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Original Article

Alterations of COX-2, HER-2/neu and E-Cadherin protein expression in the prostatic adenocarcinoma: preliminary findings

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Abstract: Altered expression of the pro-inflammatory enzyme cyclooxygenase (COX)-2, E-Cadherin cell-cell adhesion protein and Human epidermal growth factor receptor 2 (HER-2/neu, a proto-oncogene) are involved in the pathogenesis of several cancers including the prostatic adenocarcinoma (PRCa). However, to date the results of the previous studies in this neoplasm are controversial, and the relationships among expression of these molecules in benign prostatic hyperplasia (BPH) and PRCa are mostly unknown. We hypothesize that "there are alterations of COX-2, HER-2/neu and E-Cadherin protein expression in PRCa". We carried out this study to test our hypothesis and to assess the relationships among these molecules both in PRCa and BPH. We used immunohistochemistry to evaluate the expression of these proteins in the tissue specimens of both BPH (27 cases) and PRCa (45 cases). Immunohistochemical staining patterns verified over-expression of COX-2 and HER-2/neu proteins in PRCa as compared to BPH. Alternatively, there was an aberrant (reduced) E-Cadherin protein expression in PRCa. There were weak positive correlations between COX-2 versus HER-2/neu expression. A weak negative correlation was noted between COX-2 and E-Cadherin expression. In conclusion, there were alterations of COX-2, HER-2/neu and E-Cadherin proteins in PRCa. The molecular alterations of the relevant genes and the therapeutic ramifications (the development of selective inhibitors to COX-2 and HER-2/neu) of these preliminary findings are open to further investigations.

Keywords: COX-2, HER-2/neu, E-Cadherin, prostate adenocarcinoma, prostatic hyperplasia

Introduction

Benign prostatic hyperplasia (BPH) is encountered in the majority of aging men. Prostate cancer (PRCa) is a common malignancy in males and represents one of the most common causes of cancer death among them. Distant metastases are life-threatening events in patients with PRCa. Several molecular pathways and candidate genes seem to be involved in PRCa, such as Cyclooxygenase-2 (COX-2), Human epidermal growth factor receptor 2 (HER-2/neu, or ErbB) proto-oncogene, and E-Cadherin is Ca^{2+}-dependent cell-cell adhesion molecule [1].

Inflammation contributes to the pathogenesis of BPH and PRCa by enhancing the rate of cell proliferation. Cyclooxygenase (COX) is a prostaglandins endoperoxidase synthase that converts arachidonic acids to prostaglandins. COX exists in two isoforms, namely COX-1 and COX-2. The former is expressed constitutively in many tissues, and it contributes to normal cellular physiological functions. COX-2 is a pro-inflammatory molecule inducible by several factors such as tumor promoters, cytokines, oncogenes, carcinogens mitogens, and growth factors [2-4]. COX-2 enzyme converts arachidonic acid to prostaglandins. COX-2 mRNA and protein are generally undetectable in most tissues. COX-2 protein is up-regulated throughout the entire tumorigenic process, from hyperplasia to metastatic disease. In support, COX-2 protein overexpression is a common finding in the pathogenesis of several cancers such as gas-

tric carcinomas [5]. Moreover, the antineoplastic effects of non-steroidal anti-inflammatory drugs such as aspirin are related their suppressive effects on prostaglandin synthesis through inhibition of COX-2. In the prostate, COX-2 is absent in the normal prostatic epithelium. In BPH, COX-2 plays several roles in the inflammatory process which contributes to the development of both hyperplastic changes and post-inflammatory atrophy of the prostate [6]. COX-2 protein is overexpressed in the lesional tissues of these conditions, and it plays pro-inflammatory and proliferative actions on the prostatic epithelial cells. These COX-2 dependent proliferative actions are due to overexpression of Bcl-2 resulting in increased cell survival [7, 8]. In PRCa, the sequence variations in the *COX-2* gene have been reported to affect the risk of developing cancer [9].

HER-2/neu is a family of receptor tyrosine kinases [10]. *Her-2/neu* gene (a proto-oncogene) is a member of the EGF receptor family located in chromosome 17q21-22. It encodes a transmembrane receptor protein with tyrosine kinase activity. It forms heterodimers by binding to specific ligands, enhancing cell signaling and assisting in cell growth and differentiation in prostate cells [1]. To date, HER-2/neu protein expression values have been examined in the normal prostatic epithelium, BPH and PRCa by some studies and the results are contradictory (ranging from none to 100%) [11-17]. Carles and his colleagues examined HER-2/neu expression by immunohistological methods. Most of the androgen-independent bone marrow metastatic PRCa were HER-2/neu positive whereas some androgen-dependent prostate biopsies overexpressed HER-2/neu. No genomic amplification of the HER-2/neu locus was detected in any of the metastatic prostate tumors on further fluorescence in situ hybridization [16].

The epithelial-mesenchymal transition (EMT) is an essential step in tumor progression, where the invasive malignant cells switch from epithelial to mesenchymal phenotype. During this process, there is a decrease or loss of the expression of some adhesion molecules such as E-Cadherin which is essential for the regulation of cell-cell adhesion, EMT, cancer cell migration and tissue invasion. EMT is critical for acquiring invasive and metastatic phenotypes in PRCa cells. In support, the reduced expression of E-Cadherin is associated with the development and progression in PRCa [18]. Moreover, the enhancement of E-Cadherin expression in PRCa cells by using phytochemicals is associated with the prevention of EMT and ameliorated invasiveness of PRCa tumor cells [19]. To date, the expression patterns of E-Cadherin protein in PRCa are still controversial. Some studies revealed reduced expression of E-Cadherin in PRCa [20-23] whereas other investigations have not demonstrated this observation. Abdelrahman and his colleagues examined E-Cadherin protein expression in PRCa and BPH. They found an association among aberrant (reduced) E-Cadherin expression and several parameters such as high pre-treatment PSA level, Gleason score ≥7, advanced tumor stage, lymph nodal and distant metastases [24]. Ross and his colleagues examined E-Cadherin protein expression in the needle biopsy specimens of 56 PRCa, using immunohistochemistry and image analyzer. The authors found that the 51% mean positive area E-Cadherin expression in PRCa was significantly less than the 76% expression level for 15 healthy control prostate tissues. Reduced E-Cadherin protein expression was seen in high-grade versus low-grade PRCa. The 44% E-Cadherin expression level in patients with metastases was lower than the 52% level in the non-metastatic cases [23].

