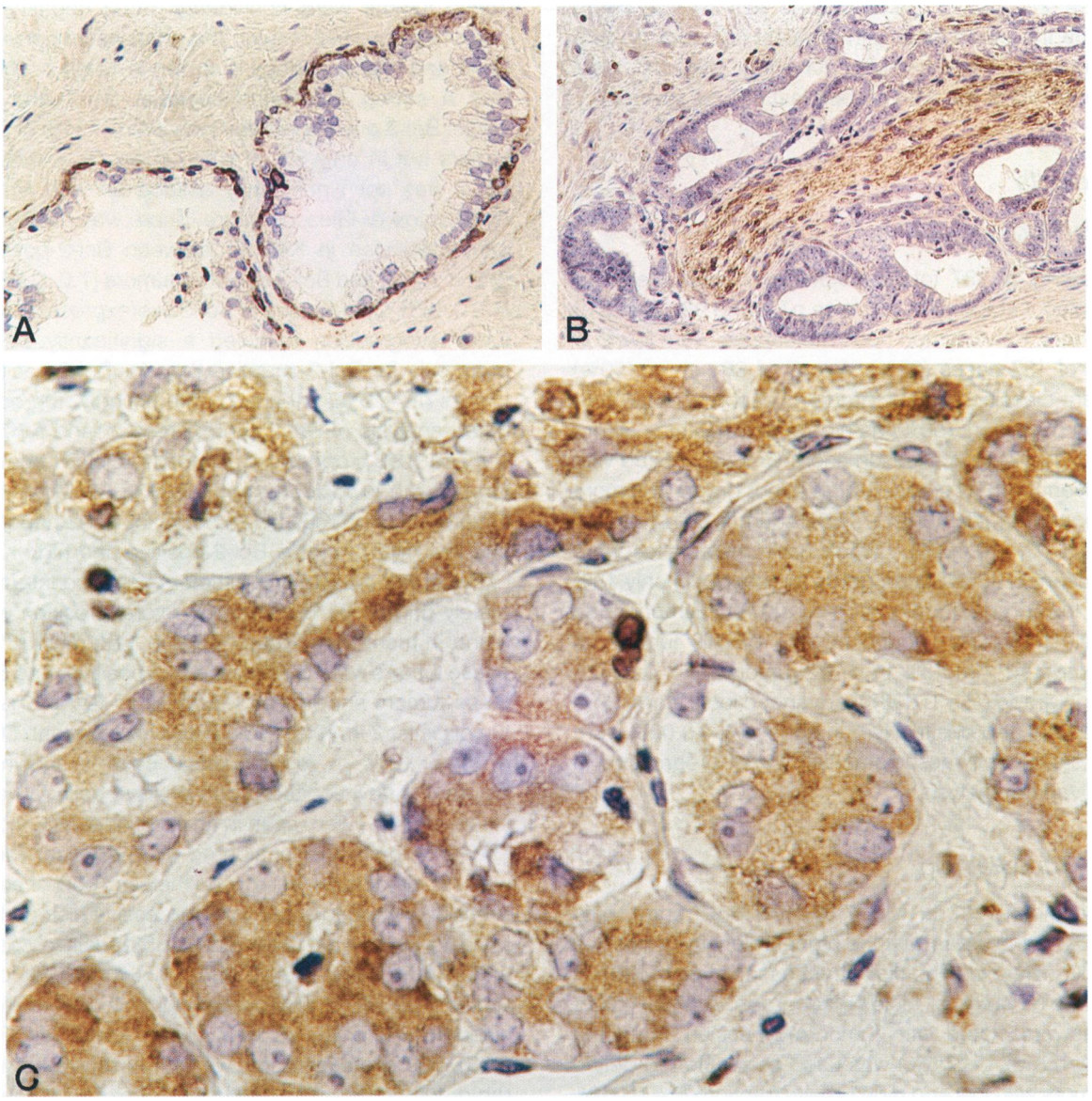

Figure 1. *Bcl-2 immunohistochemistry.* **A:** *Normal prostatic glands with a continuous layer of Bcl-2-positive basal cells. Magnification, ×80.* **B:** *Bcl-2-negative prostate cancer with infiltration of perineural space, the peripheral nerve serving as positive internal staining control. ×50.* **C:** *Prostate cancer with diffuse Bcl-2 overexpression. ×160.*

