PROGNOSTIC VALUE OF SEX-DETERMINING REGION Y-BOX 2 (SOX2) IN PROSTATE CANCER

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ABSTRACT

Objectives: The objectives of this study were to evaluate the expression of sex-determining region-Y BOX 2 (SOX2) markers and their association with clinicopathological parameters.

Methods: A cross-sectional study was carried out to involve 90 specimens from patients diagnosed with prostatic cancer (PC) and benign prostatic hypertrophy (BPH) compared with positive control (human bronchial tissue for SOX2 marker) with each run.

Results: The highest proportion of specimens of PC was graded ≤7 by Gleason grading system (53.3%). There were significant associations (p<0.05) between all this cancer stem cell marker results and prevalence of PC. Positive SOX2 marker result was significantly associated with bad prognostic sign (81.3%, p=0.001). This marker was highly sensitive (100%) specificity (82.4%), with high accuracy (90%).

Conclusion: There was a significant expression of SOX2 in prostate adenocarcinomas. SOX2 can be considered as a key regulator of tumor progression, aggressive behavior, and metastasis. Furthermore, it is a reliable marker for the early diagnosis and the designed chemotherapy of prostate adenocarcinomas.

Keywords: Prostate carcinoma, Cancer stem cell markers, Benign prostate hyperplasia.

INTRODUCTION

Prostate cancer (PC) is the second leading cause of male malignancy death throughout the world [1]. The age is the strongest risk chance of getting prostate cancer, where the incidence of prostate cancer is rising rapidly after the age of 50 [2]. PC is established as one of the most important medical problems facing the male population and is the most common male solid neoplasm [3]. It is the most common cancer in developed countries, with increasing uptake of prostate-specific antigen testing and the aging population, prostate cancer incidence is increasing, prostate cancer characterized by a generally slower progression than other cancers [4]. In recent years, cancer stem cells (CSCs) were identified in several cancers, including PC, and have been proposed to explain the metastatic capacity, recurrence, and resistance to hormone, radio and chemotherapy in established cell lines from PC origin, particularly from metastasis [5]. CSCs have been identified and isolated using different approaches such as flow cytometry, magnetic-associated cell sorting, the ability of differential cloning, and sphere growing under non-adherent culture condition [6].

The classical concept of cancer pathogenesis stipulates that tumors arise from stepwise accumulation of multiple mutations in mature somatic cells and that all cells in a cancer share similar molecular aberrations. The CSC theory is an alternative, fairly new paradigm and hypothesizes that distinct clone of cancer cells result from abnormalities that occur within specific cell types [7].

In this theory, cells in a tumor do not have the same malignant potential, but rather there are small clones of CSCs with self-renewal capabilities, high proliferative potential and pluripotency status that are responsible for tumor initiation, propagation, and metastases [8].

Enzymatic assays and immunohistochemistry have identified several proteins and surface markers that are expressed by CSCs and may be used to identify them in patients [9]. This study aimed to evaluate the expression of SOX2 markers and their association with clinicopathological parameters.

METHODS

A total number of 90 specimens were collected from tow governorate places as Al-Yarmouk Teaching Hospital and forensic medicine directorate in addition to private laboratories. The study was conducted on human prostatic tissue specimens obtained from patients attending the hospital and laboratories after surgical removal of prostate gland and from autopsy presented in forensic medicine directorate. These specimens were divided into the following: 60 of them were collected retrospectively depending on the archived files of patients diagnosed either with benign prostatic hypertrophy (BPH) (30 specimens) or with PC (30 specimens) in the years 2016 and 2017 in Al-Yarmouk Teaching Hospital and in the private laboratories. The other 30 specimens of normal prostatic tissue were collected prospectively from autopsy presented in forensic medicine directorate. All patients diagnosed with BPH or PC during 2016 and 2017 were included without any exclusion. Positive controls include the followings: Positive control: Human placenta tissue for EZH2 marker with each run, human gallbladder tissue for aldehyde dehydrogenases (ALDH) marker with each run, and human bronchial tissue for SOX2 marker with each run and negative control: It was done by deleting the primary antibody and adding antibody diluents alone in the same slide and follows the same steps in histoimmunostaining.

Patients were divided into three groups according to the pathological diagnosis.
- Group A: Included 30 patients proved to have PC.
- Group B: Included 30 patients proved to have BPH.
- Group C: Included 30 patients to have healthy looking normal prostatic tissue.