In general, COX-2, HER-2/neu and E-Cadherin proteins seem to be implicated in the development of BPH and PRCa [1]. Here we hypothesize that "there are alterations of COX-2, HER-2/neu and E-Cadherin protein expression in PRCa". To date, although some studies have examined the expression pattern of these proteins in PRCa, the results are mostly contradictory. Moreover, the relationships among the alterations of these proteins are still largely unknown. The goals of our study include i) evaluation of the expression patterns of COX-2, HER-2/neu and E-Cadherin molecules in BPH and PRCa, ii) evaluation of the relationships among the expression values of these molecules in PRCa. To achieve our goals, we used immunohistochemical staining methods to examine the expression of these proteins in the normal prostatic epithelium, BPH and PRCa.

Materials and methods

Patients

The study was carried out at King Khalid University, in the period between April 2017 and April 2018. A total of 45 cases with PRCa and 27 cases with BPH were included. The clinicopathological data were obtained from the Pathology reports. The Institutional Review Board approved the study design at King Khalid University. The formalin fixed paraffin embedded tissue specimens were derived from the archival material of the Pathology and Clinical Pathology Laboratories of King Khalid University-affiliated hospital. Full pathologic features were recorded.

Immunohistochemistry

The presence of COX-2, HER-2/neu, and E-Cadherin proteins were examined using Avidin-biotin immunoperoxidase complex following other groups [25-31]. Briefly, 4-μm thick tissue sections mounted on glass slides were deparaffinized and rehydrated through graded alcohols to water. Incubation with 3% hydrogen peroxide in methanol for 5 min was used to quench endogenous peroxidase activity. Sections were then immersed in the retrieval solution (10 mM sodium citrate buffer, pH 7.3) and subjected to heat-induced antigen retrieval for 10 minutes. The slides, in plastic Coplin jars containing retrieval solution, were microwaved in a microwave set at high (~700 watts). Non-specific protein binding was blocked with 10 minutes exposure to 10% normal goat serum.

Sections were then incubated with rabbit polyclonal antibody raised against COX-2 (at the dilution of 1:200 dilution, clone number, CX-294, Aligent-Dako, Montpellier, France), HER-2/neu (at the dilution of 1:200 dilution, clone number, polyclonal, Aligent-Dako , Montpellier, France) and E-Cadherin (at the dilution of 1:200 dilution, clone number, NCH-38, Aligent-Dako, Montpellier, France) for 30 min at room temperature. A detection system was used according to the manufacturer instructions (Universal LSAB2 Kit/HRP, Rabbit/Mouse (10 × 11 mL Link + 10 × 11 mL Streptavidin/HRP, K0675, Aligent-Dako, Montpellier, France). Sections were next treated with peroxidase-labeled streptavidin for 30 min at room temperature and incubated with 14-diaminobenzidine and 0.06% H_2O_2 for 5 min. They were counterstained with Mayer hematoxylin, dehydrated in alcohol, cleared in xylene and cover-slipped. Three Pathologists independently examined and evaluated the (Dr. Adel Osman Musalam, Dr. Mohamed Mohamed Osman and Dr. Amany Omar Elrefaie).

Positive control

Sections from colon adenocarcinoma (for COX-2) and breast ductal carcinoma (known to be HER-2/neu and E-Cadherin positive for both HER-2/neu and E-Cadherin) was included as a positive control. The positivity for COX-2 was identified as brownish cytoplasmic and membranous staining of tumor cells. The positivity for HER-2/neu and E-Cadherin appeared as membranous staining.

Negative control

Additional sections were stained in parallel with the omission of the primary antibodies as a negative control. The positive and negative controls were positive and negative, respectively indicating the validity of our results. All slides were evaluated without any knowledge of the patient's information and clinical reports.

Evaluation of COX-2 staining

Immunohistochemical evaluation of COX-2 was performed following other groups [32]. Briefly, the intensity of staining was recorded as on a scale from 0 to 3, such that 0 was considered to be negative, 1+ weak staining, 2+ intermediate staining and 3+ strong staining [32]. The percentage of positive cells was evaluated by counting at least 5 different areas at a magnification of ×400. The resulting immunoreactivity score was determined by multiplying staining intensity by the percentage of positive cells. The results were scored as negative (0-1), weak (2-3), moderate (4-6), or strong. The percentage of positive cells was evaluated [25-27].

Evaluation of HER-2/neu staining

The presence of circumferential membranous staining indicates HER-2/neu positive reactivity and was scored as follows: 0, no staining or membrane staining observed in less than 10% of tumor cells; 1+, partial membrane staining in more than 10% of tumor cells; 2+, circumferen-

Table 1. Clinicopathologic features of the patients with benign prostatic hyperplasia and prostatic adenocarcinoma

Clinicopathological features	Number of cases
I-Benign prostatic hyperplasia (n = 27)	
Age group	
Mean ± Standard Error of Mean	71.50 ± 9.72
≤ 68 years	9/27 (33%)
> 68 years	18/27 (64%)
PSA (ng/mL)	
≤ 4.0	7/27 (25%)
> 4.0	20/27 (75%)
II-Prostatic adenocarcinoma (n=45)	
Age group	
Mean ± Standard Deviation	72.4 ± 8.5
≤ 68 years	13/45 (29%)
> 68 years	32/45 (71%)
P PSA (ng/mL)	
≤ 4.0	4/45 (9%)
> 4.0	41/45 (91%)
Grade/differentiation	
Grade: 4-5 (Well differentiated adenocarcinoma)	0/45 (0.0%)
Grade: 6-7 (Moderately differentiated adenocarcinoma)	6/45 (16%)
Grade: 8-10 (Poorly differentiated adenocarcinoma)	39/45
Gleason score	
Gleason score (6)	4/45 (9%)
Gleason score (7)	2/45 (4%)
Gleason score (8-10)	39/45 (87%)
Perineural invasion	5/45 (11%)
Tumor position	
Left lobe	25/45 (56%)
Right lobe	20/45 (44%)
Metastatic lesions	1/45 (2%)

PSA: Prostatic specific antigen. There was a weak insignificant correlation between age of the patients with PRCa and the total serum PSA [Pearson (r) = 0.271, $P = 0.086$]. Similarly, in patients with BPH, we found a weak insignificant correlation between age and total PSA levels in the serum [Pearson (r) = 0.054, $P = 0.791$].

tial weak to moderate staining observed in more than 10% of tumor cells and 3+, strong circumferential membrane staining observed in more than 10% of tumor cells. Positive scores included Score of 3+ [28].

Evaluation of E-Cadherin staining

Membranous staining was recorded as reduced/low (aberrant) or high (normal) following other groups [33]. Membranous (cell membrane) staining was scored (3+, 2+, 1+, 0): 0) lack of staining, no reactivity in < 10% of the membrane 1+) faint/weak discontinuous reactivity in 10-39% of the membranes 2+) moderate, positive discontinuous reactivity in 40-90% of the membranes and 3+) strong diffuse continuous membranous reactivity (honeycomb-like pattern) [29-31].