Table 1. *Bcl-2 and p53 Immunostaining and Histopathology*

			Bcl-2			p53		
		n	Negative (%)	Positive* (%)	χ^2	Negative (%)	Positive* (%)	χ^2
Stage	pT2	40	38 (95)	2 (5)	$P = 0.001$	39 (98)	1 (2)	Not significant
	pT3	93	64 (69)	29 (31)		83 (89)	10 (11)	
	pN0	103	78 (76)	25 (24)	Not significant	95 (92)	8 (8)	Not significant
	pN1/2	34	26 (76)	8 (24)		31 (91)	3 (9)	
Gleason score†	Low	68	54 (79)	14 (21)	Not significant	66 (97)	2 (3)	$P = 0.03$
	High	69	50 (72)	19 (28)		60 (87)	9 (13)	

*Defined as clear Bcl-2 or p53 expression in >10% of tumor cells.
†Low grade, Gleason sum 2 to 6; high grade, Gleason sum 7 to 10.

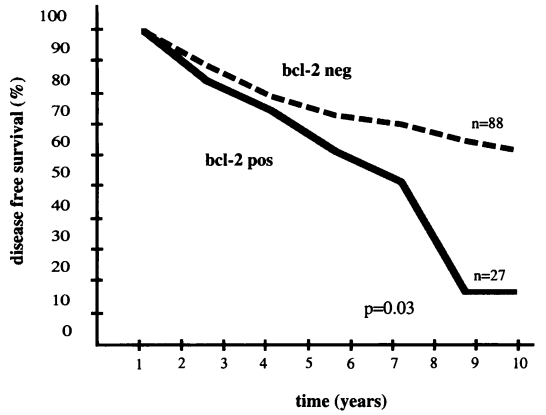

Figure 2. *Bcl-2 and disease-free survival.*

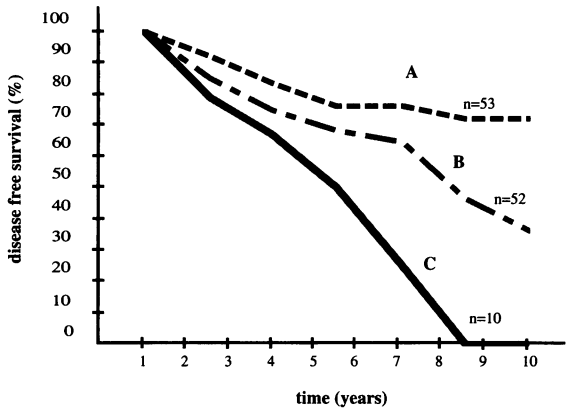

Figure 3. *Bcl-2/Ki67 LI and disease-free survival.* A: *Bcl-2-negative/low Ki67 LI (< 7.5%).* B: *Bcl-2-positive/low Ki67 LI or Bcl-2-negative/high Ki67 LI (≥ 7.5%).* C: *Bcl-2-positive/high Ki67 LI.* P = *0.03 for* A versus C.

Ki67 LI (≥7.5%) proved to be of independent prognostic value, the relative risk of progression being 3.7 ($P = 0.0001$) and 2.2 ($P = 0.013$), respectively. Lymph node status, tumor grade, and Bcl-2 overexpression provided no additional prognostic information.

Discussion

In this study, immunohistochemistry was used to examine Bcl-2 overexpression in prostate cancer. Immunohistochemistry is particularly useful for Bcl-2 analysis, as a strong positivity is always seen in lymphocytes, basal cells, and peripheral nerve tissue providing an excellent internal control for each section examined. Bcl-2 overexpression in more than 10% of cells was found in 24% of the 137 prostate carcinomas of this series. This frequency is comparable to previous reports finding Bcl-2 overexpression in 6 of 19 and 6 of 13 primary prostate cancers, respectively.[13,40] A higher frequency of Bcl-2 positivity (23 of 37) was found in another study using frozen tissue.[14] The relationship between Bcl-2 overexpression and local stage was not examined in these studies. Our results demonstrating an association between Bcl-2 overexpression and advanced local stage is consistent with a role of Bcl-2 for prostate cancer progression.

