10.1053.paor.2001.0273 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Relationship Between Apoptosis Regulator Proteins (bcl-2 and p53) and Gleason Score in Prostate Cancer

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Cellular proliferation programmed cell death (apoptosis) are associated with tumor growth in general, and prostate cancer growth in particular. The aim of this study was to examine the expression of the apoptosis regulating genes bcl-2 and p53 and Gleason score in core needle biopsy specimens of prostate cancer using immunohistochemistry. We studied bcl-2 and p53 expression in 12 cases of low grade (Gleason score 2-5), 12 cases of intermediate grade (Gleason score 6-7) and 8 cases of high grade (Gleason score 8-10) prostate cancer. Overexpression of bcl-2 was noted in 3 of 32 patients (9.32%). One of them was high grade; others were intermediate grades. Expression of p53 was observed in 3 of low grades; others were high grade. The statistical analysis of present data suggest that there is no significant relation between p53 and bcl-2 expression and Gleason score in prostate cancer. (Pathology Oncology Research Vol 7, No 3, 209–212, 2001)

Keywords: p53, bcl-2, prostate cancer, apoptosis

Introduction

Prostate adenocarcinoma (PCA) has become a major malignancy especially in industrialized countries. Annual age adjusted incidence rates for male population has increased by 178%, rising from 64/100.000 to 178/100.000 between 1973 and 1996 with mortality rate 27.3/100.000.[2] The biological behavior of PCA is unpredictable in individual patients, ranging from slowly growing, non-life-threatening to highly aggressive cancers.[19] The currently most established prognostic factors in prostate cancer are histological grade (Gleason system) and tumor stage.[7] Cellular proliferation and programmed cell death (apoptosis) are associated with tumor growth in general, and prostate cancer growth in particular. Protein expression of proto-oncogene bcl-2 (a potent inhibitor protein against apoptosis) and protein expression of the tumor suppressor gene p53 (a regulator of cellular proliferation and apoptosis) have been proved as useful prognostic indicators in PCA progression.[9,12]

Received: April 6, 2001; *accepted:* August 10, 2001

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Although several studies have strongly suggested that molecular analyses can provide useful prognostic information if largely harvested biopsy samples or the entire prostate are examined, there is little information on the clinical significance of these molecular examinations in core needle biopsies. This approach is potentially impeded by tumor heterogeneity, because small tumor fragments may not be representative of the entire carcinoma. However, molecular analyses should be performed before aggressive surgical approach, provided that treatment options could depend on molecular analyses. Prognostic factors would be particularly helpful if they could be evaluated on core needle biopsy specimens supported by transrectal ultrasonography.[10]

The goal of the present study was to evaluate the relationship between the expression of the apoptosis regulating genes bcl-2 and p53 and Gleason score in prostate cancer.

Materials and Methods

Patients

Core needle biopsy specimens of 32 previously untreated prostate cancer patients were retrospectively assessed from archives of our Department of Pathology (*Table 1*). Digital rectal examination, serum PSA (ng/ml), transrectal

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Table 1. **Gleason score in 32 patients**

Gleason score	*Number of the patients*
2–4 (Gleason grade-I)	12
5–7 (Gleason grade-II)	12
8–10 (Gleason grade-III)	8

ultrasonography, whole body bone scaning and pelvic computerized tomography – optional – were used for clinical staging of the disease.

Histopathology and immunohistochemistry

Core needle prostatic biopsy specimens prior to initiation of any treatment were submitted for this study. All slides were reviewed and a Gleason score was determined by adding the numbers for the two most predominant patterns. Immunohistochemical stains were performed on 5 µm sections of the formalin fixed, paraffin embedded biopsy specimens. Sections on poly-L-lysine coated glass slides were deparaffinized in xylene and rehydrated using ethanol gradients. The sections were pretreated three times in a microwave oven at 750 W for 10 minutes in citrate buffer (10 mmol/L citric acid monohydrate, adjusted with 2N sodium hydroxide to pH 6.0). Endogenous peroxidase activity was blocked by methanol with 0.3% hydrogen peroxide for 30 minutes. The slides were incubated for 30 minutes at 30°C with the primary antibodies: mouse monoclonal anti-bcl-2 antibody (clone 124, Dako) and anti-p53 antibody (clone DO-7, Dako). The anti p53 antibody DO-7 recognizes an epitope on the N-terminus of the p53 protein and reacts with wild type and mutant p53 proteins. The anti-bcl-2 antibody 124 reacts specifically with bcl-2 oncoprotein. The working dilution of these antibodies was 1:50 and 1:40 respectively. The slides were then incubated with a biotinylated rabbit anti-mouse immunoglobin for 30 minutes at room temperature. They were subsequently incubated with streptoavidine-biotin method.

