Expression of Bcl-2 and Bax in Advanced or Metastatic Prostate Carcinoma

Kazem Anvari,[1] Mehdi Seilanian Toussi,[1] Mahmoud Kalantari,[2] Shahram Naseri,[1] Mahdi Karimi Shahri,[1] Hassan Ahmadnia,[3] Mehrdad Katebi,[4] Abdolazim Sedighi Pashaki,[1] Mahdieh Dayani,[1] Maryam Broumand[5]

[1] Cancer Research Center, Department of Radiotherapy Oncology, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Pathology, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Urology, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Pathology, Bentolhoda Hospital, Mashhad, Iran
[5] Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author:

Mehdi Seilanian Toussi, MD
Department of Oncology, Omid Hospital, Kohsangi St., Alandasht Square, Mashhad, Iran

Tell/Fax: +98 511 842 6936
E-mail: silianm@mums.ac.ir

Received April 2011
Accepted September 2011

Purpose: To evaluate the correlation of Bcl-2 and Bax protein expressions with biochemical failure-free survival in patients with advanced or metastatic prostate carcinoma (PCa) undergoing androgen deprivation therapy.

Materials and Methods: This retrospective study was performed on patients with locally advanced (≥ T3) or metastatic PCa, who were referred to Omid Hospital between years 2003 and 2007. All subjects had undergone androgen deprivation therapy. Samples were analyzed immunohistochemically for Bax and Bcl-2 expression. The H-score was calculated for each sample based on intensity and percentage of stained cells. H-score > 50 was considered positive.

Results: Thirty-seven patients (13 metastatic and 24 locally advanced) were eligible for analysis. Thirty-six (97.3%) samples were positive for Bax and 26 (70.3%) for Bcl-2 expression. The median H-score for Bax and Bcl-2 was 200 (range, 40 to 300) and 85 (range, 0 to 220), respectively. While there was no correlation between Bax expression and Gleason score, high Bcl-2 expression (H-score > 85) was significantly associated with Gleason score > 7 (P = .004). The median time to progression in the advanced and metastatic groups was 22 (range, 10 to 37) months and 16 (range, 9 to 26) months, respectively. High Bcl-2 expression (P = .01) and prostate-specific antigen > 20 ng/mL (P = .01) were significant predictors of lower biochemical progression-free survival.

Conclusion: High Bcl-2 expression was associated with higher Gleason scores and lower biochemical-free survival in patients with advanced PCa undergoing androgen deprivation therapy.

Keywords: prostatic neoplasms, Bax protein, bcl-2, disease progression, survival

INTRODUCTION

Prostate carcinoma (PCa) has a diverse clinical behavior from indolent tumors to aggressive lethal cancers. Clinical prognostic factors, which predict recurrence after treatment, include clinical stage, grade, and pretreatment serum levels of prostate-specific antigen (PSA).[1] However, the above-mentioned prognostic factors are unable to predict the outcome in all subjects. The genetic factors play an essential role in tumor progression and in governing whether a prostate cancer is aggressive or indolent.[2-4]

Almost all tissues have a system to remove damaged cell through programmed cell death or apoptosis.[5] Disturbance of programmed cell death can lead to accumulation of cells with impaired genome and eventually cancer. A number of genes regulate apoptosis, which include Bcl-2 family and tumor suppressor p53 gene.[6] Bcl-2 family proteins include both anti-apoptotic (eg, Bcl-2 and Bcl-xL) and pro-apoptotic (eg, Bax, BAK, and BIM) members. Pro-apoptotic proteins induce permeabilization of outer mitochondrial membrane and efflux of proteins, including cytochrome c into the cytosol, which in turn activates a group of cysteine protease called caspases leading to cell death. Meanwhile, anti-apoptotic proteins prevent cell death program through preserving mitochondrial membrane integrity and releasing cytochrome c.[7,8]

A study by Tolonen and colleagues showed that normal epithelium of cancerous prostates contain multiple foci with high expression of Bax and Bcl-2.[9] In a study by Amighofran and associates, a significant correlation was found between Bcl-2 expression and Ki-67 index indicating higher proliferating rates in Bcl-2 positive tumors.[10] In some trials, high Bcl-2 expression was significantly associated with high Gleason score tumors.[11,12] These results suggest a potential role for altered apoptosis in carcinogenesis.

