

Original Article

BCL-2 and BCL-XL expression are down-regulated in benign prostate hyperplasia nodules and not affected by finasteride and/or celecoxib

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Abstract: The mechanisms involved in the development of benign prostatic hyperplasia (BPH) are poorly understood. One potential mechanism involved in BPH pathogenesis may involve altered expression of genes related to apoptosis and proliferation because reduced cell death and increased proliferation are thought to contribute to prostatic enlargement. This study examined the expression of B-cell lymphoma 2 (BCL-2) and B-cell lymphoma-extra large (BCL-XL), two important anti-apoptosis factors that are also capable of inhibiting cell proliferation via accelerated G1 arrest or delayed G1/S transition, using immunostaining in simple prostatectomy BPH specimens from patients naïve to androgen manipulation. Since androgens and inflammation are thought to play important roles in BPH pathogenesis, we tested the effect of inhibiting 5 α -reductase and/or COX-2 on the expression of BCL-2 and BCL-XL in BPH specimens from prostate cancer patients with BPH. These patients had no prior use of chronic NSAIDs and/or 5 α -reductase inhibitors and were treated with celecoxib, finasteride, celecoxib plus finasteride or no treatment for 28 consecutive days prior to surgery. In all specimens, BCL-2 and BCL-XL staining was evident in both luminal and basal epithelial cells, with more intense staining in basal cells. Both luminal and basal cells exhibited decreased BCL-2 and BCL-XL staining in BPH nodules compared to the surrounding normal prostatic tissues. In prostate cancer patients with BPH, celecoxib and/or finasteride did not affect the expression of BCL-2 and BCL-XL in luminal or basal cells in BPH nodules and normal adjacent tissues. These results suggest that BCL-2 and BCL-XL may act as anti-proliferative factors in BPH pathogenesis, and the effect of celecoxib and/or finasteride on BPH is unlikely mediated through modulating BCL-2 and BCL-XL signaling.

Keywords: BPH, BCL-2, BCL-XL, apoptosis, proliferation

Introduction

Benign prostatic hyperplasia (BPH) is one of the most common disease conditions in older men in the US [1-3]. Although BPH is in general not life threatening, it significantly impacts the quality of life of patients and costs society approximately \$4 billion annually [4]. As life expectancy increases, the prevalence of BPH is expected to increase. Additionally, the age-adjusted prevalence of BPH hospitalizations increased from 4.3% in 1998 to 8% in 2008 [5], and factors such as obesity and diabetes

are also strongly associated with increased risk of BPH [6-8]. Thus, there is an urgent need to understand the pathogenesis of BPH and search for new approaches to prevent and/or more effectively treat BPH.

BPH development and progression are poorly understood. Multiple factors are associated with BPH pathogenesis, including androgens, estrogens, growth factors and/or neurotransmitters (Reviewed in [9]). These factors are thought to cause a gradual growth involving both fibromuscular stroma and glandular epi-

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thelium, resulting in the increase of prostate volume (Reviewed in [10]). Enlarged prostate volume could cause a decrease in maximum urinary flow rate and the development of acute urinary retention, leading to the occurrence of BPH symptoms including lower urinary tract symptoms (LUTS) [11]. However, mechanisms leading to prostate enlargement remain unclear.

Dysregulation of proliferation and/or apoptosis is also thought to play an important role in the enlargement of prostate during BPH pathogenesis [12]. B-cell lymphoma 2 (BCL-2) and BCL-XL are key anti-apoptosis proteins with anti-proliferative function [13]. Overexpression of *Bcl-2* in the murine prostate induced the proliferation of both stromal and epithelial cells [14]. Increased BCL-2 expression in BPH specimens has also been reported [12, 15, 16]. Alterations in BCL-2 expression in BPH specimens suggest a potential role for BCL2 in BPH pathogenesis, and modulation of anti-apoptotic proteins such as BCL-2 or BCL-XL by therapeutic agents could be effective for BPH treatment.

Androgens and inflammation are thought to play important roles in BPH pathogenesis and 5 α -reductase II inhibitor finasteride and/or NSAIDs like celecoxib are beneficial to BPH patients [17-19]. Finasteride can indeed reduce prostate volume in BPH patients, indicating it could inhibit proliferation and/or induce cell death in BPH tissues [20-22]. Finasteride has also been shown to decrease expression of *Bcl-2* in rats [23, 24]. Although celecoxib does not induce an increase in the expression of BCL-2 in prostate cancer cells [25], the impact of celecoxib in normal prostate cells remains to be determined. Here, we evaluated the expression of BCL-2 and BCL-XL, two important regulators of apoptosis and proliferation, in BPH specimens containing both BPH and normal adjacent prostate tissues from BPH patients and prostate cancer patients with BPH treated with finasteride and/or celecoxib.

Materials and methods

Specimen acquisition

All clinical specimens were collected under an approved University of Pittsburgh Institutional Review Board protocol. To study the expression of BCL-2 and BCL-XL in BPH, 10 archival BPH

specimens from patients naïve to androgen manipulation were obtained from the Health Sciences Tissue Bank at the University of Pittsburgh Medical Center. These BPH specimens were from patients over 60 years of age with clinical symptoms of BPH and who also underwent prostatectomy because of BPH. No incidental foci of carcinoma were present in this cohort.

To evaluate the influence of celecoxib and/or finasteride on BCL-2 and BCL-XL expression in BPH, prostate cancer patients with BPH without prior use of chronic NSAIDs and/or 5 α -reductase inhibitors were recruited and treated with celecoxib, finasteride, celecoxib plus finasteride or no treatment for 28 consecutive days prior to surgery. A total of 28 BPH specimens were collected, with 7 specimens in each treatment group. Patient treatment arms included 1) celecoxib 200 mg/day with required abstention from finasteride, 2) finasteride 5 mg/day with abstention from all NSAIDs, 3) celecoxib 200 mg/day and finasteride 5 mg/day, and 4) no treatment with abstention from finasteride and all NSAIDs. Inclusion and exclusion criteria are listed below:

Inclusion criteria: 1). Evidence of BPH by transrectal ultrasound and/or digital rectal exam. For this study, prostate glands must be >30 grams to qualify; 2). No prior use of finasteride or dutasteride; 3). No prior chronic NSAID use; 4). For men with clinically localized prostate cancer, only clinical stages T1c, T2a and T2b will be eligible. Palpable tumors involving both lobes (T2c) or locally advanced (T3 or T4) will be excluded. This will assure adequate BPH and adjacent normal tissues without infiltrating prostate cancer for molecular studies; 5). For men with prostate cancer, at least 50% of the biopsy material must be non-cancerous. This will assure adequate BPH and adjacent normal tissues without infiltrating prostate cancer for molecular studies; 6). For men with prostate cancer, no Gleason score 8-10 will be enrolled. Higher grade cancers can be more infiltrative and possibly compromise the acquisition of BPH and normal tissues for analysis; 7). For men with prostate cancer, PSA must be less than 15 ng/ml. Higher PSA values are associated with more extensive cancers; 8). Subject's ability to understand this study and provide informed consent.

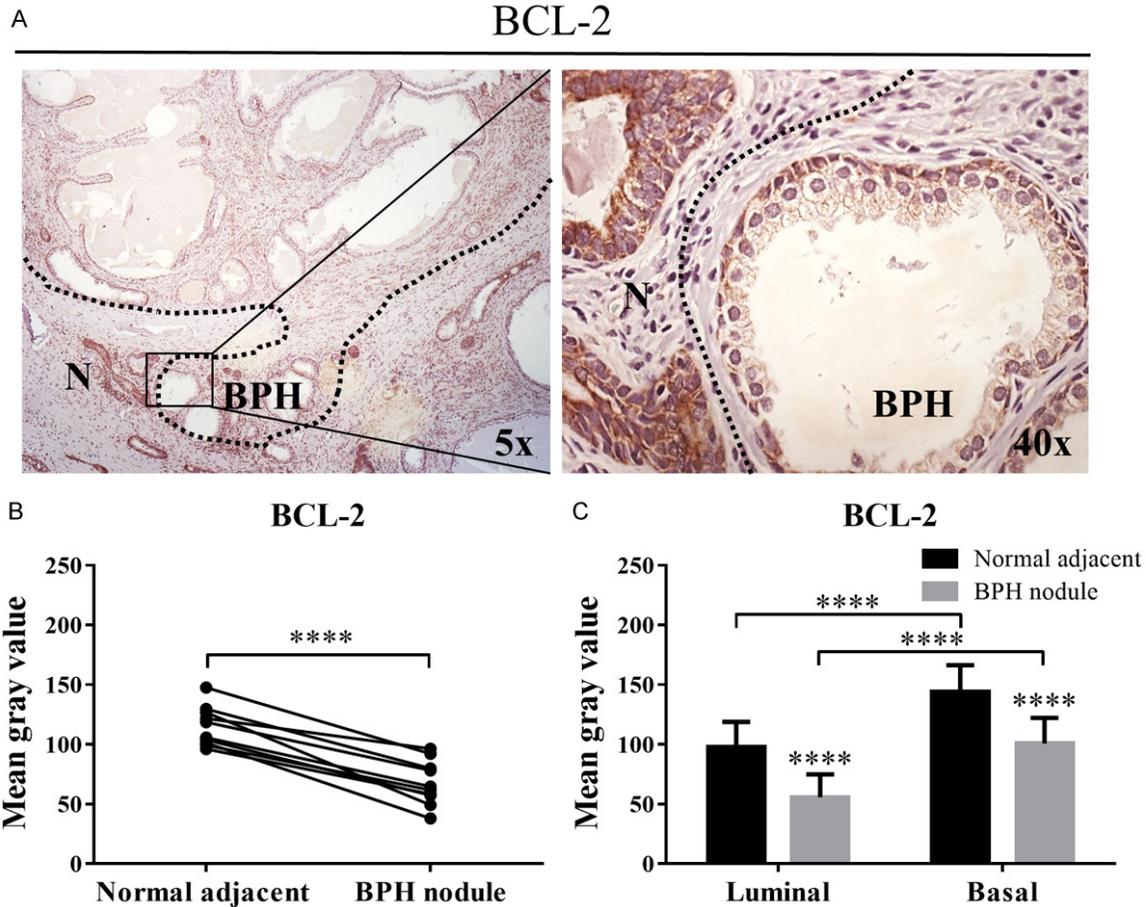


Figure 1. BCL-2 is down-regulated in BPH specimens. **A.** Representative images of BCL-2 expression detected by immunostaining in specimens from BPH patients. BPH and normal adjacent (N) areas are denoted by dotted line. Each 40× photo represented the rectangle filed in the 5× photo in the left. **B.** Quantification of BCL-2 expression in BPH nodule compared to normal adjacent prostate gland. **C.** Quantification of BCL-2 expression in luminal and basal components in BPH nodule and normal adjacent prostate gland. Immunostaining density was quantified using ImageJ and is expressed as a mean gray value for 3 individual measurements per tissue type per specimen. **** $P < 0.0001$.

Exclusion criteria: 1). Prior use of finasteride or chronic NSAIDs; 2). Peptic ulcer disease and/or asthma; 3). Previous prostate surgery for BPH (TURP or simple open prostatectomy); 4). Previous minimally invasive procedures for BPH (TUNA, laser treatments, thermotherapies such as microwave treatment); 5). Serum creatinine level greater than 2.0 mg/dl; 6). Serum ALT and/or AST level more than 1.5 times of normal upper limit; 7). History of documented bacterial prostatitis within 1 year; 8). History of active urinary tract infection within 1 month; 9). Unable to void; 10). Hypersensitivity to NSAIDs; 11). History of unstable angina, myocardial infarction, cardiac stenting or coronary artery bypass grafting within 1 year; 12). History of stroke; 13). Alcohol or drug abuse; 14). Dementia; 15). Use of lithium; 16). Use of fluconazole; 17).

Inability to understand this study and unable to provide informed consent.

Immunohistochemistry (IHC)

BCL-2 antibody was purchased from Ventana Medical Systems, Inc. (Catalog No.: 790-4464, Tucson, AZ, USA) and BCL-XL antibody from Cell Signaling Technologies (Catalog No.: 2764, Danvers, MA). IHC of formalin-fixed paraffin-embedded (FFPE) specimens was performed using rabbit ABC staining system according to the manual (sc-2018, Santa Cruz, Dallas, Texas, USA). Immunostaining was semi-quantified by Image-J software (NIH, USA) using the Colour Deconvolution [26] plugin using the “H-DAB” algorithm. For each slide, 3 areas of normal adjacent tissue and 3 areas of BPH tissue were

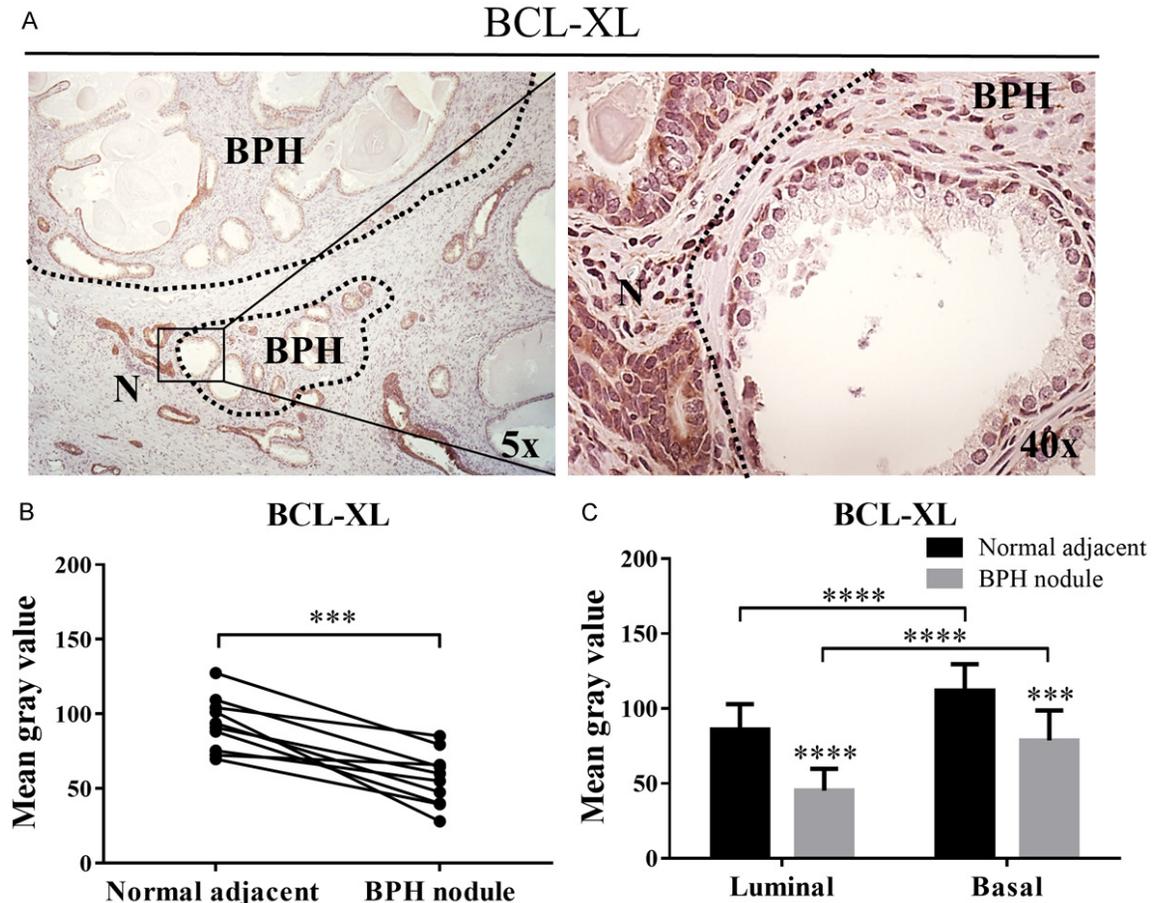


Figure 2. BCL-XL is down-regulated in BPH specimens. **A.** Representative images of BCL-XL expression detected by IHC in specimens from BPH patients. Each 40× photo represented the rectangle filled in the 5× photo in the left. **B.** Quantification of BCL-XL expression in BPH nodule compared to normal adjacent prostate gland. **C.** Quantification of BCL-XL expression in luminal and basal components in BPH nodule and normal adjacent prostate gland. Immunostaining density was quantified using ImageJ and is expressed as a mean gray value for 3 individual measurements per tissue type per specimen. *** $P < 0.001$, **** $P < 0.0001$.

measured. The mean gray value of each of the areas was calculated. Basal and luminal compartments were identified under the supervision of board certified genitourinary pathologists (AVP and RD). Immunostained sections were imaged with a Leica DM LB microscope (Leica Microsystems Inc, Bannockburn, IL, USA) equipped with an Imaging Source NII 770 camera (The Imaging Source Europe GmbH, Bremen, Germany) and NIS-Elements Documentation v 4.6 software (Nikon Instruments, Inc., Mellville, NY, USA). All tissues were examined by two board-certified genitourinary pathologists (AVP and RD) using light microscopy.

Statistical methods

All statistical graphs were generated by GraphPad Prism 7 software (GraphPad Software,

Inc. La Jolla, CA, USA). Student t test or one-way ANOVA was utilized to make statistical analysis between or among groups. A P value < 0.05 was considered to be statistically significant.

Results

BCL-2 is down-regulated in BPH specimens

To determine the expression of BCL-2 in BPH, we performed immunostaining of BCL-2 in BPH specimens from patients naïve to androgen manipulation and treated with simple prostatectomy for BPH symptoms. As shown in **Figure 1A**, BCL-2 was expressed in both luminal and basal epithelial cells and was more intense in basal cells in all specimens. BCL-2 decreased 41.23% in BPH nodule compared to normal adjacent prostate gland (**Figure 1B**, 67.64 ± 5.92 vs 115.09 ± 5.23). Next we analyzed

