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Original article

Association of ring box-1 protein overexpression with clinicopathologic prognostic parameters in prostate carcinoma

Zeliha Esin Celik[a,*], Mehmet Kaynar[b], Fatma Dobur[a], Pınar Karabagli[a], Serdar Goktas[b]

[a] *Pathology Department, Faculty of Medicine, Selcuk University Selcuklu, Konya, Turkey*
[b] *Urology Department, Faculty of Medicine, Selcuk University, Selcuklu, Konya, Turkey*

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Abstract

Aim: To determine the expression of Ring Box-1 (RBX-1) protein in prostate carcinoma (PCa) and the association between RBX-1 expression and clinicopathologic prognostic parameters.

Material and methods: Relevant data such as age, preoperative serum PSA values, and tumor stage were obtained from 51 patients' with PCa record who underwent radical prostatectomy between January 2010 and March 2014. Hematoxylin-eosin stained pathology slides were evaluated by 2 pathologists blinded to patients' data in order to determine Gleason grade groups, tumor stage, tumor volume, capsule invasion, lymphovascular invasion, perineural invasion, and seminal vesicle invasion. Immunoreactivity scoring system (IRS) was used to determine RBX-1 expressions.

Results: A statistically significant difference was determined in terms of RBX-1 expression between non tumoral prostate tissue, high grade prostatic intraepithelial neoplasia (H-PIN) and carcinoma foci ($P = 0.001$). RBX-1 expression in the Gleason pattern 4 was higher than the Gleason pattern 3 and H-PIN foci as well as non tumoral prostate tissue. Likewise, in cases with PSA levels of >10.1 ng/ml, RBX-1 expression was higher than those ≤ 10 ng/ml. Moreover, RBX-1 expression of stage II cases was higher than stage I ($P = 0.019$), RBX-1 expression of stage III higher than stage I cases ($P = 0.044$). However, RBX-1 expression was not related with clinicopathologic parameters including patient age, tumor volume, lymphovascular invasion, perineural invasion, seminal vesicle invasion, or capsule invasion.

Conclusions: RBX-1 protein is overexpressed in PCa and associated with clinicopathologic prognostic parameters related with biological potential of the aggressive disease. Further studies of basic and molecular science are needed to reveal clinical and therapeutic implications of RBX-1 in PCa.

Keywords: Ring box-1; Prostate carcinoma; Immunohistochemistry

1. Introduction

Prostate cancer (PCa) is the most common cancer among men with approximately 650,000 new cases diagnosed each year [1]. PCa differs in its clinical progression ranging from indolent to lethal course. Radical prostatectomy is among the standard treatment modalities in PCa with a life expectancy of over a decade. However, ideal management of PCa presents unique challenges such as determining disease aggressiveness and most suitable treatment modality selection due to its highly variable natural history. Models used for disease progression prediction take clinical variables such as Gleason score, tumor stage, margin status, and PSA concentration, in various combinations, into consideration [2,3]. However, these models, in which clinicians combine clinical variables to create prognostic models have limited accuracy. Thus, models with a limited performance in determining PCa aggressiveness leads to suboptimal patient management. Within the last 10 years several studies focused on the need for predicting prostate cancer progression using individual biomarkers or gene expression profiles [4].

PCa is attributed to a combination of genetic and environmental factors such as the presence of susceptibility genes, ethnicity, family history, as well as different dietary

* Corresponding author. Tel.: +90-332-224-4813; fax: +90-332-241-6065.
E-mail address: dresincelik@hotmail.com (Z.E. Celik).

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and life styles [1]. In the prostate carcinogenesis among the major factors are inflammation, oxidative stress, DNA damage, telomere shortening, and senescence [5,6]. Cellular senescence is a tumor suppressor mechanism, which irreversibly arrests the growth of cells at risk for carcinogenesis [5,7–9].

