

Increased Sox2 immunostaining in prostate cancer and associated with Gleason score and stage

Dhafer A. Alghezi^{1,2*}, Rasha Aljawher³, Entedar Alsaadi¹

Medical Microbiology and Immunology Department, College of Medicine, University of Thi-Qar,
Nasiriyah, Thi- Qar, Iraq¹

Cancer Research Unit, College of Medicine, University of Thi-Qar, Nasiriyah Thi- Qar, Iraq²
Histopathology and Forensic Medicine Department, College of Medicine, University of Thi-Qar, Nasiriyah,
Thi-Qar, Iraq³

Corresponding author: 1,2*



ABSTRACT— Prostate cancer prognostic biomarkers are few. However, clinical problems have been reported in discriminating between aggressive and non-aggressive cancers. The goal of this study is to find a more sensitive and specific PCa biomarker that can offer significant information for disease diagnosis and therapy is critical. Sox2 is a transcription factor that belongs to the SOX family and It's thought to be a potential biomarker for predicting the clinical progression and prognosis of some cancer types. However, its role in prostate cancer is uncertain. Immunohistochemistry was used to examine nuclear and cytoplasmic Sox2 staining in benign (40) and malignant (76) prostate samples. Nuclear and cytoplasmic Sox2 immunostaining was increased significantly in prostate cancer compared to benign prostate tissues. Nuclear Sox2 staining was significantly associated with poorly differentiated Gleason scores and advanced stages. Cytoplasmic Sox2 was only associated with tumor size. This finding suggests that Sox2 may promote cancer progression or aggressiveness. An additional study is also needed to better understand its function and establish whether it might be used as a prostate cancer biomarker.

KEYWORDS: Prostate cancer, Sox2, IHC.

1. INTRODUCTION

Prostate cancer (PCa) is one of the world's most significant health issues, with a high mortality rate [1], [2]. This disease can impact millions of men and is the second leading cause of cancer-related death in the United States with 300,000 cases and 41,000 deaths every year [3]. According to a recent study conducted in Iraq, the PCa rate is increased drastically from 1.85 to 4.13 between 2006 and 2016 [4]. Acinar adenocarcinoma, which is produced from the glandular areas of the prostate gland, is seen in around 95% of PCa cases [5], [6]. In contrast, only 5% of PCa patients are histopathologically classified as ductal adenocarcinoma, which starts in the cells lining the prostate gland ducts (tubes) [7].

The Gleason grade system, introduced by Dr. Donald Gleason in the 1960s and 1970s, is the most extensively used histopathological grading method for evaluating PCa progression [8], [9]. Based on an evaluation of the prostate's histopathological architecture, which indicates how much of the prostate tissue is normal or aberrant, this system may be classified into five Gleason grades (1-5) [9]. Less aggressive cancer, for example, is more likely to appear as healthy tissue, but more aggressive cancer spreads to other regions of the body and does not appear as healthy tissue [9]. PCa is a diverse illness with a variety of histological findings. A Gleason score is computed by summing the two most prevalent Gleason grades: primary and secondary, which are assigned separately for biopsy and prostatectomy [9]. The Gleason score

of 10 is the highest score in this system [8]. The first number allocated in this method represents the most common cancer grade. For example, if $3+4=7$ is used, it means that the majority of the tumor is grade 3 and just a little piece is grade 4, and the two are combined to provide a Gleason score of 7. Furthermore, a Gleason score of 7 ($3+4$) indicates that the majority of the tumor is grade 4 and few sections with grade three. This method, however, does not always discriminate between aggressive and non-aggressive tumors [10].

Another method used for PCa diagnosis and prognosis is a tumor-node-metastasis (TNM) system, which was created by the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC). This method is based on the size of the PCa and the extent to which it has spread [2], [11]. This approach has an advantage of being able to assess PCa patients' prognoses as well as predict disease progression [12], as well as serve as a guide for patient treatment planning. This system, on the other hand, is unable to predict which patients would relapse after the first treatment and which will remain in remission. Because of that, finding another technique that can help with this problem becomes a priority.

The presence of evidence of basal cell loss is critical for appropriately identifying PCa [13]. H&E staining, on the other hand, may be unable to reliably detect basal cells in prostate glands [14], necessitating the development of a biomarker that can validate the existence of basal cells in prostate glands. In addition, biomarkers that are expressed rather than lost in PCa are also employed. However, few diagnostic and/or prognostic PCa biomarkers have been identified, and clinical challenges in discriminating between prostate gland disorders such malignant vs. BPH and localized vs. metastasized PCa have been identified. As a result, finding novel PCa biomarkers has become a top goal.

One of the potential biomarkers used for prostate cancer diagnosis and prognosis is Sox2 which represents a transcription factor that belongs to the SOX (SRV-related high mobility group box) family [15]. The previous studies demonstrate that Sox2 has many functions including tumor initiation, cancer stem cell development, proliferation, sphere and colony formation, invasion, migration, metastasis, epithelial-to-mesenchymal transition (EMT), and resistance to apoptosis and therapy [16], [17]. Taken together, it was decided to study the nuclear and cytoplasmic expression of Sox2 in benign and malignant prostate tissues, Gleason score, clinical stage. This would help us better understand Sox2 expression and how it relates to PCa development as well as clinical characteristics like grades and stages.

2. Materials and methods

The ethics board of Al- Hussein teaching hospital in Thi-Qar city, Iraq (Thi-Qar 2021159 in 7/12/2022) approved this study. Seventy six malignant and 40 benign prostate (BP) tissues were collected from the histopathology laboratory of Al- Hussein teaching hospital from January 2022 to June 2022. A diagnostic H&E sections were prepared to identify the tissues architecture by histopathologists (Table 1).

Table 1: Clinical data of benign and malignant prostate tissue samples.

Clinical data		Number/ Percentage
Number of samples	Benign	40 (100%)
	Malignant	76 (100%)
Age range	Benign	30-60
	Malignant	45-81
Gleason score	Low (6)	9 (11.8%)
	Moderate (7-8)	24 (31.6%)

	High (9-10)	43 (56.6%)
T category	T1-T2	44 (57.9)
	T3-T4	32 (42.1%)
N category	N0	54 (71.1%)
	N1	22 (28.9%)
M category	M0	51(67.1%)
	M1	25(32.9%)

2.1 Immunohistochemistry

IHC was carried out to stain prostate tissue samples using anti-Sox2 antibody (mouse monoclonal, Abcam, cat. number ab79351, 1:100). