The Value of Proto-Oncogene Bcl-2 and Neuroendocrine Differentiation in Human Prostate Cancer

PROSTAT KANSERİNDE PROTO-ONKOGEN BCL-2 VE NÖROENDOKRİN FARKLILAŞMANIN ÖNEMİ

Hasan BİRİ*, Bora KÜPELİ*, Zafer SINIK*, Kenan ÎSEN*, İlker ŞEN*, Özgür TAN*, Ömür ATAOĞLU**, İbrahim BOZKIRLI*

Depts. of *Urology and **Pathology, Medical School of Gazi University, Ankara, TURKEY

Summary

The aim of this study was to investigate the role ofproto-oncogene bcl-2 over expression and neuroendocrine differentiation (NED) in prostate cancer. We evaluated 81 archival specimen from patients with prostate cancer. The relationship between age, grade, stage, and disease progression were analysed. Bcl-2 expression and NED were found in 24 (29.6 %) and 42 (51.8 %) patients, respectively. In bcl-2 positive and NED positive patients, 5 years cumulative survival were found as 4.2 % and 21.4 %, whereas these rates were 47.4 % in bcl-2 negative and 48.7 % in NED negative patients. Our results demonstrated that bcl-2 over-expression and/or occurrence of NED did not correlate with age and stage in prostate cancer. However, bcl-2 over-expression was found to be significantly related with high Gleason score and poor prognosis in terms of progression. It was observed that occurrence of NED cell in prostate cancer is associated only with progression of disease.

Key Words: Prostate cancer, Bcl-2, Neuro-endocrine cell

T Klin J Med Res 1999, 17:41-46

Özet

Bu çalışmanın amacı prostat kanserinde Protoonkogen bcl-2 tanımlanmasının ve nöroendokrin farklılaşmanın (NED) rolünü araştırmaktır. Bu amaçla prostat kanserli 81 hastanın arşiv patolojik spesimenleri yeniden incelendi ve hastaların yaşı, tümörün evresi, grade'i ve hastalıkprogresyonları analiz edildi. Sırasıyla Bcl-2 ve NED hastaların 24'ünde (%29.6) ve 42'sinde (%51.8)1espit edileli. Bcl-2 pozitif ve NED pozitif olan hastaların 5 yıllık kümülatif yaşam oranları sırasıyla %4.2 ve % 21.4 olarak bulunurken bu oranlar bcl-2 ve NED negatif hastalar için %47.4 ve %48.7 olarak hesaplandı. Bcl-2 tanımlanmasının ve/veya NED'nin bulunmasının hastanın yaşı ve tümörün evresi ile ilişkisi bulunamadı. Ancak bcl-2 tanımlanması ile Gleason skoru ve progresyon bazında kötüprognoz ile çok yakın ilişki bulundu. Prostat kanserinde nöroendolain farklılaşma gösteren hücrelerin bulunmasının ise yalnızca hastalığın progresyonu ile ilişkili olabileceği kanaatine varıldı.

Anahtar Kelimeler: Prostat kanseri, Bcl-2, Nöroendokrin hücre

T Klin Araştırma 1999, 17:41-46

Adenocarcinoma of the prostate is one of the commonest malignancy in men and accounts for. significant mortality and morbidity (1). Prostate cancer is unpredictable in its clinical course and biological behaviour. Traditional prognostic markers such as grade, clinical stage and even pre-treatment prostate specific antigen (PSA) are of limited prognostic value for individual men. The accumulation of alteration to both cellular oncogenesis and tumor-suppressor genes (TsGs) is associated with tumoriogenesis (2). The bcl-2 proto-oncogene is a gene which encodes for a protein that has been shown to act as an inhibitor of apoptosis (3,4). Neuroendocrine differentiation in the normal prostate and in prostatic carcinoma has been a focus of attention by several investigators (5,6). Neuro-endocrine cell (NC) has the same biological properties with neurons (5) and NC has been discovered in different organ systems, including gastrointestinal tract, broncho pulmonary tract and urogenital systems (5). Those cells produce various substances such as serotonin, chromogranin (Chr), cytochrome b 561 and neuron-specific enolase (NSE) (5). The most predominant constituent of secretory granules of NC is chromogranin (5,6). Recently, multiple studies have shown bcl-2 over-expression and neuro endocrin differentiation to have prognostic significance in human prostate