Androgen deprivation, which is the mainstay of treatment in metastatic PCa, can induce Bax-mediated apoptosis in androgen dependant cells and regression of tumor. However, androgen independent cancer eventually develops in all cases of metastatic disease, which is accompanied with Bcl-2, Bcl-xL, and McL-1 overexpression in most cases.[13,14] The results of some studies suggest an association between apoptotic pathway dysfunction and worse outcome.[15-17]

According to the result of a population–based cancer registry, the age-standardized incidence rate of PCa in Iran has been around 5 per 100 000 person per year,[18] which is much lower than western countries. Meanwhile, in comparison with western countries, higher proportion of Iranian patients with PCa are diagnosed with advanced stages, which can be explained partly by lack of national screening program in Iran. The aim of the current study was to investigate the association between Bax and Bcl-2 expression and time to progression in patients with metastatic or very high-risk PCa undergoing androgen deprivation therapy.

MATERIALS AND METHODS

This retrospective study was conducted in Cancer Research Center, Omid Hospital, affiliated to Mashhad University of Medical Sciences, Iran. Eligible subjects were patients with distant metastasis and/or lymph node metastasis (metastatic PCa) or patients with tumor invasion beyond prostatic capsule or more (≥ T3; locally advanced), who were referred to our department between years 2003 and 2007. We excluded patients who were not followed up properly and those without required clinicopathological information.

The usual imaging procedures were chest x-ray, abdominopelvic computed tomography scan, and bone scintigraphy. Thirty-seven eligible patients with retrievable pathological specimens and adequate clinical information were selected. The specimens were re-evaluated for determining their Gleason score by our pathologists. The Gleason

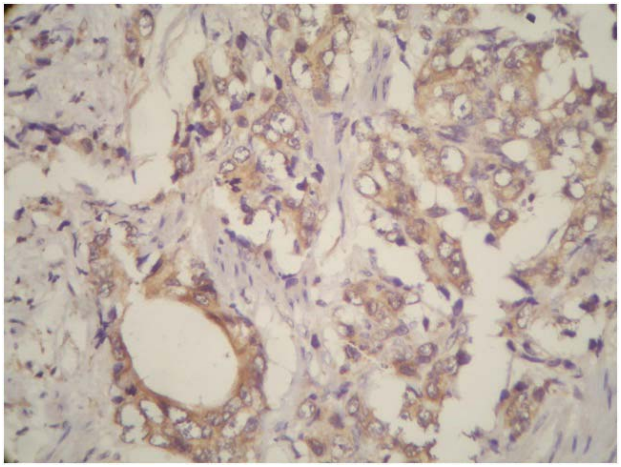

Figure 1. Positive Bax immunostaining (H-score = 240) in a prostate cancer with the Gleason score of 7.

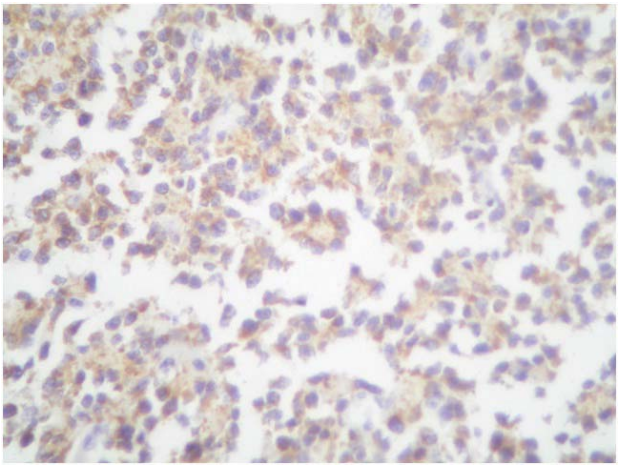

Figure 2. Positive Bcl-2 reaction (H-score = 200) in a prostate cancer with the Gleason score of 9.

scores above 7 were considered as poorly differentiated (high Gleason score). Our samples consisted of 14 low and 23 high Gleason scores.