Ring Box Protein-1 (RBX-1), also known as the regulator of Cullins-1 is a RING component of SCF (Skp-1, cullins, F-box proteins) E3 ubiquitin ligases, which regulate various cellular processes by targeting many substrates for degradation [10–12]. RBX-1 has an important function in miscellaneous cellular transactions such as DNA replication and repair, signal transduction, gene transcription, cell cycle progression, development and genome stability preservation as well as ubiquitin ligase activity [8,13]. Some recent studies revealed that RBX-1 plays a significant role in multiple human cancer types and silencing of RBX-1 by siRNA inhibits the growth of various cancer cells, including PCa, via induction of senescence and apoptosis as well as G2/M arrest. [8,10]. Though the role of RBX-1 in prostate carcinogenesis is well established, to our knowledge, any study determining RBX-1 expression in PCa by the immunohistochemical method and evaluating the association between RBX-1 expression and clinicopathologic prognostic parameters in PCa does not exist in the current literature.

This study aimed to determine the expression of RBX-1 protein in PCa, and the association between RBX-1 expression and clinicopathologic prognostic parameters.

2. Material and methods

Subsequent to the approval of the local ethics committee, 74 consecutive patients with PCa who had been treated with radical prostatectomy between January 2010 and March 2014 were planned to include in the present study.

Relevant data such as age, preoperative serum PSA values, and tumor stage were obtained from patients' records. Hematoxylin-eosin stained pathology slides were examined by 2 pathologists (E.C., F.D.) in order to determine Gleason grade group, pathologic tumor stage, tumor volume, capsule invasion, lymphovascular invasion, perineural invasion, and seminal vesicle invasion. A total of 51 cases with Gleason scores 6 to 8, available clinical data or tissue block were included to the study.

The paraffin-embedded tissue blocks with tumor foci, non tumoral prostate tissue and if present, accompanying high grade prostatic intraepithelial neoplasia (H-PIN) foci were selected for immunohistochemical staining.

The grading of the cases was based on the 2014 ISUP Consensus Conference on Gleason Grading of Prostate Carcinoma Report and categorized as Grade Groups 1 to 5. This new grading system and the terminology Grade Groups 1 to 5 have also been accepted by the World Health Organization for the 2016 edition of Pathology and Genetics: Tumors of the Urinary System and Male Genital Organs [14].

The clinical and pathological staging of the cases were based on the American Joint Committee on Cancer (AJCC) 2009 TNM PCa staging system.

2.1. Immunohistochemical staining procedure

Paraffin-embedded tissues of the chosen slides were collected and sections of 4 μm thick were prepared for immunohistochemistry. The sections were deparaffinized at 37°C oven overnight. Immunohistochemical staining was performed by using an automatic staining machine (Ventana, Benchmark XT). The sections were boiled at sodium citrate buffer at 95°C for 60 minutes and then incubated with primary antibody Anti RBX-1 (Abcam, Ab86862, Cambridge, UK) at a dilution of 1:200 for 52 minutes. The sections were incubated with the secondary antibody for 20 minutes at room temperature, incubated with Ultra I-view detection kit and counterstained with hematoxylin for 8 minutes. The sections of lung squamous carcinoma tissue were used as positive controls.

2.2. Evaluation and scoring of immunohistochemically stained slides

The immunohistochemical RBX-1 stained slides were evaluated and scored by 2 pathologists (EC, FD) who were blinded to patients' data. On light microscopic evaluation, 10 different fields of ×100 magnification for each section were reviewed. The immunoreactivity scoring system (IRS) was used to determine RBX-1 expression levels. This system, previously used by Wang et al. [11], depended on multiplication of staining intensity and percentage of RBX-1 positive cells. The percentage of positive cells was scored as: 0, negative; 1, 1% to 33%; 2, 34% to 66%; 3, 67% to 100% and the staining intensity was scored as 0 (−), 1 (+), 2 (++), 3 (+++). IR scores were obtained as 0 to 4, 6, or 9. In an individual case; cells in non tumoral prostate tissue, H-PIN and carcinoma foci were evaluated separately in terms of RBX-1 staining intensity and percentage to obtaine IRS for each. In tumor foci, IR scores of different Gleason patterns consisted the tumor were evaluated. Mean of IRS of different Gleason patterns was calculated for each case depending on percentage of each patterns.

2.3. Statistical analyses

SPSS v18.0 package program was used for statistical analysis. In order to test normality of distribution Kolmogorov-Smirnov Z test was employed. Parametric analysis methods were employed for normal distributions and in abnormal distributions nonparametrical tests were preferred. Kruskal-Wallis and One Way Analysis of Variance test were employed to determine statistical significance in terms of RBX-1 expression between non tumoral prostate tissue, Gleason pattern 3, pattern 4 groups,