Evaluation of PSA staining

Evaluation of PSA staining was carried out following other groups [25-27]. The staining intensity for PSA was scored as follows: 0-negative, 1-weak, 2-moderately positive, and 3-strongly positive staining.

Statistical analysis

Statistical analysis was performed with SAS software version 6.2 (SAS Institute, Cary, NC, USA). Proportions, median, arithmetic mean and standard deviation were used to present the data. Student “t” test, Mann-Whitney test, chi-squared and Fishers Exact test were used as tests of significance at 5% level. Pearson correlation was used.

Results

Patients

The specimen types included transurethral resection (61/72, 84.7%), transrectal resection (8/72, 11.1%) and radical prostatectomy (3/72, 4.2%) specimens. The details of the physical examination, PSA levels were obtained from the medical records. None of the patients received preoperative hormonal therapy or radiotherapy. The age of the patients ranged from 46 years to 103 years with an average of 71.50 ± 9.72 years and a median of 72.07 years. The study included specimens from 45 males with PRCa (average age of 72.4 ± 8.5 years) and 27 patients with BPH (average age of 71.5 ± 11.6

Table 2. Summary of alterations of COX-2, HER-2/neu and E-Cadherin proteins expression patterns in benign prostatic hyperplasia and prostatic adenocarcinoma

Proteins	Benign prostatic hyperplasia (number = 27)	Prostatic adenocarcinoma (number = 45)
COX-2 protein overexpression	Absent (0/27, 0.0%)	Present (18/45, 40%)
HER-2/neu protein overexpression (3+)	Absent (0/27, 0.0%)	Present (17/45, 37%)
E-Cadherin protein aberrant (reduced) expression	Absent (0/27, 0.0%)	Present (4/45, 9%)

The alterations in prostatic adenocarcinoma included overexpression of COX-2 and HER-2/neu proteins as well as aberrant (reduced) expression of E-Cadherin.

Table 3. COX-2 and HER-2/neu protein expression both in benign prostatic hyperplasia and prostatic adenocarcinoma

Protein expression	Benign prostatic hyperplasia (number = 27 cases)	Prostatic adenocarcinoma (number = 45 cases)
COX-2 protein expression		
Absent expression	8/27 (29%)	03/45 (6%)
Faint (weak) expression	19/27 (71%)	24/45 (54%)
Strong (overexpression)	0/27 (0.0%)	18/45 (40%)
Staining intensity (Mean ± SEM)	1.2 ± 0.1	2.4 ± 0.1
Immunoreactivity scores (IRS)	2.1 ± 0.1	7.7 ± 0.6
HER-2/neu protein expression		
Score +0 (interpretation negative)	10/27 (37%)	0.0/45 (0.0%)
Score +1 (interpretation negative)	17/27 (63%)	09/45 (20%)
Score +2 (interpretation negative)	0/27 (0.0%)	09/45 (20%)
Score +3 (interpretation positive)	0/27 (0.0%)	17/45 (37%)
Staining intensity (Mean ± SEM)	2.0 ± 0.1	0.6 ± 0.1

years). The age difference among the patients with PRCa and BPH was not statistically significant (t = 0.370, *P* = 0.712). The Gleason score ranged from 6 to 10. The mean Gleason score was 6.4, with the majority of PRCa cases were of Gleason score of 9 followed by a score of 8. The total serum PSA was statistically significantly high in the patients with PRCa as compared to BPH (Z = 4.357, P = 0.001, Mann-Whitney test of significance). The average value of PSA in the serum of the patients with PRCa (55.875 ± 47.199 ng/mL) was significantly higher (Z = 4.357, *P* = 0.001, Mann-Whitney test of significance) when compared to that of patients with BPH (13.183 ± 15.11 ng/mL). There was a weak insignificant correlation between age of the patients with PRCa and the total serum PSA [Pearson (r) = 0.271, *P* = 0.086]. Similarly, in patients with BPH, we found a weak insignificant correlation between age and total PSA levels in the serum [Pearson (r) = 0.054, *P* = 0.791]. A summary of these findings is shown in **Table 1**.

Overexpression of COX-2 protein in PRCa

COX-2 protein expression was absent in the normal prostatic epithelium. The expression was faint (19 cases) or completely absent (8 cases) in BPH. Alternatively, strong COX-2 protein overexpression was observed in 18 cases of PRCa. The expression was faint (24 cases) or completely absent (3 cases) in PRCa. The mean expression values were statistically significantly high in PRCa (SI: 2.4 ± 0.1 and IRS: 7.7 ± 0.6 as compared to BPH (SI: 1.2 ± 0.1 and IRS: 2.1 ± 0.1, *P* = 0001 and *P* = 0001). There was no significant correlation between serum total PSA and COX-2/neu protein expression in PRCa [Pearson (r) = -0.058, P = 0.721] or BPH [Pearson (r) = - 0.051, P = 0.799]. Similarly, there was no significant correlation between serum free PSA and COX-2 protein expression in PRCa [Pearson (r) = -0.352, P = 0.152]. A summary of these findings is shown in **Tables 2**, **3**; **Figures 1**, **2**.

Overexpression of HER-2/neu protein in PRCa

HER-2/neu protein expression was absent (score 0) in the normal prostatic epithelium and was faint (1+ score in 17 BPH cases) or completely absent (score 0 in 10 cases) in BPH. A strong protein expression (overexpression, 3+ score) was observed in 17 cases of PRCa. The remaining cases of PRCa showed faint (1+ score in 9 cases), moderate (2+ score in 9 cases) or completely absent (score 0 in cases).