As the entire prostate is removed at radical prostatectomy, the PSA value goes to zero after radical surgery in most cases. Serological controls of the PSA values are therefore ideally suited for the clinical follow-up of patients after radical prostatectomy. Several studies have shown that a PSA increase after radical prostatectomy is indicative of a tumor recurrence.[41,42] PSA measurements have been widely used to define prostate cancer progression after radical prostatectomy.[32,43,44] Also in this study, the majority of tumor recurrences were detected by a PSA increase. The analysis of disease-free survival in this patient set showed a strong association between Gleason score, local tumor stage, nodal status, and clinical outcome. This is consistent with previous reports[44,45] and also provides indirect evi-

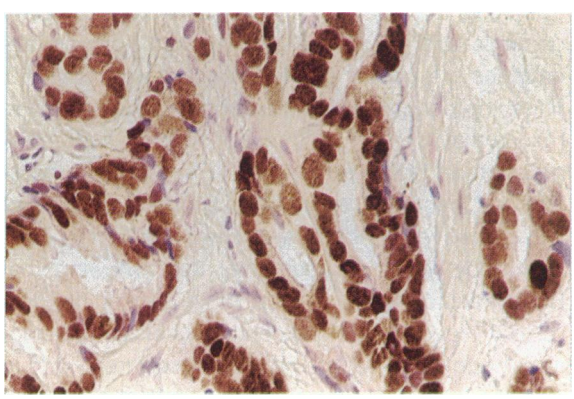

Figure 4. *p53 immunohistochemistry. Prostate cancer shows intense nuclear positivity. Magnification, ×100.*

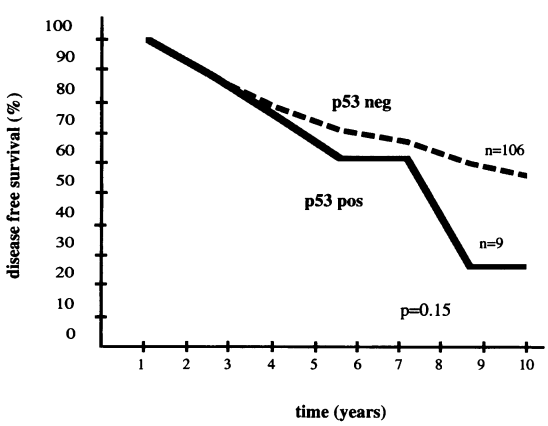

Figure 5. *p53 and disease-free survival.*

dence for the quality of our clinical follow-up data as well as for the selected approach to define tumor progression in this study.

Bcl-2 overexpression was significantly associated with shortened disease-free survival in our series. Given the postulated role of Bcl-2 overexpression for hormone resistance in prostate cancer,[13–16] such a result would be expected after anti-androgen treatment. However, as only six of our patients had undergone anti-androgen therapy, our result cannot be explained by this mechanism. Considering the role of Bcl-2 as an inhibitor of apoptosis, it could also be expected that Bcl-2-positive tumors might be less sensitive to radiation therapy as radiation exerts its anti-neoplastic effect through the induction of apoptosis.[46] However, there was no evidence for a different effect of Bcl-2 expression on prognosis in the subgroups of patients with or without radiation therapy in this study (data not shown).

Given the strong inhibitory effect of Bcl-2 on apoptosis,[4] Bcl-2 overexpression is likely to facilitate prostate cancer progression through an increased net tumor growth due to a prolonged tumor cell survival. In addition, cells that are unable to undergo apoptosis may be prone to accumulate secondary genomic aberrations[47,48] as cells with genomic aberrations are frequently eliminated by apoptosis.[3,46] Thus, the accumulation of additional genomic aberrations may be ultimately responsible for tumor progression and poor prognosis of Bcl-2-positive prostate carcinomas. The finding of a poor prognosis of Bcl-2-positive prostate carcinomas is strongly supported by recent experimental data showing that Bcl-2 transformation of a prostate cancer cell line (LNCaP) leads to a significantly higher tumorigenicity in nude mice that are not hormonally treated.[16] In addition, previous clinical studies have suggested a poor prognosis of hormonally treated Bcl-2-positive prostate carcinomas.[13,14]