Treatment and Follow-up

The diagnosis was based on histologic evaluation of the prostate biopsy specimens. All non-metastatic cases underwent local and regional external beam radiotherapy. All the patients received androgen deprivation therapy, which included luteinizing hormone-releasing hormone (LHRH) agonist in 17 and bilateral orchiectomy in 20 patients. Twenty-eight subjects received anti-androgen plus medical or surgical castration. All the patients were followed up every 3 months for the evidence of biochemical progression. Three consecutive rises in PSA was considered as biochemical progression.[19]

Immunostaining for Bax and Bcl-2

Multiple 4-µm-thick sections of representative formalin-fixed, paraffin-embedded tissues were cut for immunohistochemical studies. A Polymer-Based (EnVision™) immunohistochemical method was used for the detection of Bax (Polyclonal Rabbit Anti-Human, Dako) and Bcl-2 (Monoclonal Mouse Anti-Human, Clone 124, Dako). Reactive lymph node with follicular hyperplasia was used as positive control. Immunostaining without adding antibody was used as the negative control. All immunostained sections were examined by the two observers with a ×40 objective and 10 × 10 eyepiece under the light microscope (Olympus CH30, Olympus optical Co, Ltd, Tokyo, Japan) for evaluating Bax and Bcl-2 expression. Bax and Bcl-2 staining was cytoplasmic. Protein expression was scored as negative, weak; faint cytoplasmic staining, moderate; diffuse cytoplasmic stain, and strong; diffuse intense cytoplasmic stain. Furthermore, proportion of malignant cells which had positive staining was considered in reporting. Mild, moderate, and strong staining was considered as positive.

The immunostaining was quantified as H-score, which considers both the intensity and percentage of cells stained in each intensity.[20] H-score was calculated as follows: (% of cells stained at intensity 1 × 1) + (% of cells stained at intensity 2 × 2) + (% of cells stained at intensity 3 × 3). A H-score between 0 and 300 was obtained where 300 was equal to 100% of tumor cells stained strongly. When the H-score was above 50, the sample was considered as positive for Bax or Bcl-2 expression (Figures 1 and 2). The median H-score values were selected for distinction between the groups of high and low Bax or Bcl-2 expression.

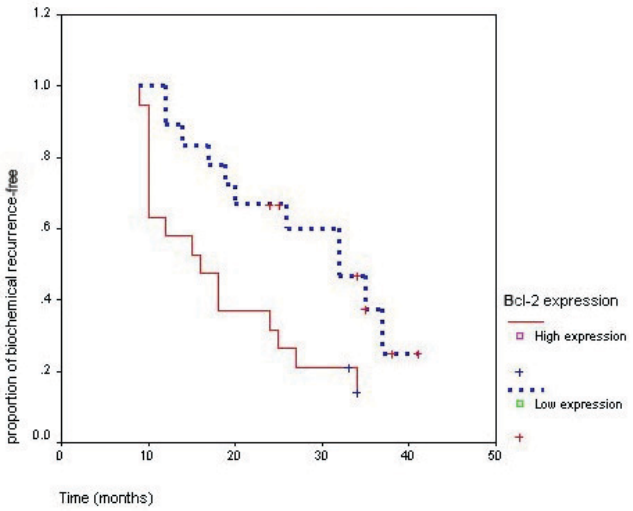

Figure 3. Association between Bcl-2 expression and biochemical recurrence- free survival in patients with advanced or metastatic prostatic carcinoma.

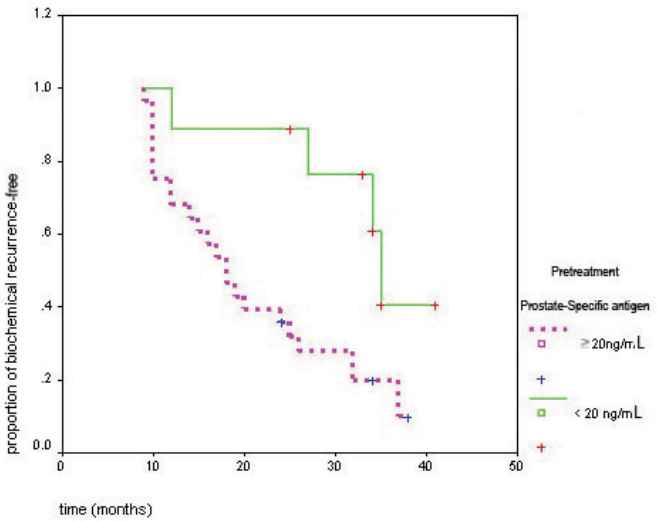

Figure 4. Association between pretreatment prostate-specific antigen and biochemical recurrent-free survival in patients with advanced or metastatic prostatic carcinoma.

Statistical Analysis

Progression-free survival was determined from the time of diagnosis to the time of biochemical failure or the last visit using Kaplan-Meier method. We used Log-rank test for univariate comparing survival curves between groups. A Cox regression model with backward stepwise selection of co-variates was used for multivariate analysis. Chi-Square test was used to evaluate the association between the Bax and Bcl-2 expression and the Gleason score.

RESULTS

The participants included 13 patients with metastasis (9 distant and 4 lymph node metastasis) and 24 with locally advanced tumors. The median age of the patients was 73 years (range, 52 to 87 years).

The median pretreatment PSA value was 17 ng/mL (range, 7.5 to 96 ng/mL) for high-risk advanced patients and 40 ng/mL (range, 19 to 100 ng/mL) for metastatic cases. Prostate-specific antigen values > 20 ng/mL were detected in 41.6% (10/24) of high-risk advanced and 84.6% (11/13) of metastatic cases.

The median Gleason score was 8 (range, 6 to 10) for all the subjects. We recorded Gleason scores > 7 in 23 (62.7%) patients, including 16 (66.6%) of high-risk advanced and 7 (53.8%) of metastatic cases.

Treatment Results

With a median follow-up period of 32 months (range, 12 to 80 months), 27 patients experienced biochemical progression, including 13 out of 13 subjects (100%) in metastatic and 14 out of 24 (58.3%) in the locally advanced group. The median time to the biochemical progression was 22 months (range, 10 to 37 months) in the locally advanced group and 16 months (range, 9 to 26 months) in the metastatic group.

The median biochemical recurrence-free survival rates for locally advanced and metastatic groups were 34 months [95% confidence interval (CI): 25.23 to 42.77] and 16 months (95% CI: 10.13 to 21.87), respectively.

Bax and Bcl-2 Expression

Of 37 samples, 36 (97.3%) were positive for Bax expression. The median Bax H-scores was 200 (range, 40 to 300). Twenty-six cases were positive for Bcl-2 (70.3%) with a median H-score of

Table 1. Correlation between Gleason score and Bax or Bcl-2 expression

	High Bax expression, n (%)	High Bcl-2 expression, n (%)
Gleason score:		
G 2 to 7, n = 14	8 (57.1%)	3 (21.4%)
G 8 to 10, n = 23	9 (39.1%)	16 (69.6%)
	P = .28	*P* = .004*

*Statistically significant.

85 (range, 0 to 220). As it is displayed in Table 1, while there was no significant correlation between Bax expression and Gleason score ($P = .28$), high Bcl-2 expression was significantly associated with high Gleason score ($P = .004$).

Table 2 shows the effect of different parameters on biochemical failure-free survival. Gleason Score and Bax expression did not affect biochemical progression-free survival. Meanwhile, as shown in Figures 3 and 4, patients with high Bcl-2 expression ($P = .01$) and those with pretreatment values > 20 ng/mL ($P = .01$) were significantly associated with lower biochemical progression-free survival. In our series, although lower Gleason score was associated with relatively higher biochemical failure-free survival, the difference did not reach statistical significance. In multivariate analyses, Bcl-2 expression ($P = .02$) and pretreatment PSA ($P = .01$) remained significant predictors of biochemical progression-free survival.

DISCUSSION

In the present study, we selected a relatively homogeneous group of patients with PCa undergoing androgen deprivation therapy. About 41.6% of high-risk locally advanced group had pretreatment PSA values > 20, which implies existence of hidden metastasis in significant number of these subjects that could not be found via our imaging methods, such as bone scintigraphy and computed tomography scan.

Hormonal treatment was the main treatment modality for prolongation of survival in most of our patients. We used the median H-score for the distinction between high and low expression of each of these two proteins. In this study, lower biochemical progression-free survival was shown for subjects with higher pretreatment PSA values (> 20 ng/mL) and those with high Bcl-2 expression.

The contribution of Bcl-2 to prostate carcino-

Table 2. The pathological features and progression-free survival rate

	Median biochemical failure-free survival (95% confidence interval), months	*P*
Bax		
High expression	24 (14.59 to 33.41)	.45
Low expression	20 (09.60 to 39.41)	
Bcl-2		
High expression	16 (09.60 to 22.30)	.01*
Low expression	32 (22.35 to 41.03)	
Gleason score		
High (G 8 to 10)	18 (07.8 to 35.22)	.54
Low (G 2 to 7)	24 (15.54 to 32.46)	
Pretreatment prostate-specific antigen, ng/mL		
> 20	18 (14.11 to 21.88)	.01*
≤ 20	35 (23.02 to 36.98)	

*Statistically significant.

genesis and hormone independence has been documented.[21] As the Bcl-2 family modulates apoptosis, altered expression of Bcl-2 might affect response to genotoxic stresses, including radiotherapy, hormone deprivation, or cytotoxic agents. Expression of Bcl-2 was shown to be associated with higher failure rate after radiotherapy in localized prostate cancer.[22-25] Bcl-2 expression has also been associated with higher biochemical recurrence after radical prostatectomy.[26]

Androgen deprivation therapy is the first-line treatment in advanced and metastatic PCa. The predicting factors for time to progression included Gleason score, pretreatment PSA value, and nadir PSA value after treatment initiation.[27-29] Tissue biomarkers might also be helpful in prediction of response duration in metastatic or advanced diseases. Following androgen ablation therapy, increased expression of Bcl-2 develops in tumor cells, which eventually leads to an androgen independent state.[14,30]

Zhou and colleagues used in-vivo model of androgen-sensitive LNCaP human prostate cancer cell in SCID mice to investigate the cellular and molecular biology of progression from a hormone sensitive to hormone resistant state following castration. Hormone resistant tumors had decreased apoptosis accompanied with augmented expression of p53, p21/waf1, Bcl-2, Bax, and the Bcl-2/Bax ratio compared to androgen-sensitive tumors.[31] It has also been shown that tumors with high Bcl-2 expression in newly diagnosed metastatic patients might be less responsive to hormone manipulation.[13]

As Bax is a pro-apoptotic agent, its expression is expected to be associated with increased tumor sensitivity to radiotherapy or systemic therapy. However, the results of studies have been inconsistent. In a study on 62 Pca samples from non-Japanese Asian population, Chia and associates reported worse prognosis for patients with Bax expression.[17] Meanwhile, in a group of patients undergoing external beam radiotherapy, lower Bax expression was associated with worse prognosis; however; the difference was not statistically significant. Patients with increased Bcl-2/Bax ratio had higher risk of failure following radiotherapy.[22] In a study by Amirghofran and colleagues, Bax expression was not correlated with apoptosis index suggesting presence of nonfunctional Bax protein.[10] Mutated or dysfunctional Bax may appear overexpressed, while negatively affect apoptotic response to stress factors. In some studies, altered Bax expression in a tumor specimen was considered as under- or overexpression relative to the normal epithelium. In a group of patients with T1 to T3 PCa undergoing external beam radiotherapy, Pollack and associates showed that higher Bcl-2 expression and altered Bax expression were associated with higher biochemical failure rates.[16] In a group of patients with locally advanced prostate cancer who underwent radiotherapy, more favorable outcome were associated with negative Bcl-2 and normal Bax, especially in those who received short-term androgen ablation therapy plus radiotherapy.[15] Altered Bax expression seems to be a more reliable predictor of outcome compared to Bax overexpression.