Received: Feb. 18, 1999

Correspondence: Bora KÜPELİ
Beyazgül Sitesi A-1/21
06530 Ümitköy, Ankara, TURKEY

cancer (7,8). In this study, the value of proto-oncogene bcl-2 and neuro-endocrine differentiation were evaluated as a prognostic biomarker in prostatic adenocarcinoma and the relationships between age, clinical stage, pathological grade and tumor progression were investigated.

Materials and Methods

In this study, the records of 81 patients who had histologically proven prostate adenocarcinoma between January 1988 and October 1996 were reviewed. Patients' ages ranged from 50 to 86 years, with a mean age of 61.1 years. The bcl-2 over-expression and NED and their implication in prostatic adenocarcinoma, available archival pathologic material and accurate clinical follow up of these patients as of March 1997 were evaluated retrospectively and the findings were analysed according to patient age, tumor grade, tumor stage and disease progression. Patients' characteristics were shown in Table 1.

Patients were evaluated with digital rectal examination, transrectal ultrasonography with systematic biopsy, bone scan, abdominal ultrasonography and computerized tomography and serum PSA. Twentytwo (27.2%) patients had positive bone scans at presentation whilst 27 (33.3%) were considered clinically to have localised disease. Fifteen (18.51‰) patients underwent radical retropubic prostatectomy whereas hormonal therapy and radiotherapy were performed in 44 (54.3%) and 22 *(27.2%)* patients, respectively. The follow-up ranged from 5 to 9 years. An increase in prostate specific antigen, progressive increase in the size of the primary lesion or in the volume of existing metastatic sites or development of additional metastases in patients was considered evidence of disease progression. During follow-up, 37 (44.0%) patients had progression and of these patients 9 (11.1%) died from their disease.

Table 1. Patients' characteristics.

Variable	Patient N (%)
Age:	
<60	21 (25.9)
60-70	37 (45.7)
>70	23 (28.4)
Stage:	
T1a-b(A1,A2)	2 (2.4)
T2a(B1)	7 (8.6)
T2b(B2)	18 (22.2)
>T3	54 (66.6)
Gleason Score:	
2-4	14 (17.3)
5-7	42 (51.8)
8-10	25 (28.4)
Total	81

Histological examination: The prostatic tissue samples were fixed in 10 % buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin for routine histological examination. With these findings, the patients clinically staged, and pathologically graded according to modified Jewett classification and Gleason grading system. Prostate cancer was considered as well differentiated cancer in those with 2,3,4 Gleason numbers, as moderately with 5,6 and poorly with 7,8,9,10. After review of all available haematoxylin and eosin stained slides, the archival block corresponding to the most representative area of tumor was chosen for bcl-2 and NED analysis, defined as the highest concentration of tumor containing blocks. For immunohistochemical demonstration of bcl-2 and NED, 5 micrometer paraffin wax sections from corresponding paraffin block were deparaffmized and rehydrated. Endogenous peroxide was blocked by hydrogen peroxide for 5 minutes followed by a 5-minute-PBS-wash. The tissue sections were incubated with monoclonal anti-bcl-2 antibody (Clone no: bcl-2 100,catolog # B3170, Sigma Aldrich, St Louis, USA) dilutions of 1:1000 and both monoclonal mouse neuron spesific enolase (Immunon, Lipshow company, USA) and monoclonal mouse-chromogranin A (Immunon, Lipshow company, USA) in PBS for 2 hours. The procedure was completed with streptavidin-biotinylated peroxidase complex. Peroxidase activity was detected using 3.3-diaminobenzidine as the chromogen with hydrogen peroxide as the substrate. The sections were counterstained lightly with Harris' haemotoxylin (4).