BCL-2 and BCL-XL are not affected by finasteride or celecoxib in BPH

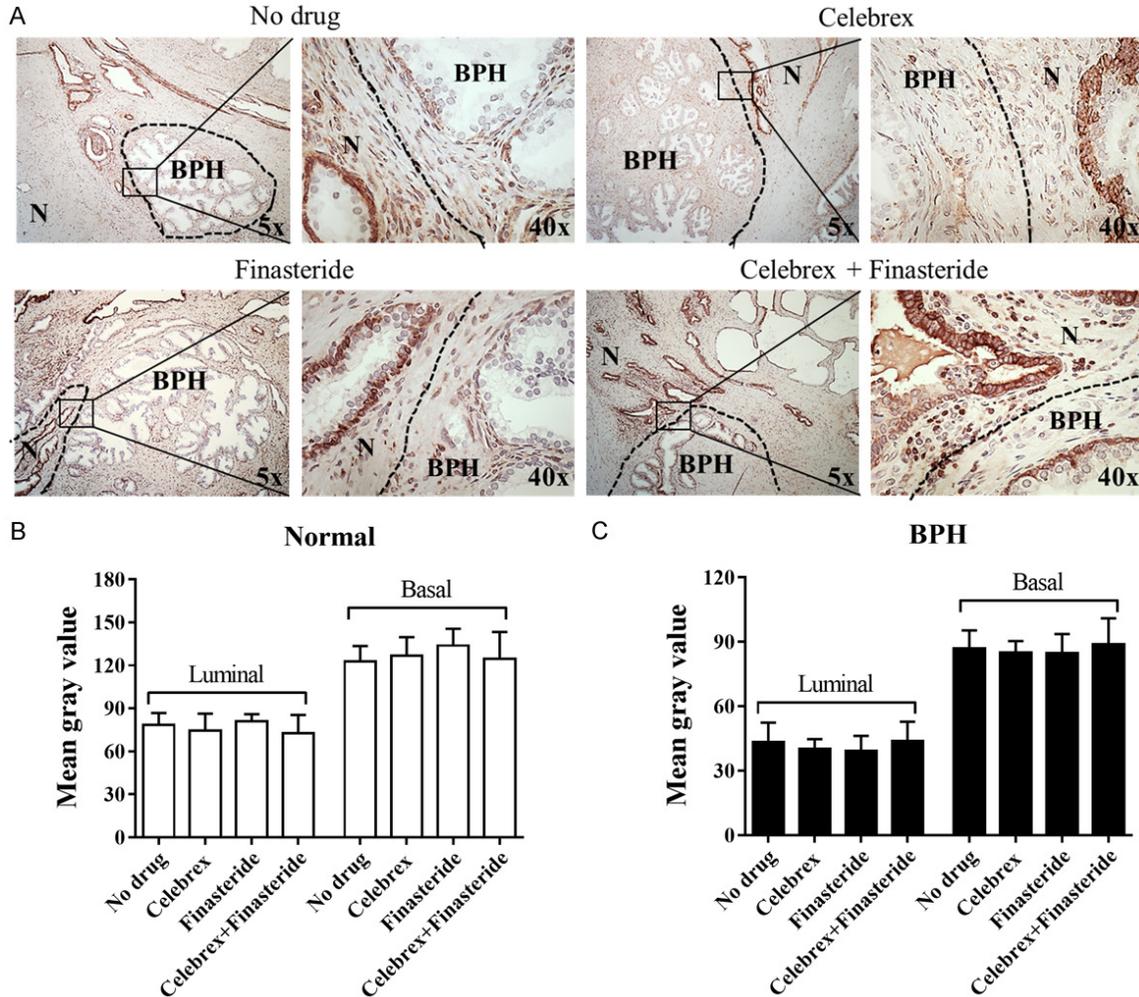


Figure 3. Finasteride and/or Celebrex do not affect BCL-2 down-regulation in BPH specimens. A. Representative images of BCL-2 expression detected by IHC in specimens from prostate cancer patients with BPH. Each 40× photo represented the rectangle filed in the 5× photo in the left. B. Quantification of BCL-2 expression in luminal and basal components in normal adjacent prostate gland. C. Quantification of BCL-2 expression in luminal and basal components in BPH nodule. Immunostaining density was quantified using ImageJ and is expressed as a mean gray value for 3 individual measurements per tissue type per specimen.

BCL-2 expression in luminal and basal cells separately for both the BPH nodule and normal adjacent prostate gland. BCL-2 showed a 43.19% decrease in luminal cells and 30.15% decrease in basal cells in BPH nodules compared to surrounding normal tissues (**Figure 1C**, 55.68 ± 6.06 vs 98.00 ± 6.624 and 100.82 ± 6.78 vs 144.4 ± 6.96 respectively).

BCL-XL is down-regulated in BPH specimens

In addition to BCL-2, another important anti-apoptosis protein is BCL-XL. We thus also performed immunostaining of BCL-XL in the same set of BPH specimens from patients naïve to androgen manipulation. Similar to the expression pattern of BCL-2, BCL-XL was stained in

both luminal and basal epithelial cells, with more intense staining in basal cells in all specimens (**Figure 2A**). BCL-XL decreased 39.33% in BPH nodules compared to normal adjacent prostate glands (**Figure 2B**, 56.59 ± 5.74 vs 93.28 ± 5.73). BCL-XL decreased 47.30% in luminal cells and 29.63% in basal cells in BPH nodule compared to surrounding normal tissues (**Figure 2C**, 45.28 ± 4.60 vs 85.91 ± 5.40 and 78.67 ± 6.37 vs 111.90 ± 5.60 respectively).