As the role of apoptotic modulator, especially Bcl-2, has been documented in development of hormone independent prostate cancer, these genes were considered as a target for development of novel treatments. Bcl-2 antisense oligonucleotides might counteract anti-apoptotic survival mechanisms and enhance hormone and chemotherapy sensitivity.[32-36]

CONCLUSION

We concluded that high Bcl-2 expression is associated with worse biochemical progression-free rates in locally advanced and metastatic PCa in patients undergoing hormone manipulation therapy.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Vice-chancellorship for Research, Mashhad University of Medical Sciences.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Kattan MW, Eastham JA, Stapleton AM, Wheeler TM, Scardino PT. A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. J Natl Cancer Inst. 1998;90:766-71.
2. Qian J, Jenkins RB, Bostwick DG. Determination of gene and chromosome dosage in prostatic intraepithelial neoplasia and carcinoma. Anal Quant Cytol Histol. 1998;20:373-80.
3. Xu J, Zheng SL, Isaacs SD, et al. Inherited genetic variant predisposes to aggressive but not indolent prostate cancer. Proc Natl Acad Sci U S A. 2010;107:2136-40.
4. Kehinde EO, Maghrebi MA, Anim JT. The importance of determining the aggressiveness of prostate cancer using serum and tissue molecular markers. Can J Urol. 2008;15:3967-74.
5. Hartwell LH, Kastan MB. Cell cycle controland cancer. Science. 1994;266:1821-8.
6. Lane DP. Cancer. p53, guardian of the genome. Nature. 1992;358:15-6.
7. Harada H, Grant S. Apoptosis regulators. Rev Clin Exp Hematol. 2003;7:117-38.
8. Antonsson B. Bax and other pro-apoptotic Bcl-2 family "killer-proteins" and their victim the mitochondrion. Cell Tissue Res. 2001;306:347-61.
9. Tolonen TT, Tommola S, Jokinen S, Parviainen T, Martikainen PM. Bax and Bcl-2 are focally overexpressed in the normal epithelium of cancerous prostates. Scand J Urol Nephrol. 2007;41:85-90.
10. Amirghofran Z, Monabati A, Gholijani N. Apoptosis in prostate cancer: bax correlation with stage. Int J Urol. 2005;12:340-5.
11. Iacopino F, Angelucci C, Lama G, et al. Apoptosis-related gene expression in benign prostatic hyperplasia and prostate carcinoma. Anticancer Res. 2006;26:1849-54.
12. Hering FL, Lipay MV, Lipay MA, Rodrigues PR, Nesralah LJ, Srougi M. Comparison of positivity frequency of bcl-2 expression in prostate adenocarcinoma with low and high Gleason score. Sao Paulo Med J. 2001;119:138-41.
13. Apakama I, Robinson MC, Walter NM, et al. bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. Br J Cancer. 1996;74:1258-62.
14. Lin Y, Fukuchi J, Hiipakka RA, Kokontis JM, Xiang J. Up-regulation of Bcl-2 is required for the progression of prostate cancer cells from an androgen-dependent to an androgen-independent growth stage. Cell Res. 2007;17:531-6.
15. Khor LY, Moughan J, Al-Saleem T, et al. Bcl-2 and Bax expression predict prostate cancer outcome in men treated with androgen deprivation and radiotherapy on radiation therapy oncology group protocol 92-02. Clin Cancer Res. 2007;13:3585-90.
16. Pollack A, Cowen D, Troncoso P, et al. Molecular markers of outcome after radiotherapy in patients with prostate carcinoma: Ki-67, bcl-2, bax, and bcl-x. Cancer. 2003;97:1630-8.
17. Chia SJ, Tang WY, Elnatan J, Yap WM, Goh HS, Smith DR. Prostate tumours from an Asian population: examination of bax, bcl-2, p53 and ras and identification of bax as a prognostic marker. Br J Cancer. 2000;83:761-8.
18. Sadjadi A, Nooraie M, Ghorbani A, et al. The incidence of prostate cancer in Iran: results of a population-based cancer registry. Arch Iran Med. 2007;10:481-5.
19. Cox JD, GallagherMJ, Hammond EH, Kaplan RS, Schellhammer PF. Consensus statements on radiation therapy of prostate cancer: guidelines for prostate re-biopsy after radiation and for radiation therapy with rising prostate-specific antigen levels after radical prostatectomy. American Society for Therapeutic Radiology and Oncology Consensus Panel. J Clin Oncol. 1999;17:1155.
20. Huang C, Kohno N, Inufusa H, Kodama K, Taki T, Miyake M. Overexpression of bax associated with mutations in the loop-sheet-helix motif of p53. Am JPathol. 1999;155:955-65.
21. Bruckheimer EM, Brisbay S, Johnson DJ, Gingrich JR, Greenberg N, McDonnell TJ. Bcl-2 accelerates multistep prostate carcinogenesis in vivo. Oncogene. 2000;19:5251-8.
22. Mackey TJ, Borkowski A, Amin P, Jacobs SC, Kyprianou N. bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. Urology. 1998;52:1085-90.