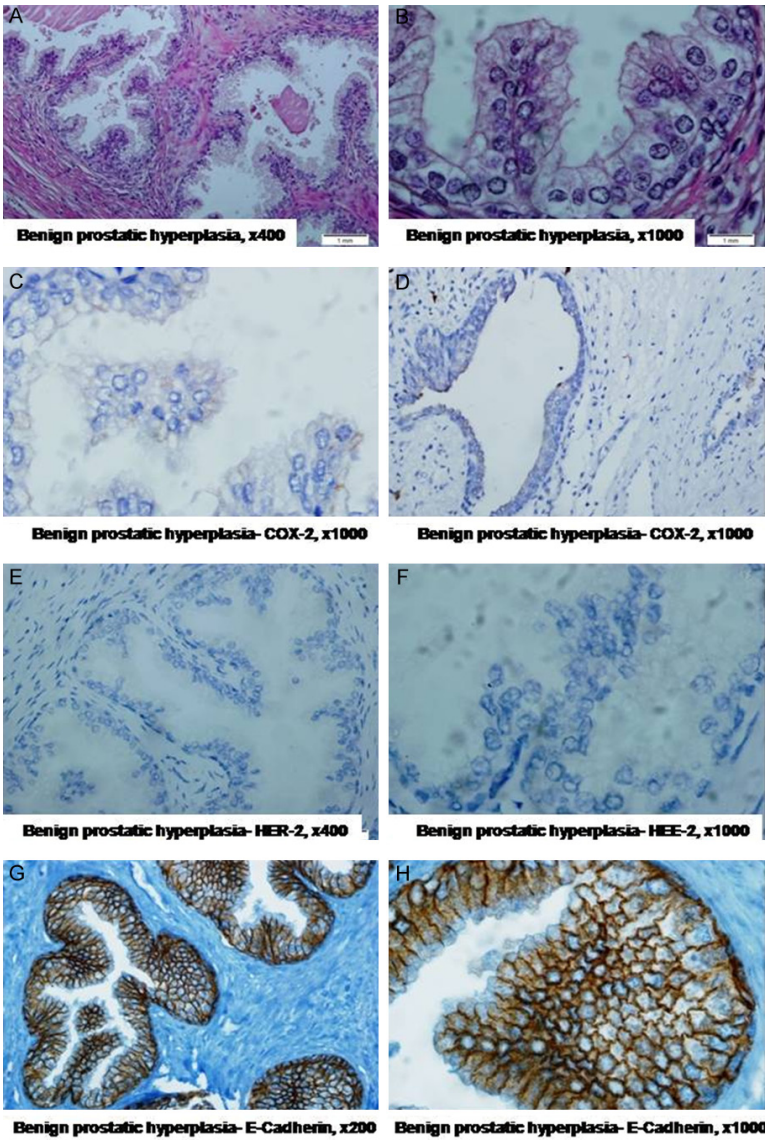

Figure 1. COX-2, HER-2 and E-Cadherin protein expression in the benign prostatic hyperplasia. A, B: Histologically, there is hyperplasia of both stromal tissue to the left and glandular elements to the right. The glands are large in size with more prominent papillary infoldings. The lining cells have preserved tall columnar arrangement near the gland lumina is preserved. There are several pink corpora amylacea in gland lumens. The basal cells are apparent. C, D: The glandular and stromal cells of benign prostatic hyperplasia show absent or focally faint cytoplasmic staining for COX-2. E, F: The glandular and stromal cells of benign prostatic hyperplasia show absent expression of HER-2/neu. G, H: A strong diffuse, continuous membranous staining for E-Cadherin protein is seen in the luminal cells lining the enlarged glands. The stromal and inflammatory cells are negative for E-Cadherin protein expression.

The mean expression values of HER-2/neu were statistically significant high in PRCa (SI: 2.0 ± 0.1) as compared to BPH (SI: 0.6 ± 0.1, *P* = 0001). There was no significant correlation between serum total PSA and HER-2/neu protein expression in PRCa [Pearson (r) = -0.009, P = 0.956] or BPH [Pearson (r) = -0.242, P = 0.225]. Similarly, there was no significant correlation between serum free PSA and HER-2/neu protein expression in PRCa [Pearson (r) = -0.079, P = 0.754]. A summary of these findings is shown in **Tables 2**, **3**; **Figures 1**, **3**.

Aberrant (reduced) expression of E-Cadherin protein in PRCa

Normal (strong membranous staining) E-Cadherin protein expression was seen in the normal prostatic epithelium, BPH and the majority of PRCa. E-Cadherin protein expression was strong (3+ score in 25 cases) or moderate (score 2+, in 2 cases) in BPH. We found E-Cadherin protein aberrant (reduced) expression (0 score) in 4 cases of PRCa. E-Cadherin expression was strong (3+ score in 38 cases), moderate (2+ score in 3 cases) in the remaining cases of PRCa. The mean expression values were almost similar both in PRCa (SI: 2.9 ± 0.05) as compared to BPH (SI: 2.6 ± 0.1, *P* = 0.17). There was no significant correlation between serum total PSA and E-Cadherin protein expression in PRCa [Pearson (r) = - 0.2909, P = 0.070] or BPH [Pearson (r) = -0.104, P = 0.606]. Similarly, there was no significant correlation between serum free PSA and E-Cadherin protein expression in PRCa [Pearson (r) = -0.2909, P = 0.070]. A summary of these findings is shown in **Tables 2**, **4**; **Figures 1**, **4**.

Strong PSA protein expression in the benign prostatic epithelium, BPH and PRCa

We found normal (strong cytoplasmic staining) PSA protein expression in the normal prostatic epithelium. The majority of BPH cases had

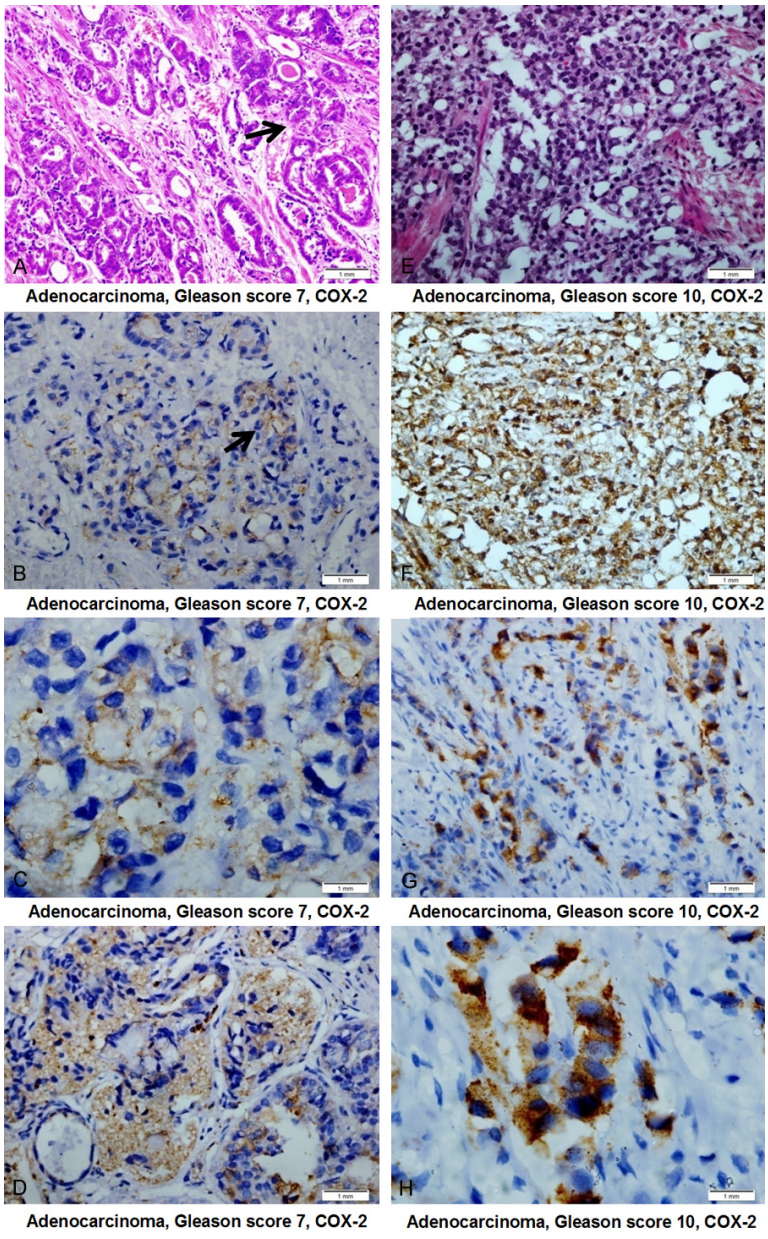

Figure 2. COX-2 protein expression in the prostatic adenocarcinoma. A, B: On histology, prostatic adenocarcinoma Gleason score 7 (left panel) is composed of malignant small and medium-sized acini with irregular outlines and variation in acinar spacing, size, and shape. The acini are haphazardly and randomly scattered in the stoma. Some abortive acini with primitive lumens are seen (arrow). B-D: The malignant cells show mild to moderate cytoplasmic staining of COX-2 protein. E: Histologically, prostatic adenocarcinoma Gleason score 10 (right panel) is composed of malignant cells arranged in single cells, cords, sheets, and fused closely packed acini. F-H: The neoplastic cells show strong and diffuse cytoplasmic staining of COX-2 protein.

strong PSA (3+ in 24 cases), followed by a weak expression (1+ in 3 cases). Similarly, the majority of PRCa cases had strong PSA (3+, 35 cases), followed by a moderate expression (2+, 10 cases). The distribution of PSA tissue marker scores among malignant and benign cases was not statistically different (Pearson Chi-square = 2.108, P = 0.055). The mean expression values were almost similar both in PRCa (SI: 2.8 ± 0.06) as compared to BPH (SI: 2.7 ± 0.06, *P* = 0.23). There was no significant correlation between serum free PSA and PSA protein expression in PRCa (F = 0.2413, P = 0.123). A summary of these findings is shown in **Tables 2**, **4**; **Figures 1**, **5**.

Correlation among COX-2 and HER-2/neu protein expression in PRCa

We found some weak correlations among the expression values of COX-2, E-Cadherin and HER-2/neu proteins in PRCa. There was a positive correlation between COX-2 and HER-2/neu protein. Alternatively, the overexpression of COX-2 in PRCa was found to be associated with aberrant/reduced expression of E-Cadherin. A summary of these findings is shown in **Table 5**.

Discussion

PRCa is common cancer worldwide, and as such, it is responsible for nearly 6% of all male cancer deaths. At the time of diagnosis, about 95% of PRCa patients have either advanced local disease or metastatic deposits, and therefore hormonal therapy or chemotherapy have little or even no impact on patient survival. Therefore, new therapeutic targets for PRCa are strongly required [34, 35]. It is well known that HER-2/neu, COX-2, and E-Cadherin molecules contribute to the pathogenesis of several malignant tumors including prostate, breast, colorectal and gas-

