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Original article

Over-expression of β-catenin is associated with high grade of prostatic cancer in Libyan patients

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Abstract

Objectives: At present, sufficient prognostic markers for prostate cancer (PCa) progression are still lacking, in spite of thorough investigation. The aim of this study was to evaluate abnormalities of β-catenin protein expression, subcellular localization and determine its relation to different clinicopathological features and disease free survival in prostate cancer patients.

Patients and methods: Forty prostate cancer specimens, obtained from patients with different stages of prostate cancer (83% stage IV) who underwent a radical prostatectomy or TURP flanked by 2006 and 2011, β-catenin was determined by immuno-histochemistry (IHC). The membranous expression was semi-quantitatively evaluated in four scores (0, 1+, 2+, 3+). Clinical records of these patients were studied for follow up data.

Results: β-Catenin immune staining results show over-expression of β-catenin in PCa Libyan patients. There was no statistically significant difference in β-catenin immune expression as regards histopathological type, perineural invasion, tumor stage, biological recurrence. However, β-catenin over-expression showed significant correlation with old age ($p < 0.014$).

Conclusions: We concluded that changes in expression and cell distribution of β-catenin correlated with the progression degree of prostate adenocarcinoma, signifying a role of this molecule as a marker of progression and prognosis. Further investigations, on a larger and more heterogeneous population, should be carried out to validate and extend our results.

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Introduction

Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer deaths among men worldwide with an estimated 899,000 new cases and 258,000 new deaths in 2008. The global PCa burden is anticipated to grow to 1.7 million new cases and 499,000 new deaths by 2030 basically due to the growth and aging of the global population [1]. Recent statistics reveal that PCa continues to remain the most commonly diagnosed lethal malignancy in men in the United States with 1 out of 6 men developing PCa and 1 out of 35 dying from it [2].

There are now more than 240,000 men in the United States diagnosed with PCa each year [3], and 90% of PCa are clinically localized and occult disease at time of diagnosis [4].

In patients with localized PCa, the 5-year survival approximates 100%; however, in patients in whom distant metastasis have occurred, the 5-year survival drops to 31% [5]. Like most other solid malignancies, PCa can metastasize to distant organs such as the liver, lungs and brain, but it has an extraordinarily high propensity for metastasizing to the bone. In one autopsy study, ~80% of the men who had died from PCa possessed bone metastasis [6].

Gleason grade is one of the most widely used grading systems for predicting the progression of PCa. A more aggressive disease is associated with higher Gleason sum scores. Nevertheless, the pathological grade, serum Prostate Specific Antigen (PSA) value and clinical stage have some restrictions that hinder evaluation of the prognosis of PCa, although the PSA level combined with the Gleason grading system is still considered the most reliable prognostic marker [7,8]. Presently, adequate prognostic or predictive markers for tumor progression are still deficient. However, some molecules are involved in diverse processes such as cell proliferation, apoptosis, cellular adhesion, tumor suppression and cell cycle-related factors have been linked to PCa outcome [9,10].

Several genes and signaling pathways have been implicated in PCa initiation and progression, such as p53, C-MYC, Nkx3.1, PTEN, androgen receptor (AR), and Wnt/β-catenin [11]. Wnt/β-catenin signaling has been implicated in both normal prostate development and in PCa progression [12]. β-Catenin forms part of the adherent junction with E-cadherin and is also a component of canonical Wnt signaling. However, the function of β-catenin in human PCa is unclear [13]. It has been observed that β-catenin expression and localization change during human PCa progression. However, results are inconsistent. Several studies have seen an increase in β-catenin expression and nuclear localization in late stage cancer samples, while others have reported a loss in nuclear expression in advanced tumors [14–18].

In the current study, we examined the expression of β-catenin in a series of Libyan PCa by IHC. We tried to determine its value as predictive marker for metastatic potential. Additionally, the relationship between this molecular marker and known prognostic factors of serum PSA and Gleason grade was evaluated.

Patients and methods

Clinicopathological features and follow up data

Archival samples of 40 prostatic adenocarcinoma were examined in the present study. All the tumor samples were collected from Pathology Department, Faculty of Medicine, Benghazi University between January 2006 to December 2011 depending on accessibility of representative paraffin blocks.

The patient's clinical files were read in the hospital archives in order to gather the appropriate clinical information and follow up data for current study. For each patient, we obtained the following information: age, histological diagnosis, grading, staging, pre-treatment PSA level, date of diagnosis, treatment, cause and date of death. All patients were followed up until death or when last seen alive at their clinical visit (Dec-2012) with the mean followup time of 25 months (range: 6–72 months). The duration of follow-up was determined for each patient from hospital and clinic charts.

Clinical stages were determined according to the International Union against Cancer (UICC) classification of 2009. Clinical staging routinely included abdominal and pelvic computerized tomography (CT), chest radiograph or thoracic CT, isotope bone scanning, and extended/extensive prostate biopsy, as described elsewhere. PSA levels at diagnosis ranged between 0.1 and 500 ng/ml (mean: 113 ng/ml), and Gleason score at biopsy ranged between 6 and 10.

A skilled pathologist confirmed all diagnosis, and the following histopathological features were recorded which include; histological type, histological grading determined in accordance with the Gleason grading system, lymphovascular invasion, perineural invasion. All tumors were classified using the histopathological criteria of WHO classification. The key clinicopathological data of patients are summarized in Table 1.

Immuno-histochemical method

β-*Catenin immunostaining*

Paraffin embedded blocks of PCa have been obtained from pathology department archive. Sections were cut in sequence at 5 μm for immune-histochemical staining. IHC analysis has been done using the automatic system (Bench-Mark XT, Ventana Medical System, Inc., Tucson, Arizona, USA). This fully automated processing for bar code labeled slides included baking of the slides, solvent free deparaffinization, antigen retrieval in a cell conditioning buffer CC1 (Mild: 36 min conditioning, and standard: 60 min conditioning), incubation with Mouse monoclonal anti-β-catenin antibody (clone: 4, isotype: IgG1, Catalog no.: 760-4242 Ventana Medical Systems), for 32 min at 37 °C. Application of ultra-view TM universal DAB has been applied. Ultra view DAB includes: ultra-view universal HRP, ultra view universal DAB inhibitor, ultra view universal DAB chromogen, ultra view universal DAB H2O2, and ultra view universal DAB copper. Counterstaining with haematoxylin (2021) for 4 min, and post-counterstaining with bluing reagent (2037) for 4 min. After staining, the sections were dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of **β**-*catenin staining*

The assessment of staining was performed with a light microscope at the magnification of ×40, blinded by the information on tumor grade, stage or clinical outcome. Membranous and cytoplasmic staining was evaluated separately. For cell membrane staining, four categories were used, (+++, ++, +, 0), where 0: no expression, no detectable staining in <10% of the membrane, +: weak but detectable discontinuous staining present in 10–39% of the membrane, ++: moderate, clearly positive discontinuous staining present in 40–90% of the membrane, and +++: intense continuous staining