23. Scherr DS, Vaughan ED, Jr., Wei J, et al. BCL-2 and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. J Urol. 1999;162:12-6; discussion 6-7.

24. Szostak MJ, Kaur P, Amin P, Jacobs SC, Kyprianou N. Apoptosis and bcl-2 expression in prostate cancer: significance in clinical outcome after brachytherapy. J Urol. 2001;165:2126-30.

25. 25.Vergis R, Corbishley CM, Thomas K, et al. Expression of Bcl-2, p53, and MDM2 in localized prostate cancer with respect to the outcome of radical radiotherapy dose escalation. Int J Radiat Oncol Biol Phys. 2010;78:35-41.

26. Cho IC, Chung HS, Cho KS, et al. Bcl-2 as a predictive factor for biochemical recurrence after radical prostatectomy: an interim analysis. Cancer Res Treat. 2010;42:157-62.

27. Morote J, Esquena S, Abascal JM, et al. Usefulness of prostate-specific antigen nadir aspredictor of androgen-independent progression of metastatic prostate cancer. Int J Biol Markers. 2005;20:209-16.

28. Ross RW, Xie W, Regan MM, et al. Efficacy of androgen deprivation therapy (ADT) in patients with advanced prostate cancer: association between Gleason score, prostate-specific antigen level, and prior ADT exposure with duration of ADT effect. Cancer. 2008;112:1247-53.

29. Choueiri TK, Xie W, D'Amico AV, et al. Time to prostate-specific antigen nadir independently predicts overall survival in patients who have metastatic hormone-sensitive prostate cancer treated with androgen-deprivation therapy. Cancer. 2009;115:981-7.

30. McDonnell TJ, Troncoso P, Brisbay SM, et al. Expression of the protooncogene bcl-2 in the prostate and its associationwith emergence of androgen-independent prostate cancer. Cancer Res. 1992;52:6940-4.

31. Zhou JR, Yu L, Zerbini LF, Libermann TA, Blackburn GL. Progression to androgen-independent LNCaP human prostate tumors: cellular and molecular alterations. Int J Cancer. 2004;110:800-6.

32. Gleave ME, Zellweger T, Chi K, et al. Targeting anti-apoptotic genes upregulated by androgen withdrawal using antisense oligonucleotides to enhance androgen- and chemo-sensitivity in prostate cancer. Invest New Drugs. 2002;20:145-58.

33. Yamanaka K, Rocchi P, Miyake H, et al. Induction of apoptosis and enhancement of chemosensitivity in human prostate cancer LNCaP cells using bispecific antisense oligonucleotide targeting Bcl-2 and Bcl-xL genes. BJU Int. 2006;97:1300-8.

34. Rubenstein M, Guinan P. Bispecific antisense oligonucleotides have activity comparable to monospecifics in inhibiting expression of BCL-2 in LNCaP cells. In Vivo. 2010;24:489-93.

35. Rubenstein M, Tsui P, Guinan P. Treatment of prostate and breast tumors employing mono- and bi-specific antisense oligonucleotides targeting apoptosis inhibitory proteins clusterin and bcl-2. Med Oncol. 2010;27:592-9.

36. Karnak D, Xu L. Chemosensitization of prostate cancer by modulating Bcl-2 family proteins. Curr Drug Targets. 2010;11:699-707.