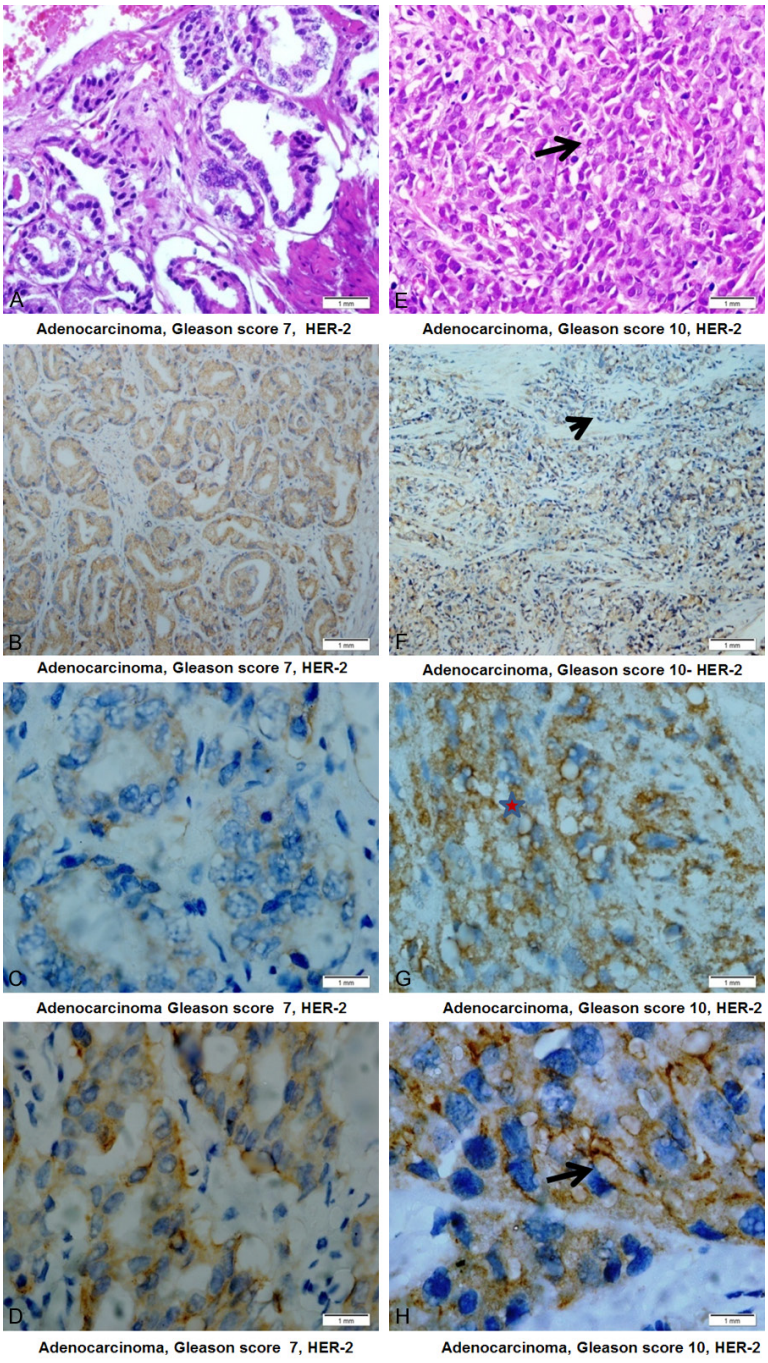

Figure 3. HER-2/neu protein expression in the prostatic adenocarcinoma. (A) The neoplastic cells of the prostatic adenocarcinoma Gleason score 7 (left panel) are arranged in medium-sized acini with variations in shape and spacing. (B-D) The neoplastic cells of shows mild cytoplasmic staining of HER-2/neu. No detectable membranous staining was seen. (E, F) The prostatic adenocarcinoma Gleason score 10 (right panel) is composed of patently malignant cells with pleomorphic hyperchromatic nuclei and pink cytoplasm arranged in solid (arrow), cording (arrowhead) and patternless patterns, amid desmoplastic stroma. (E-H) The malignant cells show strong and diffuse cytoplasmic (G: 5 point star) and membranous (H: arrow) staining of HER-2/neu protein.

tric cancers. The development of selective inhibitors to COX-2, E-Cadherin, and HER-2/neu, may be beneficial chemotherapeutic agents in malignant tumors including prostatic adenocarcinoma. Although several studies have examined COX-2, HER-2/neu and E-Cadherin protein expression in PRCa, there is no available data that bears directly on the correlations among the expression of these molecules in BPH and PRCa. Here we hypothesize that that "there are alterations of COX-2, HER-2/neu and E-Cadherin protein expression in PRCa". We carried out this preliminary investigation to test our hypothesis and to fill this existing gap in the literature. Our preliminary observations include (i) overexpression of COX-2 and HER-2/neu proteins in PRCa, (ii) aberrant (reduced) E-Cadherin protein expression in PRCa and (iii) correlations among the expression of these proteins.

Overexpression of COX-2 protein in PRCa

In agreement with the previous studies, we found COX-2 protein overexpression in PRCa whereas it was absent or weakly expressed both in normal prostatic epithelium and in BPH [36-38]. Zang and his colleagues examined the expression values of COX-2 both in BPH and PRCa using immunohistochemical staining methods and western blot. At the immunohistochemical level, COX-2 protein expression was seen in both BPH and PRCa. Similarly, Western blot analysis revealed COX-2 protein overexpression in PRCa as

Table 4. E-Cadherin and PSA protein expression in benign prostatic hyperplasia and prostatic adenocarcinoma

E-Cadherin protein expression (SI)	Benign prostatic hyperplasia (number = 27 cases)	Prostatic adenocarcinoma (number = 45 cases)
Score 0 (negative)	0.0/27 (0.0%)	04/45 (9%)
Score +1 (mild expression)	0.0/27 (0.0%)	0.0/45 (0.0%)
Score +2 (moderate expression)	2/27 (7%)	03/45 (7%)
Score +3 (strong expression)	25/27 (93%)	38/45 (84%)
Staining intensity (Mean ± SD)	2.6 ± 0.1	2.9 ± 0.05
PSA protein expression (SI)		
Score 0 (negative)	0.0/27 (0.0%)	0.0/45 (0.0%)
Score +1 (mild expression)	03/27 (11%)	0.0/45 (0.0%)
Score +2 (moderate expression)	0.0/27 (0.0%)	10/45 (22%)
Score +3 (strong expression)	24/27 (89%)	35/45 (78%)
Staining intensity (Mean ± SD)	2.8 ± 0.06	2.7 ± 0.06

compared to BPH [36, 39]. Yoshimura and his colleagues examined the expression of COX-2 protein in PRCa using reverse transcriptase-polymerase chain reaction and immunohistological staining methods. They found strong COX-2 protein expression in PRCa whereas the expression was faint both in BPH and in the normal prostatic epithelium [38]. Similarly, reverse transcriptase-polymerase chain reaction data revealed enhanced expression of COX-2 protein in PRCa versus weak expression in BPH [38]. Taken together, our findings along with those of others indicate that the malignant cells of the human PRCa over generate COX-2 protein. These findings also support an essential role for COX-2 protein in the proliferation of the neoplastic cells in PRCa.

The role of COX-2 in the development of PRCa is supported by several observations. The administration of combination therapy of rofecoxib (COX-2 inhibitor) together with finasteride was associated with an improvement in International Prostate Symptom Score as compared to finasteride treatment alone [8]. Induction of COX-2 occurs in response to several stimuli [3, 4, 40] and are mediated via tyrosine kinase, protein kinase C or/and protein kinase A signal transduction pathways [40]. The prostaglandin biosynthetic pathways are linked with the activation of MAPK signaling cascades [41]. The level of activated MAPK increases with increasing Gleason score and tumor stage in PRCa [42]. In general, it is tempting to propose that anti-COX-2 agents (COX-2 inhibitors) may represent promising therapeutic strategies in the treatment of BPH and PRCa.

Overexpression of HER-2/neu protein in PRCa

In agreement with other studies, we found overexpression of HER-2/neu in PRCa and lack of its expression both in normal prostatic epithelium and BPH [12-17]. Bai and his colleagues reported HER-2/neu protein expression in both in the androgen-dependent and independent groups. HER-2/neu expression was significantly higher in the androgen-independent group, cases with Gleason score > 7 group and the clinical stage > T2 group [17]. Gu and his colleagues examined the expression pattern of HER-2/neu protein in radical prostatectomy tissue specimens from patients with PRCa and BPH. All cases of PRCa showed positive immunostaining of variable degree while two cases only of BPH specimens showed positive HER-2/neu staining [13]. We found two staining patterns (cytoplasmic and membranous reactivity) in our series. The cytoplasmic reactivity may be reasoned to the binding of the antibodies used to the cytoplasmic portion of the HER-2/neu transmembrane protein. The presence of HER-2/neu protein overexpression in PRCa as compared to its lack in the normal prostatic epithelium and BPH suggest that HER-2/neu oncoprotein contributes to the pathogenesis of PRCa. Our findings also suggest possible therapeutic values (selective HER-2/neu inhibitors) for HER-2/neu as a treatment option in PRCa.