Finasteride and/or celebrex did not affect BCL-2 and BCL-XL down-regulation in BPH

To evaluate the influence of finasteride and/or celecoxib on BCL-2 and BCL-XL expression in

BCL-2 and BCL-XL are not affected by finasteride or celecoxib in BPH

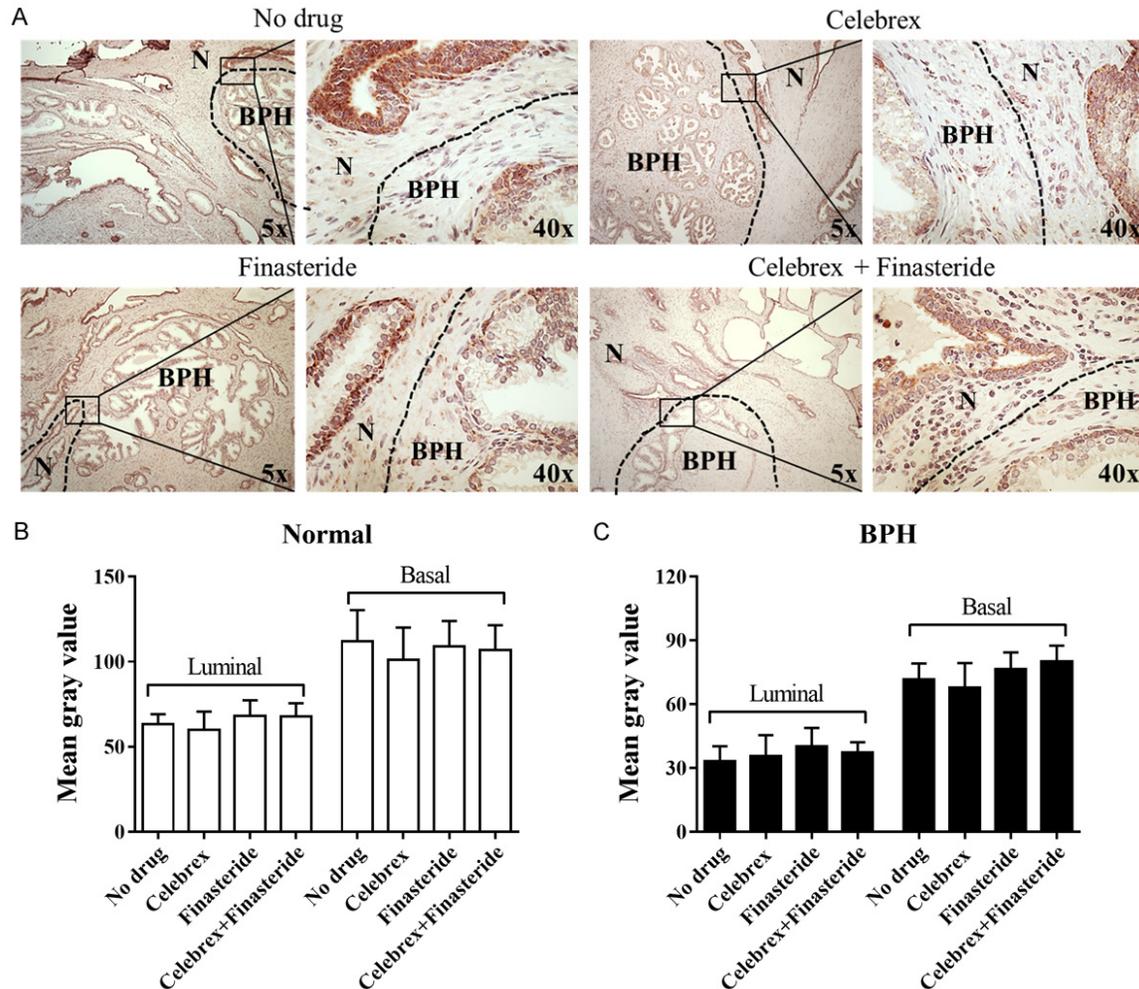


Figure 4. Finasteride and/or Celebrex do not affect BCL-XL down-regulation in BPH specimens. A. Representative images of BCL-XL expression detected by IHC in specimens from prostate cancer patients with BPH. Each 40× photo represented the rectangle filed in the 5× photo in the left. B. Quantification of BCL-XL expression in luminal and basal components in normal adjacent prostate gland. C. Quantification of BCL-XL expression in luminal and basal components in BPH nodule. Immunostaining density was quantified using ImageJ and is expressed as a mean gray value for 3 individual measurements per tissue type per specimen.

BPH, we used specimens from prostate cancer patients with BPH without prior use of chronic 5 α -reductase inhibitors and/or NSAIDs and treated them with celecoxib, finasteride, celecoxib plus finasteride or no treatment for 28 consecutive days prior to surgery. As shown previously (see **Figure 1**), BPH nodules displayed decreased immunostaining of BCL-2 compared to normal adjacent tissues (**Figure 3**). Finasteride and/or celecoxib treatment had no effect on the expression of BCL-2 in either the luminal or basal compartments (**Figure 3B, 3C**). Furthermore, BCL-XL expression was also decreased in BPH nodules compared to normal adjacent tissues (**Figure 4**). As with BCL-2, BCL-XL immunostaining was not impacted by treatment with finasteride and/or celecoxib.

