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 İSEN*, İlíker 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 of proto-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

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 tumorigenesis (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 cancer.
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.

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 deparaffinized and rehydrated. Endogenous peroxidase 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, catalog # B3170, Sigma Aldrich, St Louis, USA) dilutions of 1:1000 and both monoclonal mouse neuron specific 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' haematoxylin (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%),

**Table 1. Patients' characteristics.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient N (%)</th>
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<tbody>
<tr>
<td>Age:</td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>21 (25.9)</td>
</tr>
<tr>
<td>60-70</td>
<td>37 (45.7)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>23 (28.4)</td>
</tr>
<tr>
<td>Stage:</td>
<td></td>
</tr>
<tr>
<td>T1a-b(A1,A2)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>T2a(B1)</td>
<td>7 (8.6)</td>
</tr>
<tr>
<td>T2b(B2)</td>
<td>18 (22.2)</td>
</tr>
<tr>
<td>&gt;T3</td>
<td>54 (66.6)</td>
</tr>
<tr>
<td>Gleason Score:</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>14 (17.3)</td>
</tr>
<tr>
<td>5-7</td>
<td>42 (51.8)</td>
</tr>
<tr>
<td>8-10</td>
<td>25 (28.4)</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
</tr>
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</table>
(++)= 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 streptavidin-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-square test was used for correlations between markers (bcl-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 cancer, the present study provides new insights into the role of bcl-2 and NED in the progression of human prostate cancer. The results suggest that bcl-2 and NED could be useful markers for predicting disease progression and survival in prostate cancer patients.
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).

Table 3. Five year cumulative survival and mean progression time.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Five year cumulative survival (%)</th>
<th>Mean progression time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 (-)</td>
<td>47.4</td>
<td>54.5</td>
</tr>
<tr>
<td>Bcl-2 (+)</td>
<td>4.2</td>
<td>39.4</td>
</tr>
<tr>
<td>NED (-)</td>
<td>48.7</td>
<td>53.0</td>
</tr>
<tr>
<td>NED (+)</td>
<td>21.4</td>
<td>47.2</td>
</tr>
<tr>
<td>Bcl-2 (+) NED (-)</td>
<td>48.7</td>
<td>53.0</td>
</tr>
<tr>
<td>Bcl-2 (-) NED (+)</td>
<td>5.9</td>
<td>42.4</td>
</tr>
</tbody>
</table>

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.
- Bcl-2 negative
- Bcl-2 positive

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
- NED 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
- Bcl-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