Aberrant (reduced) expression of E-Cadherin in PRCa

E-Cadherin is a cell adhesion molecule associated with cell-cell and cell-matrix interaction, leukocyte function, and tumor invasion and metastases [23]. It is an “invasion suppressor” gene. Here, we found homogenous uniform diffuse and strong E-Cadherin protein expression both in the benign prostatic epithelium, BPH and in most cases of PRCa. We found few cases of PRCa with an aberrant (reduced) expression of E-Cadherin. Our finding concurs with previous investigations in human [20, 22, 23, 43]

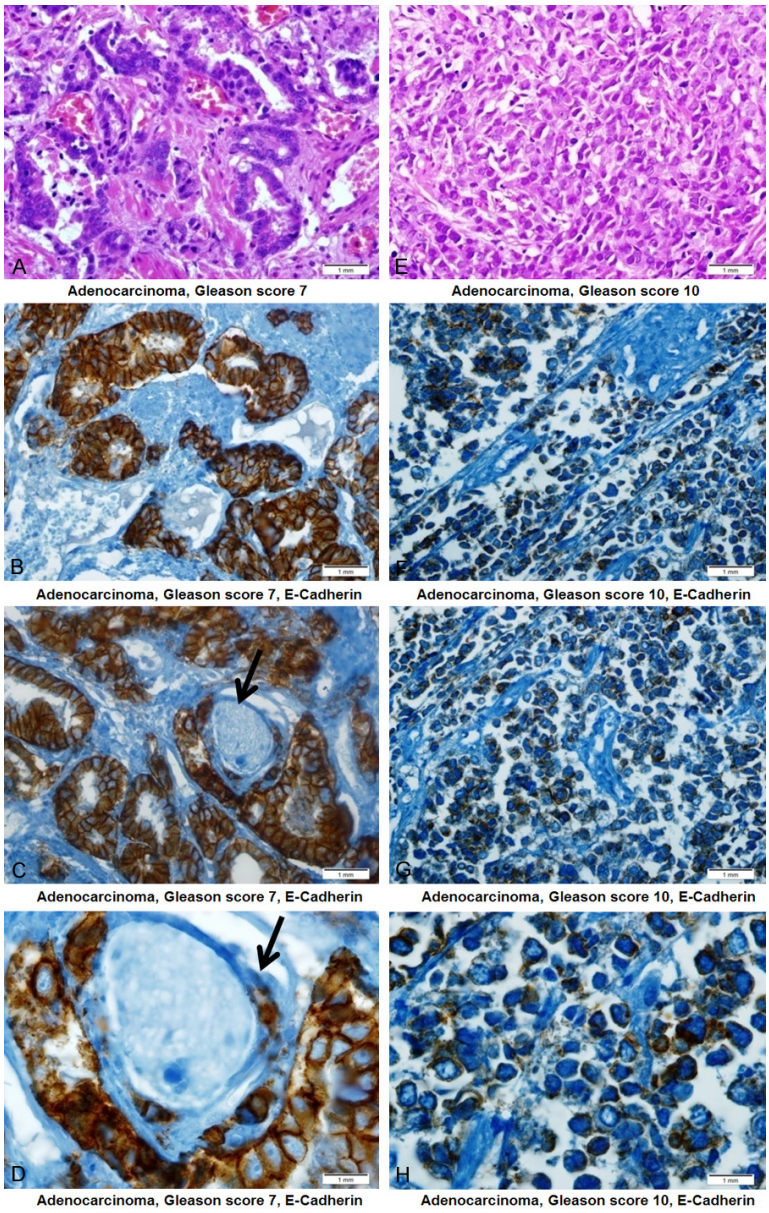

Figure 4. E-Cadherin protein expression in the prostatic adenocarcinoma. (A) Prostatic adenocarcinoma Gleason score 7 (left panel) composed of variable small to medium sized malignant acini with some luminal necrotic materials (arrow), arranged haphazardly amid congested stroma. The malignant cells have hyperchromatic nuclei and pink cytoplasm. (B-D) The neoplastic cells show strong diffuse homogenous membranous staining for E-Cadherin protein. Perineural invasion is apparent (arrows, C, D). (E) Prostatic adenocarcinoma Gleason score 10 (right panel) composed of malignant cells with abundant eosinophilic cytoplasm arranged in a solid pattern. (F-H) The tumor cells show heterogeneous reactivity for E-Cadherin with admixtures of cells lacking E-Cadherin expression together with some malignant cells with mild cytoplasmic and membranous staining.

and animals [21]. Koksal and his colleagues examined the expression of E-Cadherin in 58 radical prostatectomy specimens using immunohistological methods. Aberrant (reduced) staining patterns of E-Cadherin protein were observed in 18 and in 25 of the cases with pathological stages pT2 and pT3a, respectively. Aberrant E-Cadherin staining patterns were seen in 16 cases with Gleason score > or = 7 and in 27 cases of cases with Gleason score < 7 [20]. Rubin and his colleagues examined E-Cadherin protein expression in prostatic tissue specimens using tissue microarrays and immunohistochemistry. High (normal) E-Cadherin expression was seen both in benign (87% of cases) and PRCa (82% of cases). Aberrant E-Cadherin expression was associated with positive resection margins, higher Gleason score, and PSA failure [33]. Umbas and his colleagues examined the expression patterns of E-Cadherin protein in 92 tumor tissue specimens of primary and metastatic deposits of PRCa. Forty-six specimens of PRCa had reduced or absented E-Cadherin staining when compared to the benign prostatic epithelium. The reduced E-Cadherin reactivity was associated with the loss of tumor differentiation. The percentage of tumors with aberrant E-Cadherin expression was high among recurrent or metastatic tumors as compared to clinically localized tumors [43, 44]. Barber and his colleagues investigated the expression of E-Cadherin protein using immunofluorescence in 50 normal prostate tissues and 414 PRCa patients. Reduced expression of E-Cadherin was significantly associated with earlier biochemical recurrence of PRCa. The dog (Canis lupus familiaris) is the only other mammal besides humans that develop spontaneous PRCa. Fonseca-Alves and his colleagues examined E-Cadherin expression in the canine normal prostatic epithelium, BPH, proliferative