Preparation of tissue section (Group C)

Bancroft, 2008, ordered procedure was used to prepared paraffin-embedded tissue blocks of prostate tissue sample (Group C) in the following order: Fixation, dehydration, clearing, impregnation, embedding, sectioning, dewaxing, and hydration followed by staining and mounting.

Quantitative scoring method

The cells were scored as positive or negative staining depending on the presence of distinct brown cytoplasm or nuclear staining. All tissue sections of the three groups were correlated with age, Gleason score, and bad prognostic sign. The slides were examined with low-power microscopy 10× to determine the regions of highest staining, if they show no staining at low power, reexamination was done by high power 40× to determine area of weak staining, five fields of each slide were examined and scored semi-quantitatively by calculating the proportion of positive stained cells over the total number of malignant cells (%).

SOX2

Slides were reviewed to evaluate staining expression of the marker under light microscope; it shows nuclear staining.

RESULTS

Scoring and intensity of SOX2 marker in the study groups are shown in Table 1. It was obvious that in Group A, SOX2 marker scored 2 in half of the specimens and in 75% of specimens of Group B, while the two positive results in Group C scored 1.

Concerning intensity, moderate intensity was the most common intensity of SOX2 marker in Group A (56.3%) and in all the positive result specimens in Groups B and C (Table 2).

The sensitivity = 100%, specificity = 82.4%, and accuracy of SOX2 marker was 90% as shown in Table 3.

In this study, the highest prevalence of bad prognostic sign was seen among patients with positive SOX2 marker result (81.3%) with a significant association (p=0.001) between prevalence of bad prognostic sign and SOX2 marker result (Fig. 3).

DISCUSSION

CSC theory is an alternative, fairly new hypothesis that distinct clones of cancer cells result from abnormalities occurred within specific cell [10]. In this theory, there are small clones of CSCs with self-renewal capabilities, high proliferative potential and pluripotency status that are responsible for tumor initiation, propagation, and metastases [11].

Enzymatic assays and immunohistochemistry have identified many proteins and surface markers that are expressed by CSCs and may identify them in patients.

SOX genes can regulate a number of developmental processes including lens, hair follicle, gut, B-cell, muscle, and blood vessel development [12].

In prostate cancer, SOX2 is overexpressed in CSCs where it mediates tumorigenesis and has been linked to poor prognosis [13].

The SOX genes comprise a family of genes that are related to the mammalian sex-determining gene. These genes similarly contain sequences that encode for the high mobility group-box domain, which is responsible for the sequence-specific DNA-binding activity. SOX genes encode putative transcriptional regulators implicated in the decision of cell fates during development and the control of diverse developmental processes [14].

Half of the specimens of Group A were positive for SOX2, while positive in 13.3% of Group B specimens and only 6.7% of specimens of Group C were positive.
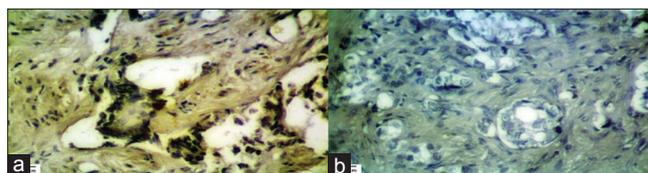

Fig. 1: Histopathological sections of prostate carcinoma. (a) Microphotograph shows +ve expression of SOX2 marker in prostate adenocarcinoma, brownish discoloration of nuclear stain, moderate intensity, ×40. Gleason score 4+4=8 (b) Microphotograph shows -ve IHC expression of SOX2 marker in prostate adenocarcinoma, bluish discoloration of nuclear stain, ×40. Gleason score 4+4=8

Table 1: Scoring and intensity of SOX2 marker in the study groups

SOX2 marker	Group A n=16 (%)	Group B n=4 (%)	Group C n=2 (%)	Total n=22 (%)
Scoring				
Score 1	6 (37.5)	1 (25.0)	2 (100.0)	9 (40.9)
Score 2	8 (50.0)	3 (75.0)	0	11 (50.0)
Score 3	2 (12.5)	0	0	2 (9.1)
Intensity				
Weak	4 (25.0)	0	0	4 (18.2)
Moderate	9 (56.3)	4 (100.0)	2 (100.0)	15 (68.2)
Strong	3 (18.7)	0	0	3 (13.6)