Although an association between Bcl-2 overexpression and unfavorable prognostic factors has also been found for neuroblastomas,[9] these results are at variance with reports suggesting a favorable prognostic impact of Bcl-2 positivity in several other tumors including breast, lung, and thyroid carcinomas.[10–12,35] This discrepancy may be explained by a different role of Bcl-2 in different types of neoplasm. This hypothesis is supported by recent reports suggesting significant differences in the relationship of Bcl-2 overexpression with hormonal tumor growth regulation between prostate and breast cancer. Although there is evidence for an association between Bcl-2 overexpression and hormone resistance in prostate cancer,[13–16] Bcl-2 overexpression is associated with estrogen receptor positivity in breast cancer,[36] explaining the suggested association with response to hormone therapy in this tumor.[35] Moreover, it has been shown that Bcl-2 expression lowers the sensitivity against CD95-mediated apoptosis in B-CLL cells[49] but not in prostate or colon cancer cell lines.[50] It could be speculated that such differences in the biological significance of a Bcl-2 overexpression between different tumors might be due to a tissue-specific expression of Bcl-2 binding proteins such as Bax or Bcl-x, which are known to determine the functional activity of Bcl-2.[51–53]

It is now generally accepted that disturbed regulation of both cell proliferation and programmed cell death (apoptosis) are important for net tumor growth.[3,4] Several reports have demonstrated that an increased tumor cell proliferation is of prognostic relevance in prostate cancer.[54,55] In a previous study, we have shown that Ki67 LI is an independent prognostic factor in clinically localized prostate cancer.[39] The lack of a significant relationship between Ki67 LI and Bcl-2 overexpression suggests that alterations leading to an increased cell proliferation may be independent from mechanisms affecting the control of programmed cell death. Interestingly, the combined analysis of both cell proliferation and Bcl-2 overexpression showed that prognosis was best in Bcl-2-negative tumors with low Ki67 LI and worst in Bcl-2-positive tumors with high Ki67 LI (whereas the remaining tumors with either Bcl-2 overexpression or high Ki67 LI behaved intermediately). This finding suggests that a combined analysis of apoptosis and cell proliferation could provide clinically relevant information in prostate cancer.

Several previous studies have shown that alterations of the p53 tumor suppressor gene are less frequent in prostate cancer than in other common tumors.[20,21,28–31,56–60] The frequency of 8% p53-positive tumors is in line with previous reports.[20,21,28–31,57–60] The association found between p53 alterations and advanced stage, high grade, and high proliferative activity is consistent with the results of previous studies[20,21,28–31,57,58,60] and provides additional evidence that p53 alterations, although rare in prostate cancer, confer a highly aggressive phenotype to these tumors. The potential clinical relevance of p53 alterations is also suggested by several authors[30–32] finding an independent prognostic significance of p53 overexpression in prostate cancer. Also in this study, there was a tendency toward a worse prognosis in p53-positive tumors. The lack of a significant association between p53 overexpression and prognosis may be due to

the low number of p53-positive cases. The analysis of a large number of p53-positive tumors may also be necessary to evaluate the relationship between Bcl-2 and p53 in prostate cancer. The tendency toward a more frequent p53 positivity in Bcl-2-overexpressing tumors (15.2%) than in Bcl-2-negative tumors (5.8%) is consistent with a functional relationship between Bcl-2 and p53.[61,62]

Multiparameter analysis by the Cox proportional hazard model showed that only pT3 stage and a high Ki67 LI were independent predictors of tumor progression, whereas Gleason grade, nodal status, Bcl-2, and p53 overexpression provided no additional prognostic information. This finding further stresses the potential usefulness of Ki67 LI as an easy to determine prognostic parameter in prostate cancer. Considering the fact that pTN stage is not available before radical prostatectomy and the frequently discrepant results between clinical and pathological staging,[63] the potential value of novel prognostic markers such as Ki67 LI and Bcl-2, and p53 overexpression may be even higher in prostate cancer biopsies where the results of the immunohistochemical analyses may influence therapeutic strategies. Additional studies are necessary to evaluate the prognostic significance of Ki67 LI and Bcl-2 and p53 overexpression in transurethral and needle biopsies of prostate carcinomas.

In summary, overexpression of both Bcl-2 and p53 are associated with tumor progression in clinically localized prostate cancer. In particular, the combination of high growth fraction (Ki67 LI) and inhibited apoptosis as evidenced by Bcl-2 overexpression seems to confer a poor prognosis. The lack of an association between Bcl-2 overexpression and Ki67 LI suggests that altered mechanisms of both proliferation and apoptosis control may independently contribute to the development of a highly malignant tumor phenotype.

Acknowledgments

The authors thank Ms. R. Epper and the staff of the Pathology Department, University of Basel, for their technical support.