All sections were scored objectively by the same observer. An average of 2000 malignant cells was counted in each case. For bcl-2 activity, cytoplasmic staining for bcl-2 in tumor cell was categorized as (+)= focal areas of strong staining(<5%),

(++)= diffuse staining (5-50%) and (+++)= diffuse staining (>50%). Basal cells and lymphocytes were used as positive controls. Negative controls consisted of normal prostate epithelial and stromal cells. For NED, stomach neuro-endocrine carcinoma was simultaneously stained as a positive control for each antibody. Staining procedure was completed with stteptavidin-biotin complex. Diaminobenzidin (DAB) was used as chromogen substrate, and hematoxylin as staining substrate. Staining degree was determined with the criteria described by di Sant Agrese and Schmid (6,9). Cells are accepted as positive for both showing tumor cell characteristics and exist within tumor groups. We accepted either neuron- specific- enolase (NSE) positive or chromalin A (ChA) positive as NED.

Kaplan-Meier analysis and log rank test were used to assess the cumulative survival rate or bcl-2 and NED. Chi-squarc test was used for correlations between markers (bel- 2,NED) and age, pathological stage and Gleason score. Student's t test was used for statistical analysis of disease progression time

Results

Bcl-2 over-expression and NED were found in 24 *(29.6%)* and 42 (51.8%) patients, respectively. Of these patients 17 (20.9 %) had both bcl-2 expression and NED. The pathologic features of 81 patients according to patients' age, tumor's stage and Gleason score are shown in Table 2. There were no statistically significant difference between age groups in both bcl-2 (+) and (-) and NED (+) and (-) patients (p= 0.75, p= 0.59). Tumor stage had also no effect on bcl-2 expression and NED (p= 0.47, p= 0.99). However bcl-2 expression was found to be increased significantly with the increasing Gleason score (p= 0.0026). Again there were some increase in NED (+) cell population with the increasing Gleason score, however no statistically significant relation was found (p= 0.82).

When we evaluate the patients who had disease progression (n=37), the time elapsed until the progression was found significantly longer in bcl-2 (-) and NED (-) patients (p< 0.01, p< 0.01). Mean progression time and five-year cumulative survival rates are shown in Table 3. The rates were found to be higher in patients with bcl-2 (-) and NED (-) (p< 0.01). Cumulative survival curves for bcl-2 (-) and (+), NED (-) and (+) and combination of both markers are shown in Figure 1, 2 and 3, respectively. There were highly significant difference in cumulative survival between bcl-2 (-)/NED (-) patients versus bcl-2 (+)/NED (+) patients (p< 0.01).

Discussion

Although numerous methods have been proposed for the establishment of prognosis in prostate

Table 2. Pathological features of the patients.

Variable	BCL-2(+) N (%)	BCL-2(-) N (%)	BCL-2 (+) Progr. N (%)	BCL-2(-) Progr. N (%)	NED(+) N (%)	NED (-) N (%)	NED(+) Progr. N (%)	NED(-) Progr. N (%)
Age:								
<60	5(20.8)	16(28.1)	2(10)	2(11.7)	10(23.8)	11(28.2)	6(20.7)	2(13.4)
60-70	13(54.7)	24(42.1)	8(40)	8(47.1)	23(54.7)	14(35.9)	17(58.6)	3(20)
>70	6(25)	17(29.8)	10(50)	7(41.2)	9(21.4)	14(35.9)	6(20.7)	3(20)
Stage:								
T1a-b(A1,A2)	1(4.2)	1(1.7)	1(5)	0	1(2.4)	1(2.7)	0	0
T2a(B1)	2(8.3)	5(8.7)	1(5)	0	3(7.1)	4(10.3)	1(3.4)	- 1 ^
T2b(B2)	2(8.3)	16(28.2)	2(10)	4(23.5)	10(23.8)	8(20.5)	9(17.3)	4(26.7)
>T3	19(79.2)	35(61.4)	16(80)	13(76.5)	28(66.6)	26(66.6)	19(51.7)	4(26.7)
Gleason Score:								
2-4	1(4.7)	13(22.8)	0	2(17.7)	6(14.2)	8(20.5)	1(3.44)	2(25)
5-7	7(29.2)	35(61.4)	8(40).	4(23.5)	22(52.4)	20(51.2)	18(62.1)	4(50)
8-10	16(66.6)	9(115.8)	12(60)	11(64.7)	14(33.3)	11(28.2)	10(34.5)	2(25)
Total	24	57	20	17	42	39	29	8

Table 3. Five year cumulative survival and mean pro£session time.

Variables	Five year cumulative survival (%)	Mean progression time (months)	
Bcl-2 (-)	47.4	54.5	
Bcl-2 (+)	4.2	39.4	
NED (-)	48.7	53.0	
NED (+)	21.4	47.2	
Bcl-2 (-) NED (-)	48.7	53.0	*
Bcl-2 (+) NED (+)	5.9	42.4	

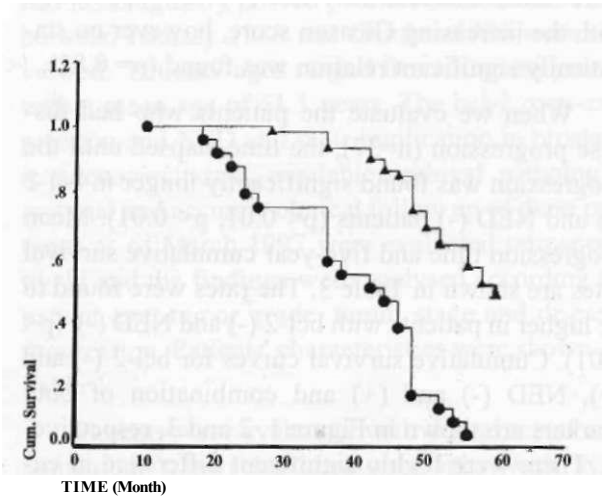

Figure 1. Survival curves for study patients based on presence or absence of bcl-2 over-expression (Kaplan-Meier Analysis). Log rank statistics = 0.000, p<0.01.
ABcl-2 negative
•Bcl-2 positive

cancer, none of them proved to have a definite role. Besides clinical stage and pathological grade; advanced age, chronic diseases, status of lower performance, pain, anemia and preoperatively high serum PSA and alkaline phosphatase levels could contribute to prognosis of prostate cancer (10). However, determination of prognosis is still controversial because of biological heterogeneity. Therefore, novel prognostic parameters are needed due to overcoming the uncertainty of prognosis.

In most studies, bcl-2 over-expression and the occurrence of neuro - endocrine -cell have been investigated for the prognostic markers of prostatic carcinoma. In these tumors, bcl-2 over expression showed frequencies from 26.9 to 41% (8,12,13). The incidence of neuro-endocrine differentiation in prostate carcinoma has steadily increased from 10 % to 100 % from the first to the current reports, with the use of special tissue fixatives (7,14).

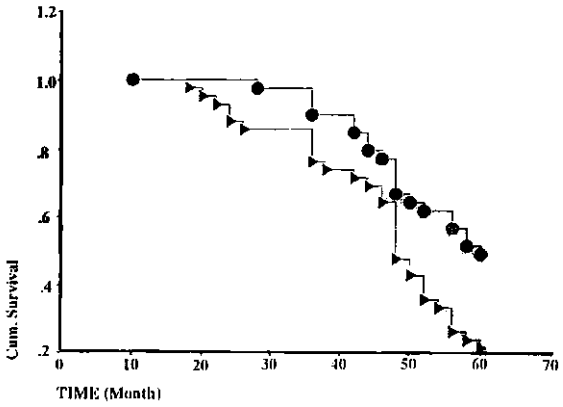

Figure 2. Survival curves for study patients based on presence or absence of NED cell (Kaplan-Meier Analysis). Log rank statistics = 0.0075, p<0.01.
•NED negative
TNED positive