Quantification of the BCL-2 and BCL-XL staining in these specimens further indicated that treatment with finasteride and/or celecoxib had no effect on BCL-2 and BCL-XL expression in BPH tissues. As shown in **Figure 5A** and **5B**, BCL-2 decreased 43.39% in BPH nodule compared to normal adjacent prostate glands (51.67 ± 1.37 vs 91.28 ± 2.78) while BCL-XL decreased 38.61% (47.43 ± 1.89 vs 77.27 ± 2.33). The expression of both BCL-2 and BCL-XL were lower in luminal epithelial cells compared to basal epithelial cells. Also, the down-regulation of BCL-2 and BCL-XL in BPH nodules was more significant in luminal epithelial cells than in basal cells. BCL-2 showed a 45.51% decrease in luminal cells and a 31.85% decrease in basal cells (41.54 ± 1.50 vs 76.24 ± 2.16 and

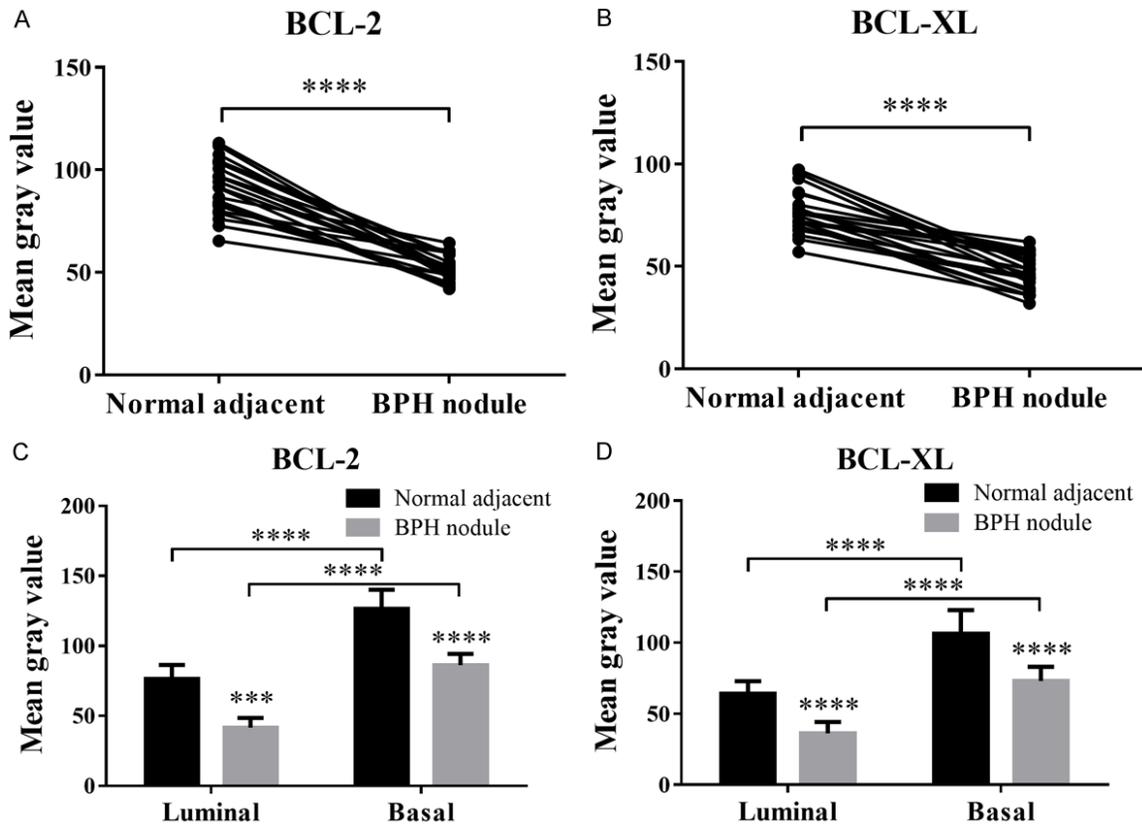


Figure 5. Overall analysis of the expression of BCL-2 and BCL-XL in prostate cancer patients with BPH. Quantification of BCL-2 (A) and BCL-XL (B) expression in BPH nodule and normal adjacent prostate gland. Quantification of BCL-2 (C) and BCL-XL (D) expression in luminal and basal components in BPH nodule and normal adjacent prostate gland. Immunostaining density was quantified using ImageJ and is expressed as a mean gray value for 3 individual measurements per tissue type per specimen. *** $P < 0.001$, **** $P < 0.0001$.

86.30 ± 1.701 vs 126.63 ± 2.86 respectively) while BCL-XL decreased 43.30% in luminal cells and 31.22% in basal cells in BPH nodule compared to surrounding normal tissues (36.31 ± 1.68 vs 64.05 ± 1.91 and 73.12 ± 2.12 vs 106.31 ± 3.53 respectively) (Figure 5C and 5D).