The sections were counterstained with hematoxylin. p53 positive colon adenocarcinoma served as a positive control for p53 immunostaining, and prostatic basal cells as an internal control for bcl-2 immunostaining.

All slides were evaluated by two pathologists. Specimens were considered to be bcl-2 positive if greater than 10% of the tumor cells stained for bcl-2. We scored p53 positive as positive if greater than 10% of prostate tumor cells demonstrated nuclear reactivity.[8]

Statistic analysis

Statistics were performed by Fisher's x^2 test. The patient population was divided into several groups according to bcl-2 immunostaining, p53 immunostaining and Gleason score.

Results

The mean age and serum PSA values of 28 patients that were retrospectively obtained for their clinical archives were 65.4±12.8 years (54-83 years) and 27.5±26.3 ng/ml. (5.94-120.00 ng/ml.), respectively. Clinical stages of these patients ranged from A2 to D2.

Overexpression of bcl-2 was determined in 3 of 32 patients (9.32 %). One of them was Gleason grade III; others were Gleason grade II (*Figure 1*). Positive bcl-2 staining was not observed in the patients with Gleason grade I. Expression of p53 was noted in 3 cases (9.32 %); one of them was Gleason grade I; others were Gleason grade III (*Figure 2*). There was no staining with p53 in Gleason grade II. There was no statistically approved relationship between increased levels of apoptosis regulator proteins (p53 and bcl-2) and Gleason score (*Table 2*).

Discussion

Estimates indicate that in 1998 approximately 184.500 new case of prostate cancer were diagnosed in the United States (10). While pathological stage, grade, positive sur-

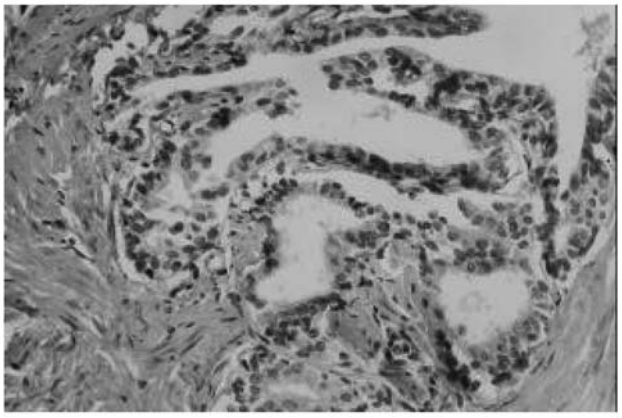

Figure 1. *Bcl-2 positivity.*

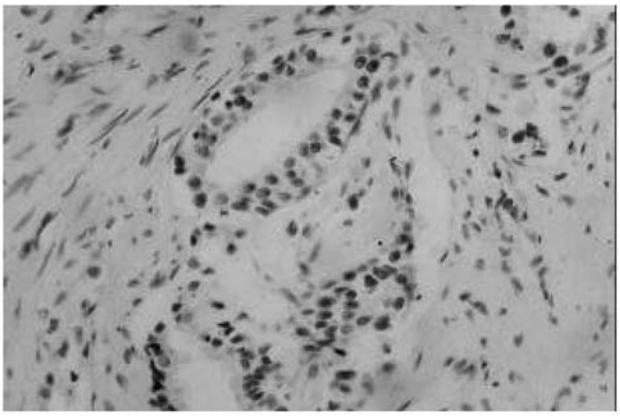

Figure 2. *p53 positivity.*

Table 2. **Relationship of bcl-2 and p53 positivities compared with Gleason score**