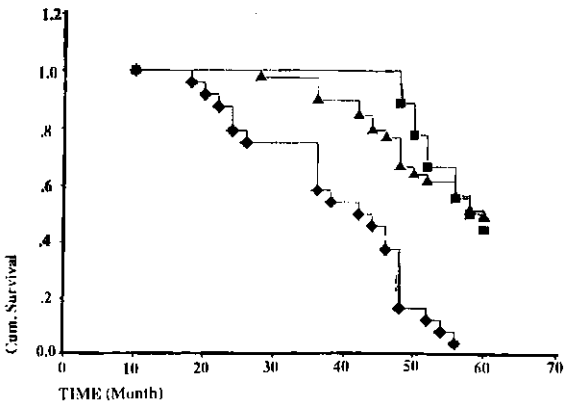

Figure 3. Survival curves for study patients based on presence or absence of bcl-2 over-expression and NED (Kaplan-Meier Analysis). Log rank statistics = 0.000, p<0.01.
•Bcl-2 positive, NED positive
ABcl-2 positive, NED negative
•Bcl-2 negative, NED negative

Neuroendocrine differentiation in the normal prostate and in prostatic carcinomas has been a focus of attention by several investigators (5-7).

In this study, bcl-2 over expression was observed 29.6% and occurrence of NED cell was demonstrated in 51.8%. Our results suggest that bcl-2 over expression and occurrence of NED cell were not significant with age and stage (p>0.01). In other studies, the similar results were observed (8).In our study, there was statistically significant relationship between bcl-2 and Gleason score, while no statistically significance was found between occurrence of NED cell and Gleason score (p>0.01).

Several reports also confirm that bcl-2 over-expression correlate with gene mutation advancing stage, Gleason score and hormone-refractory prostate cancer (8,15). In addition bcl-2 over-expression in prostate cancer appeared to be significant as independent prognostic bio-markers (8,12,15). On the other hand, numerous studies have been performed for investigating whether NED is of prognostic importance in prostate cancer. Although some authors reported that NED indicated poor prognosis (9,10,16), others suggested that NED did not have any prognostic value in prostate cancer (17). In our study, bcl-2 expression and NED were observed in 7 (8.6 %) and 8 (12.3%) cases, respectively. Also, 17 cases (29.6 %) showed both bcl-2 over-expression and NED.

When we focus on the disease progression, most studies have shown that time elapsed until progression was significantly lower in bcl-2 (+) and NED (+) patients (8,18). However, patient survival and the extension of metastatic disease were not affected by the expression of bcl-2 (19). We demonstrated that a significantly higher 5-year cumulative survival rate was found in bcl-2 negative patients (39.4 %) versus bcl-2 positive patients (4.2 %). Likewise the NED negative and positive 5-year cumulative survival were 43.4 % and 17.9 %, respectively. Our 5-year follow-up study indicated that both markers were correlated highly with tumor progression so, bcl2 and NED can be used as a prognostic factor.

As a conclusion, our data demonstrate that either bcl-2 or NED can be useful in predicting poor prognosis in prostate cancer. Also, combined detection of these markers seems to have more prognostic value. However, studies including larger patient groups, and longer follow-up need to make a more accurate decision about the prognosis of patients with prostate cancer.