References

1. Muir CS, Nectoux J, Staszewski J: The epidemiology of prostatic cancer: geographical distribution and time-trends. Acta Oncol 1991, 30:133–140
2. Rinker-Schaeffer CW, Partin AW, Isaacs WB, Coffey DS, Isaacs JT: Molecular and cellular changes associated with the acquisition of metastatic ability by prostatic cancer cells. Prostate 1994, 25:249–265
3. Kerr JFR, Winterford CM, Harmon BV: Apoptosis. Cancer 1993, 73:2013–2026
4. Korsmeyer SJ: Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 1992, 80: 879–886
5. Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ: Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990, 348:334–336
6. Bronner MP, Culin C, Reed JC, Furth EE: The *bcl-2* proto-oncogene and the gastrointestinal epithelial tumor progression model. Am J Pathol 1995, 146:20–26
7. Bosari S, Moneghini L, Graziani D, Lee AKC, Murray JJ, Coggi G, Viale G: Bcl-2 oncoprotein in colorectal hyperplastic polyps, adenomas, and adenocarcinomas. Hum Pathol 1995, 26:534–540
8. Chandler D, El-Naggar AK, Brisbay S, Redline RW, McDonnell TJ: Apoptosis and expression of the *bcl-2* protooncogene in the fetal and adult human kidney: evidence for the contribution of *bcl-2* expression to renal carcinogenesis. Hum Pathol 1994, 25:789–796
9. Castle VP, Heidelberger KP, Bromberg J, Ou X, Dole M, Nunez G: Expression of the apoptosis-suppressing protein Bcl-2 in neuroblastoma is associated with unfavorable histology and N-*myc* amplification. Am J Pathol 1993, 143:1543–1550
10. Joensuu H, Phylkkänen L, Toikkanen S: Bcl-2 protein expression and long-term survival in breast cancer. Am J Pathol 1994, 145:1191–1198
11. Viale G, Roncalli M, Grimelius L, Graziani D, Wilander E, Johansson H, Bergholm, U, Coggi G: Prognostic value of Bcl-2 immunoreactivity in medullary thyroid carcinoma. Hum Pathol 1995, 26:945–950
12. Pezzella F, Turley H, Kuzu I, Tungekar MF, Dunnill MS, Pierce CB, Harris A, Gatter KC, Mason DJ: bcl-2 protein in non-small-cell lung carcinoma. N Engl J Med 1993, 329:690–694
13. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LWK, Hsieh JT, Tu SM, Campbell ML: Expression of the protooncogene *bcl-2* in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res 1992, 52:6940–6944
14. Colombel M, Symmans F, Gil SO, TMK, Chopin D, Benson M, Olsson CA, Korsmeyer S, Buttyan R: Detection of the apoptosis-suppressing oncoprotein Bcl-2 in hormone-refractory human prostate cancers. Am J Pathol 1993, 143:390–400
15. Westin P, Stattin P, Damber J, Bergh A: Castration therapy rapidly induces apoptosis in a minority and decreases cell proliferation in a majority of human prostatic tumors. Am J Pathol 1995, 146:1368–1375
16. Raffo AJ, Perlman H, Chen MW, Day ML, Streitman JS, Buttyan R: Overexpression of *bcl-2* protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. Cancer Res 1995, 55:4438–4445