	bcl-2 positivity(%)	*p53 positivity(%)*
Gleason score 2–4		1(3.12 %)
Gleason score 5–7	2 (6.25 %)	
Gleason score 8–10	1 (3.12 %)	2(6.25 %)
Total	3 (9.32 %)	3(9.32 %)

gical margins and tumor volume are perhaps the most commonly accepted prognostic factors after radical prostatectomy, they can not be used preoperatively. An approach to develop prognostic markers correlating with biological aggressiveness wozuld be highly appreciated. Among the several portential targets, regulators of the apoptotic pathway, including bcl-2 and p53, have been at the forefront of prostate cancer research and they have recently been evaluated as prognostic markers.

p53 and bcl-2 overexpression have been investigated independently in a large number of different malignancies for their potential value as prognostic markers. In high grade B-lymphomas Piris et al. have suggestred that simultaneous expression of bcl-2 and p53 protein is associated with poor prognosis than p53 accumulation alone.[16] Soini et al. investigated the extent of apoptosis in a set of testicular and ovarian germ cell tumors and compared the results with the expression of p53 and bcl-2. Their results revelaed that the extent of apoptosis was highest in embryonal carcinomas followed by seminomas, choriocarcinomas and immature teratomas. Embryonal carcinomas also showed quantitatively the strongest p53 expression. Bcl-2 was only expressed in teratomas and might partly counteract apoptosis in these tumors.[18]

There are several studies of the prognostic significance of bcl-2 and p53 in prostate cancer. Nearly all of studies evaluated only p53 positivity in needle biopsy, transurethral prostate resection or radical prostatectomy specimens, to draw prognostic conclusions. In the present study, we analyzed only pretreatment prostate needle biopsies.

Shurbaji et al examined the expression of p53 in 109 prostate cancers of stage A1-D1. They concluded that mutation of p53 might be involved in the development of some prostate cancers. Patients whose prostate cancers showed p53 immunoreactivity had significantly worse prognosis than patients with p53-negative cancers.[17] Prendergast et al studied 18 patients with locally recurrent prostate carcinoma after radiotherapy (RT) and found that 72% had p53 nuclear immunoreactivity; while all 5 patients with available pre-RT biopsies had p53 immunoreactivity.[15] Cheng et al. examined p53 abnormalities by immunohistochemistry in lymph node positive prostate cancer. They found that a significiant proportion of primary tumors (52%) and matched lymph node metastases (58%) showed nuclear accumulation of p53 protein (4). However, Fox et al examined the expression of p53 in 55 stage A1 prostate cancers and concluded that it was not a useful prognostic indicator.[6] Masuda et al also found no association between p53 expression and patient outcome.[13]

Kallakury et al. reported that only 3 of 40 (7.5%) adenocarcinoma specimens of the prostate exhibited combined p53 and bcl-2 positivity.[11] However, Bauer et al studied p53 and bcl-2 immunoreactivity in 175 radical prostatectomy specimens. Aberrant bcl-2 was observed in 27% of cases with cancer. Sixty-seven per cent of these patients had relapse within 5 years, while only 31% of those with bcl-2 negative cancers had relapse. Aberrant p53 expression was observed in 65% of cancer cases, of which 51% had relapse, while 78% of patients with p53 negative cancers did not have disease progression. When expression rates for p53 and bcl-2 were combined, the 5-year failure rate was 75.3%. They showed a statistically significant difference between p53 and bcl-2 positive and negative patients. They have suggested that overexpression of bcl-2 is not directly associated with p53 protein accumulation in adenocarcinoma of the prostate.[1]

There are also some studies of Gleason score and apoptosis regulator proteins in prostate cancer. Matsushima et al studied p53 and bcl-2 immunoreactivity in 146 prostatic carcinomas. Bcl-2 and p53 positivity was found in 20% and 27% of 146 prostate cancers, respectively. Both bcl-2 and p53 positivity were found only in 5% of the cases. They reported not only that p53 positivity was associated with advancing Gleason grade but also bcl-2 positivity was found exclusively in moderately to poorly differentiated (Gleason score 6 to 10) tumors. However, there was no statistically significant correlation between bcl-2 positivity and Gleason score.[14] Budendolf et al. also reported that there was no correlation between bcl-2 positivity and Gleason grade.[3]

Similarly, we could not find a correlation between the expression of the apoptosis regulating genes bcl-2 and p53 and Gleason score in adenocarcinoma of the prostate. Further confirmation of the significance of these molecular examinations in more homogeneous and larger patient groups are required.