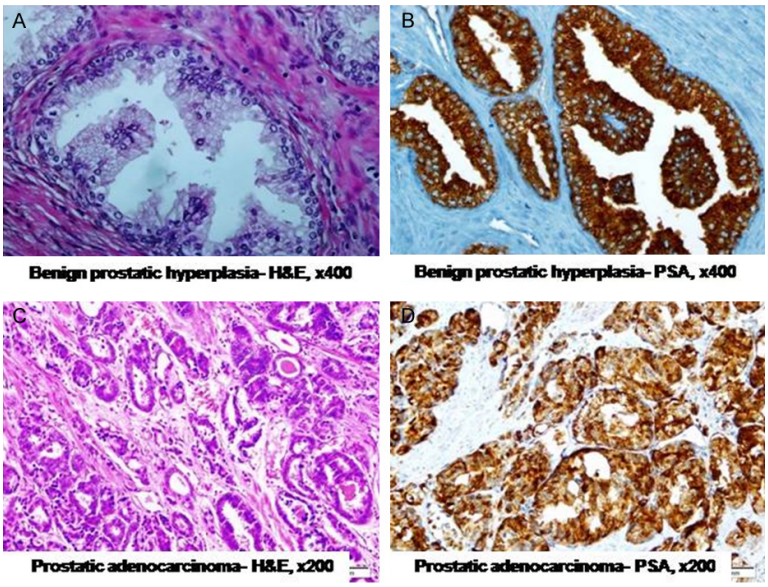

Figure 5. Prostatic specific antigen (PSA) protein expression in the benign prostatic hyperplasia and prostatic adenocarcinoma. A: Benign prostatic hyperplasia with large sized benign acinus with prominent papillary infoldings. B: The benign acinar cells are strongly positive for PSA. The staining pattern appears as diffuse homogenous cytoplasmic staining. C: Prostatic adenocarcinoma composed of variable sized small and medium-sized acini with the random arrangement, amid desmoplastic stroma. Luminal necrotic materials are noted. D: Similarly, the malignant cells show strong diffuse homogenous cytoplasmic staining for PSA.

Table 5. Correlations among the expression values (staining intensity) of PSA, COX-2, HER-2/neu and E-Cadherin proteins

Protein expression	*r* value	*p* values
PSA serum level versus COX-2	0.2214	0.2098
PSA serum level versus HER-2/neu	-0.099.	0.9461
PSA serum level versus E-Cadherin	0.7174	< .0001
COX-2 versus HER-2/neu	0.0756	0.8931
COX-2 versus E-Cadherin	0.3587	0.1488
HER-2/neu versus E-Cadherin	-0.1496	0.8398

inflammatory atrophy and PRCa using tissue microarray and immunohistochemistry. They found reduced expression of E-Cadherin PRCa when compared to the normal prostatic epithelium and proliferative inflammatory atrophy [21].

Impaired E-Cadherin gene function is usually associated with aberrant (reduced) expression of the protein. The aberrant (reduced) E-Cadherin protein expression in PRCa in our series may be reasoned to an allelic loss at the genetic locus at which the human E-Cadherin gene is located [43, 45] or due to of the E-cad promoter region leading to silencing E-Cadherin gene [46]. In support, in E-Cadherin negative breast and PRCa cell lines, the treatment with demethylating agents such as 5-aza-2'-deoxycytidine, partially restored E-Cadherin RNA and protein levels [47].

Strong PSA protein expression in the benign prostatic epithelium, BPH and PRCa

PSA has commonly used tumor marker for the diagnosis of PRCa. PSA is a 33-kDa serine protease secreted by prostatic epithelium and non-prostate tissues such as the perianal glands, epithelial lining of the periurethral and urachus remnant and Cowper's glands. PSA contributes to the liquefaction of seminal coagulum to allow the release of spermatozoa. In our series, the strong PSA protein expression in the benign prostatic epithelium, BPH and PRCa is in agreement with other studies [48-50]. The relationships among PSA and the other molecules involved in the development of PRCa are mostly unknown. Krill and his colleagues examined the relationships between PSA and E-Cadherin expression in both primary cultures and the prostate tumor cell line (LNCaP) using both immunohistological staining methods and Western blot. They found higher expression of E-Cadherin in normal epithelial cells than tumor-derived prostate cells. The incubation of normal prostate epithelial cells with E-Cadherin antibody was associated with enhanced amounts of PSA released into the culture media of both the normal cells and the LNCaP tumor cell line [49].

Correlation among COX-2 and HER-2/neu protein expression in PRCa

We found some correlations among the expression values of COX-2, E-Cadherin and HER-2/neu proteins in PRCa. The overexpression of

COX-2 protein was associated with aberrant (reduced) expression of E-Cadherin and overexpression of HER-2/neu proteins in PRCa. These correlations (although weak) suggest possible interactions between these genes. The positive correlations between COX-2 and HER-2/neu protein in our study concur with previous studies in colon and breast carcinomas [51] and suggest cross-talks between the COX-2 and HER-2/neu in the development of tumors. It is well-known that HER-2/neu and transforming growth factor-β/Smad molecular pathways are associated with COX-2 protein expression. Several studies support this notion. Wu and his colleagues examined COX-2 and HER-2/neu protein expression in 1026 colorectal carcinomas. A total of 350 carcinomas were negative for HER-2/neu out of 800 COX-2 positive specimens [51]. In breast carcinomas, the positive rates of COX-2 in invasive breast cancer were 87%, and that of HER-2 in invasive breast cancer was 34%, suggesting that HER-2 and COX-2 may regulate each other [51, 52]. The overexpression of HER-2/neu in the epithelium of the bile ducts of transgenic mice was associated with the induction of upregulation of COX-2 protein expression [53]. In human colorectal cancer cell lines, HER-2/neu activated factors were able to promote COX-2 leading to COX-2 mRNA and protein expression [54]. Several genes interact with each other during the development of PRCa.

In agreement with other studies [37, 55, 56], we found a negative correlation between COX-2 and E-Cadherin protein expression. Our findings together with others indicate possible interactions between these proteins [55, 56]. Some studies have indicated that Cyclooxygenase-2 (COX-2) contributes to the invasive and metastatic potentials of the malignant cells by downregulating the expression of E-Cadherin protein [55, 56]. In support, the overexpression of COX-2 in the gastric cancer tissues was found to be associated with downregulation of E-Cadherin protein expression. Moreover, treatment of the SGC7901 cells that overexpress COX-2 with COX-2 inhibitors such as celecoxib was associated with decreased expression of COX-2 and increased expression of E-Cadherin with amelioration of cell invasion [55, 56].

To conclude, our preliminary investigation reports the expression patterns of COX-2, HER-2/neu, E-Cadherin in the BPH and PRCa. We found over-expression of COX-2 and HER-2/neu proteins together with the aberrant expression of E-Cadherin in PRCa. COX-2 and HER-2/neu proteins were absent or weakly expressed in normal prostatic epithelium and BPH. E-Cadherin protein was strongly expressed in normal prostatic epithelium and BPH whereas this expression was weak or almost lost in few cases of PRCa. The therapeutic ramifications of our study await further investigations. To gain more insights into the pathogenesis of BPH and PRCa, further molecular studies are mandated to examine the expression patterns (especially, RT and western blot assay) of COX-2, HER-2/neu, E-Cadherin genes, and their relevant upstream and downstream genes.

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Disclosure of conflict of interest

None.

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