Discussion

The present study showed that BCL-2 and BCL-XL expression were downregulated in the epithelial compartment of BPH nodules compared to adjacent normal prostatic tissues. In both BPH and normal adjacent prostate, basal epithelial cells expressed more intense immunostaining of both BCL-2 and BCL-XL than luminal epithelial cells. Higher expression of BCL-2 in basal epithelial cells than in luminal epithelial cells was also reported by other investigators [15]. In contrast to our findings, Colombel et al., reported that Bcl-2 expression was higher in

BPH than in the transition and peripheral zones of normal prostate (mean age 43.7 years). In their study, BPH specimens contained only BPH glands with no surrounding normal adjacent tissues so it may be that the expression of BCL-2 in normal tissues adjacent to BPH is significantly higher than in the normal prostate tissues of younger men. Our finding of BCL-2 and BCL-XL downregulation in BPH nodules as compared to normal adjacent prostate tissue is novel. BPH can have both non-nodular and nodular histology as well as stromal nodules. Our studies focused on nodular BPH, which is the predominant form of BPH. This study did not address whether the expression of BCL-2 and/or BCL-XL was altered in BPH tissues as compared to normal donor prostates. Future studies will need to determine whether BCL-2 and/or BCL-XL expression in normal epithelial cells adjacent to BPH nodules are different from their expression in donor normal prostatic epithelial cells.

BCL-2 and BCL-XL are not affected by finasteride or celecoxib in BPH

Downregulation of BCL-2 and BCL-XL in the epithelial compartment of BPH nodules suggests that the major role of these proteins in BPH epithelial cells may be related to their anti-proliferation activities rather than their anti-apoptotic functions. BCL-2 family proteins were reported to inhibit cell cycle progression. The anti-proliferative activity of BCL-2 can be dissociated from its anti-apoptotic functions in some cases [27]. Further studies will be needed to determine whether BCL-2 and BCL-XL indeed affects BPH epithelial cells through its anti-proliferative activities.

Our studies showed that BCL-2 and BCL-XL downregulation in BPH nodules as compared to the normal adjacent prostatic tissue was not affected by 5 α -reductase inhibitor finasteride in a Phase II clinical trial in prostate cancer patients with BPH. Previous studies showed that androgen deprivation therapy (ADT) can upregulate BCL-2 in BPH, but not in normal and cancerous prostate [28]. Thus, the effect of ADT on BCL-2 expression in BPH is different from finasteride. Since finasteride did not affect BCL-2 downregulation in BPH nodules, the efficacy of finasteride on treatment of nodular BPH is unlikely mediated through impact on BCL-2 signaling pathways.

Our studies also showed that BCL-2 and BCL-XL downregulation in epithelial compartment of BPH nodules was not affected by the COX-2 inhibitor, celecoxib, either in the absence or presence of finasteride in prostate cancer patients with BPH. This observation suggests that BCL-2 and BCL-XL downregulation in BPH nodules was also unlikely mediated through COX-2. According to the literature, COX-2 expression is associated with BCL-2 immunostaining, particularly in atrophic prostatic lesions [29]. One possibility for the lack of any effect on BCL-2 or BCL-XL staining is that the patient cohort in our study may be low in chronic inflammation in the prostate. The downregulation of BCL-2 and BCL-XL in BPH nodules may not be related to chronic inflammation. The mechanisms of BPH downregulation in the epithelial component of BPH nodules will need to be explored in the future.

In summary, our studies showed that BCL-2 and BCL-XL expression were downregulated in both basal and luminal epithelial cells in BPH nodules as compared to normal adjacent prostatic

tissues and the downregulation was not inhibited by 5 α -reductase inhibitor finasteride and COX-2 inhibitor celecoxib. Our findings suggest that BCL-2 may play an anti-proliferative function in BPH pathogenesis and the efficacy of finasteride and COX-2 inhibitor in BPH may not act through BCL-2 family proteins. Future studies will be needed to define the role of BCL-2 and BCL-XL in nodular BPH and whether BCL-2 and BCL-XL exerts different activities in different types of BPH.

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Disclosure of conflict of interest

None.

Abbreviations

BPH, benign prostatic hyperplasia; BCL-2, B-cell lymphoma 2; BCL-XL, B-cell lymphoma-extra large; LUTS, lower urinary tract symptoms; AR, androgen receptor; ER, estrogen receptor; TNF- α , tumor necrosis factor alpha; TGF- β 1, transforming growth factor beta 1; IHC, immunohistochemistry; SD, sexual dysfunctions; BPE, benign prostatic enlargement; BPO, benign prostatic obstruction; ECM, extracellular matrix; IPSS, international prostate symptom score; IL-8, interleukin-8; IL-6, interleukin-6; COX-2, cyclooxygenase 2.

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