17. Vogelstein B, Kinzler KW: p53 function and dysfunction. Cell 1992, 70:523–526
18. Levine AJ, Momand J, Finlay CA: The p53 tumour suppressor gene. Nature 1991, 351:453–456
19. Finlay C, Hinds P, Tan T, Eliyahu D, Oren M, Levine A: Activating mutations for transformation by p53 produce a gene product that forms a hsc70-p53 complex with an altered half-life. Moll Cell Biol 1988, 8:531–539
20. Navone NM, Troncoso P, Pisters LL, Goodrow TL, Palmer JL, Nichols WW, Von EAC, Conti CJ: p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. J Natl Cancer Inst 1993, 85:1657–1669
21. Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC: p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res 1993, 53:3369–3373
22. Hall PA, Lane DP: p53 in tumor pathology: can we trust immunohistochemistry? Revisited! J Pathol 1994, 172: 1–4
23. Thor AD, Yandell DW: Prognostic significance of p53 overexpression in node-negative breast carcinoma. J Natl Cancer Inst 1993, 85:176–177
24. Dalquen P, Sauter G, Torhorst J, Schultheiss E, Jordan P, Lehmann S, Soler M, Stulz P, Mihatsch MJ, Gudat F: Nuclear p53 overexpression is an independent prognostic parameter in node-negative non-small-cell lung cancer. J Pathol 1996, 178:53–58
25. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen S, Nichols PW, Skinner DG, Jones PA, Cote RJ: Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994, 331:1259–1264
26. Lee JS, Yoon A, Kalapurakal SK, Ro JY, Lee JJ, Tu N, Hittelmann W, Hong WK: Expression of p53 oncoprotein in non-small-cell lung cancer: a favorable prognostic factor. J Clin Oncol 1995, 13:1893–1903
27. Rosen PP, Lesser ML, Arroyo CD, Milicent C, Borgen P, Norton L: p53 in node-negative breast carcinoma: an immunohistochemical study of epidemiologic risk factors, histologic features, and prognosis. J Clin Oncol 1995, 13:821–830
28. Myers RB, Oelschlager D, Srivastava S, Grizzle WE: Accumulation of the p53 protein occurs more frequently in metastatic than in localized prostatic adenocarcinomas. Prostate 1994, 25:243–248
29. Thomas DJ, Robinson M, King P, Hasan T, Charlton R, Martin J, Carr TW, Neal DE: p53 expression and clinical outcome in prostate cancer. Br J Urol 1993, 72:778–781
30. Visakorpi T, Kallioniemi OP, Heikkinen A, Koivula T, Isola J: Small subgroup of aggressive, highly proliferative prostatic carcinomas defined by p53 accumulation. J Natl Cancer Inst 1992, 84:883–887
31. Shurbaji MS, Kalbfleisch KH, Thurmond TS: Immunohistochemical detection of p53 protein as a prognostic indicator in prostate cancer. Hum Pathol 1995, 26:106–109
32. Bauer JJ, Sesterhenn IA, Mostofi KF, McLeod DG, Srivastava S, Moul JW: p53 nuclear protein expression is an independent prognostic marker in clinically localized prostate cancer patients undergoing radical prostatectomy. Clin Cancer Res 1995, 1:1295–1300
33. International Union Against Cancer (UICC): Classification of Malignant Tumours, ed 4, revision 2. Berlin, Springer, 1992
34. Gleason DF: Histologic grading and clinical staging of prostatic carcinoma. Urologic Pathology: The Prostate. Edited by M Tannenbaum. Philadelphia, Lea and Febiger, 1977, pp 171–197
35. Gasparini G, Barbareschi M, DallaPalma P, Mauri FA, Boracchi P, Bevilacqua P, Caffo O, Morelli L, Verderio P, Pezzella F, Harris AL: Expression of Bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer. Clin Cancer Res 1995, 1:189–198
36. Bhargava V, Kell DL, van de Rijn M, Warnke RA: Bcl-2 immunoreactivity in breast carcinoma correlates with hormone receptor positivity. Am J Pathol 1994, 145: 535–540
37. Sauter G, Deng G, Moch H, Kerschmann R, Matsumura K, DeVries S, George T, Fuentes J, Carroll P, Mihatsch MJ, Waldman FM: Physical deletion of the p53 gene in bladder cancer: detection by fluorescence *in situ* hybridization. Am J Pathol 1994, 144:756–766
38. Moch H, Sauter G, Epper R, Mihatsch M, Gudat F, Waldman F: p53 but not *erb*-B2 expression is associated with rapid tumor cell proliferation in urinary bladder cancer. Hum Pathol 1994, 25:1346–1351
39. Bubendorf L, Sauter G, Moch H, Schmid HP, Gasser TC, Jordan P, Mihatsch JM: Ki67 labeling index: an independent predictor of progression in prostate cancer treated by radical prostatectomy. J Pathol (in press)
40. Segal NH, Cohen RJ, Haffejee Z, Savage N: BLC-2 proto-oncogene expression in prostate cancer and its relationship to the prostatic neuroendocrine cell. Arch Pathol Lab Med 1994, 118:616–618
41. Lange PH, Ercole CJ, Lightner DJ, Fraley EE, Vessella R: The value of serum prostate specific antigen determinations before and after radical prostatectomy. J Urol 1989, 141:873–879
42. Partin AW, Pound CR, Clemens JQ, Epstein JI, Walsh PC: Serum PSA after anatomic radical prostatectomy: the Johns Hopkins experience after 10 years. Urol Clin North Am 1993, 20:713–725
43. Catalona WJ: Surgical management of prostate cancer. Cancer 1995, 75:1903–1908
44. Epstein JI, Pizov G, Walsh PC: Correlation of pathologic findings with progression after radical retropubic prostatectomy. Cancer 1993, 71:3582–3593
45. Humphrey PA, Walther PJ: Adenocarcinoma of the prostate. II. Tissue prognosticators. Am J Clin Pathol 1993, 100:256–269
46. Strasser A, Harris AW, Jacks T, Cory S: DNA damage can induce apoptosis in proliferating lymphoid cells via