REFERENCES

1. Waxman J and Saini A. The current status of scientific research and hormonal treatments for carcinoma of the prostate.Br J Cancer 1991;64:419-21.
2. Marshall C. Tumor suppressor genes. Cell 1991; 64:313-26.
3. Bissonnette RP, Echeverry F, Mahboubi A, Green DR: Apoptotic cell death induced by c-myc is inhibed by bcl-2. Nature 1992; 359: 552-4 .
4. Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 1992; 80:879-86.
5. De Lellis RA, Dayal L. Neuroendocrine system. Histology for pathologists(ed.Stephen S Sternberg), Raven pres Ltd Newyork,Chapter, 1992; 17: 347-61.
6. Schmid W, Helpap P, Totsch M, Kirchmair M, Dockhorn-Dwniczack Bocker W, Fischer-Colprie. Immunohistochemical localization of chromogranins A and B and secretogranin 2 in normal, hyperplastic and neoplastic prostate. Histopathology 1994; 24:233-9.
7. Cohen RJ, Glezerson G, Haffejee Z, Africa D. Prostatic carcinoma histological and Immunohistological Factors Affecting Prognosis. Br J Urol 1990; 66: 405-10.
8. Bauer JJ, Sesterhenn IS, Mostofy FK, Mcleod DG, Srivastava S, Moul JW. Elevated levels of apoptosis regulator proteins p53 and Bcl-2 are independent prognostic biomarkers in surgically treated clinically localized prostate cancer. J Urol 1996; 156:1511-6.
9. Di SantAgnese PA. Neuroendocrine differentiation in carcinoma of prostate: Diagnostic, prognostic and therapeutic implications. Cancer 1992;(1 supl): 254-68.
10. Denis LJ. Staging and prognosis of prostate cancer. Eur Urol 1993; 24: 13-28.
11. Salem CE, Tomasic AN, Elmajian DA, EsrigD, Nichols PW, Taylor CR, Skinner DG, Roy-Burman P, Lieskovsky G and Cote RJ. p53 protein and gene alterations in pathological stage C prostate carcinoma. J Urol 1997;158:510-4.
12. McDonnel T J, Troncoso P, Brisbay S M, Logothetis C, Chung LWK, Hsier JT, Tu SM and Campbell M L. Expression of the proto- oncogene bcl-2 in the prostate and its association with emergence of androgen -independent prostate cancer.Cancer Res 1992; 52:6940-4 .
13. Kalkakury BVS, Figgie J, Leihovich B, Rifkin M, Kaufman R, Figgie HL, Nazeer T and Ross JS. Increased bcl-2 protein levels in prostatic carcinomas are not associated with rearrangements in the 2.8 kb major break-point region or with p53 protein accumulation.Mod.Pathol 1995; abstract447,8:78A.

14. Aprikian AG, Cordon- Cardo C, Fair WR, Reuter VE. Characterization of neuroendocrine differentiation in benign prostate and prostatic adenocarcinoma. Cancer 1993;71(12):3953-65.

15. Apakama I, Robinson MC, Walter NM, Charlton RG, Royds JA, Fuller CE, Neal DE and Hamdy FC. bcl-2 overexpression combined with p53 protein accumulation correlates with hormone- refractorc prostate cancer. Br J Cancer 1996;74:1258-62.

lö. Falkmer S, Askcnsten U, Grimelius P, Abrahamsson PA. Cytochemical markers and DNA content of neuroendocrine cells in carcinoma of prostate gland tumor progression. Acta Histochemica 1990; 18:127-32.

17. Cocket AT, di Sant'Agnese PA, Gopinath P, Schoen SE, Abrahamsson PA. Relationship of neuroendocrine cells of prostate and serotonin to benign prostatic hyperplasia. Urology 1993; 42: 512-9.

18. Cohen RJ, Glezerson G, Haffejee Z. A new prognostic parameter in carcinoma of prostate. Br J Urol 1991; 68: 258-62.

19. McDonnell TJ, Navone NM, Troncoso P, Pisters LL,'Conti C, Eschenbach ACV, Brisbay S, Logothetis C.J: Expression of Bcl-2 oncoprotein and P53 protein accumulation in bone marrow metastases of androgen independent prostate cancer. J Urol 1997; 157: 569-74.