References

1.[2]*Bauer JJ, Sesterhenn IA, Mostofi FK, et al:* Elevated levels of apoptosis regulator proteins p53 and bcl-2 are independent prognostic biomarkers in surgically treated clinically localized prostate cancer. J Urol 156:1511-1516, 1996.

2.[2]*Boring CC, Squires T:* Cancer statistics 1994. CA Cancer J Clin 44:7, 1994.

3.[2]*Bubendorf L, Sauter G, Moch H, et al:* Prognostic significance of bcl-2 in clinically localized prostate cancer. Am J Pathol 148:1557, 1996.

4.[2]*Cheng L, Leibovich BC, Bergstralh EJ, et al.* P53 Alteration in regional lymph node metastases from prostate carcinoma. Cancer 85:2455-2459, 1999.

5. *Cheng L, Sebo JT, Pisansky T, et al:* P53 Protein Overexpression Is Associated with Increased Cell Proliferation in Patients with Locally Recurrent Prostate Carcinoma after Radiation Therapy. Cancer 85:1293-1299, 1999.
6. *Fox SB, Persad RA, Royds J, et al:* p53 and c-myc expression in stage A1 prostatic adenocarcinoma: Useful prognostic determinants? J Urol 150:490-494, 1993.
7. *Grignon DJ, Hammond EH:* Collage of American pathologists conferance XXVI on clinical relevance of prognostic markers in solid tumors: Report of prostate cancer working group. Arch Pathol Lab Med 119:1122-1126, 1995.
8. *Grossfeld GD, Olumi AF, Connolly JA, et al:* Locally recurrent prostate tumors following either radiation therapy or radical prostatectomy have changes in Ki-67 labelling index, p53 and bcl-2 immunoreactivity. J Urology 159:1437-1443, 1998.
9. *Hockenbery D, Nunez G, Milliam C, et al:* Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334, 1990.
10. *Issacs JT:* Molecular markers for prostate cancer metastasis: Devoloping diagnostic methods for predicting the aggressiveness of prostate cancer. Am J Pathol 150:1511-1521, 1997.
11. *Kallakury BV, Figge J, Ross JS, et al:* Association of p53 immunoreactivity with high Gleason tumor grade in prostatic adenocarcinoma. Human Pathol 25:92,1994.
12. *Levine AJ:* P53, the cellular gatekeeper for growth and division. Cell 88:323, 1997.
13. *Masuda M, Takano Y, Iki M, et al:* Prognostic Significance of Ki-67, p53 and bcl-2 expression in prostate cancer patients with lymph node metastases: A retrospective immunohistochemical analysis. Pathol Int:48:41-46,1998.
14. *Matsushima H, Kitamura T, Goto T, et al:* Combined anaysis with bcl-2 and p53 immunostaining predicts poorer prognosis in prostatic carcinoma. J Urology 158:2278-2283, 1997.
15. *Prendergest NJ, Atkins MR, Schatte EC, et al:* p53 immunohistochemical and genetic alterations are associated at high incidence with post-irradiated locally persistent prostate carcinoma. J Urol 155:1685-92,1996.
16. *Piris MA, Pezzella F, Martinez-Montero JC, et al:* p53 and bcl-2 expression in high grade B-cell lymphomas. Br J Cancer, 69:337, 1994.
17. *Shurbaji MS, Kalbfleisch JH, Thurmond S:* Immunohistochemical detection of p53 protein as a prognostic indicator in prostate cancer. Human Path 26:106-109, 1995.
18. *Soini Y, Paakko P:* Extent of Apoptosis in Relation to p53 and bcl-2 Expression in Germ Cell Tumors. Hum Pathol 27:1221-1226, 1996.
19. *Von Eschenbach AC:* The biologic dilemma of early carcinoma of prostate. Cancer 78:326-329, 1996.