p53-independent mechanisms inhibitable by Bcl-2. Cell 1994, 79:329–339
47. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD: Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 1992, 70:923–935
48. Vaux D, Cory S, Adams J: Bcl-gene promotes haemopoietic cell survival and cooporates with c-*myc* to immortalize pre-B cells. Nature 1988, 335:440–442
49. Mapara M, Bargou R, Zugck C, Döhner H, Ustaoglu F, Krammer PH, Dörken P: APO-1 mediated apoptosis or proliferation in human chronic B lymphocytic leukemia: correlation with *bcl-2* oncogene expression. Eur J Immunol 1993, 23:702–708
50. Owen-Schaub L, Radinsky R, Kruzel E, Berry K, Yonehara S: Anti-Fas on non-hematopoietic tumors: levels of Fas/APO-1 and Bcl-2 are not predictive of biological responsiveness. Cancer Res 1994, 54:1580–1586
51. Oltvai ZN, Millimann CL, Korsmeyer SJ: Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993, 74:609–619
52. Boise LH, Gonzáles-García M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nuñez G, Thompson CB: *bcl*-x, a *bcl*-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993, 74:597–608
53. Takayama S, Takaaki S, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC: Cloning and functional analysis of BAG-1: a novel Bcl-2 binding protein with anti-cell death activity. Cell 1995, 80:279–284
54. Visakorpi T: Proliferative activity determined by DNA flow cytometry and proliferating cell nuclear antigen (PCNA) immunohistochemistry as a prognostic factor in prostatic carcinoma. J Pathol 1992, 168:7–13
55. Harper ME, Glynne JE, Goddard L, Wilson DW, Matenhelia SS, Conn IG, Peeling WB, Griffiths K: Relationship of proliferating cell nuclear antigen (PCNA) in prostatic carcinomas to various clinical parameters. Prostate 1992, 20:243–253
56. Hollstein M, Sidransky D, Vogelstein B, Harris CC: p53 mutations in human cancers. Science 1991, 253:49–53
57. Aprikian AG, Sarkis AS, Fair WR, Zhang ZF, Fuks Z, Cordon CC: Immunohistochemical determination of p53 protein nuclear accumulation in prostatic adenocarcinoma. J Urol 1994, 151:1276–1280
58. Thompson SJ, Mellon K, Charlton G, Marsh C, Robinson M, Neal DE: p53 and Ki-67 immunoreactivity in human prostate cancer and benign hyperplasia. Br J Urol 1992, 69:609–613
59. Vesalainen SL, Lipponen PK, Talja MT, Alhava EM, Syrjanen KJ: Proliferating cell nuclear antigen and p53 expression as prognostic factors in T1–2M0 prostatic adenocarcinoma. Int J Cancer 1994, 58:303–308
60. Kallakury BVS, Figge J, Ross JS, Fisher HAG, Figge HL, Jennings TA: Association of p53 immunoreactivity with high Gleason tumor grade in prostatic adenocarcinoma. Hum Pathol 1994, 25:92–97
61. Wang Y, Szekely L, Klein G, Wiman KG: Wild-type p53-triggered apoptosis is inhibited by *bcl*-2 in a v-*myc*-induced T-cell lymphoma line. Oncogene 1993, 8:3427–3431
62. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffmann B, Reed JC: Tumor suppressor p53 is a regulator of *bcl*-2 and *bax* gene expression *in vitro* and *in vivo*. Oncogene 1994, 9:1799–1805
63. Ohori M, Wheeler TM, Scardino PT: The new American joint committee on cancer and international union against cancer TNM classification of prostate cancer. Cancer 1994, 74:104–114