Table 2: Association between prevalence of bad prognostic sign and CSC marker result

CSC marker	Bad prognostic sign		Total (%) n=30	p-value
	Yes (%) n=13	No (%) n=17		
SOX2				
Positive	13 (81.3)	3 (18.8)	16 (53.3)	0.001
Negative	0 (0)	14 (100.0)	14 (46.7)	

Table 3: Sensitivity, specificity, and accuracy of SOX2 marker

SOX2 marker result	Bad prognosis		Total
	Yes	No	
Positive	13	3	16
Negative	0	14	14
Total	13	17	30

Concerning scoring and intensity of SOX2 marker, half of the SOX2 marker specimens scored 2 in Group A and in three-quarter of specimens of Group B, while two positive results observed in Group C scored 1.

In addition, moderate intensity was the most common intensity of SOX2 marker in Group A (56.3%) and in all the positive result specimens in Groups B and C. Regarding sensitivity, specificity, and accuracy of SOX2 marker, results were 100%, 82.4%, and 90%, respectively, in consistent to results yielded in Australia 2015, SOX2 immunohistochemistry showed nuclear positivity in epithelial tumor cells in 61 of the 142 (46.38%) [15].

Many types of SOX genes have been described which named after a shared motif called the SRY-box, a region homologous to the DNA-binding domain of SRY, the mammalian sex-determining gene. One of these genes encodes for a transcription factor SOX2 which has a well-known role in the maintenance and acquisition of stem cell features [16]. Overexpression of SOX2 gene in CSCs was a predictor of prognosis and mediates tumorigenesis [17].

The SOX genes have multiple applications other than prostate carcinoma, as approved by a study conducted in Germany, in 2011, which concluded that embryonic stem cell factor SOX2 is expressed in a variety of early-stage postmenopausal breast carcinomas and metastatic lymph nodes and also suggested that SOX2 plays an early role in breast carcinogenesis and high expression may promote metastatic potential [18]. In addition, in Germany (2017), researchers found that SOX2, a transcription factor that is well characterized as a marker for stem cells, is upregulated in both mouse and human bladder carcinoma. SOX2 expression is absent in normal urothelial cells, but it begins to be expressed in preneoplastic bladder tumors and continues to be expressed in invasive mouse bladder carcinoma [19].

In addition, SOX2 is also associated with lymph node metastases [20]. Loss of SOX2 expression is strongly associated with poor prognosis in patients with cervical cancer, as concluded by a Korean study in 2015 [21]. In Slovenia (2014), the study approved that SOX2 amplifications are common in esophageal squamous cell carcinoma and the detection of SOX2 amplifications in the early stages of disease may be crucial for early disease detection and a more accurate prognosis [11].

SOX2 is an important stem cell marker that is crucial for embryonic development and to maintain the differentiation potential of stems cells. SOX2 is one of the key transcription factors involved in inducing pluripotent stem cells. In the past decade, SOX2 has been established as one of the hallmark participants of the developmental process in cancer, including in skin squamous cell carcinoma, prostate carcinoma, glioblastoma, colorectal cancer, lung cancer, breast cancer, and esophageal squamous cell carcinoma. Furthermore, percentages of SOX2-positive tumors are increasing with Gleason score and metastases [22]. More than half of the study patients in Group A did not show bad prognostic signs (56.7%). With regard to association between the prevalence of bad prognostic sign and CSC marker result, the only significant association was noticed among 81.3% of patients with positive SOX2 marker result. In contrary to Australian study in 2015, authors noticed that CSC marker ALDH1 is expressed significantly in prostate carcinomas, and more likely, so in cases with adverse pathological parameters such as extraprostatic extension and lymphovascular invasion, while in agreement to the present result a statistically significant association between SOX2 and bad prognostic sign. Differently, other studies had shown similar significant correlations between this marker and poor prognosticators as well as disease-free survival, as shown by studies done in the USA, in 2010 [23,24].

CONCLUSION

There was a significant expression of SOX2 in prostate adenocarcinomas. SOX2 can be considered as a key regulator of tumor progression, aggressive behavior, and metastasis. Furthermore, it is a reliable marker for the early diagnosis and the designed chemotherapy of prostate adenocarcinomas.

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CONFLICTS OF INTEREST

None.

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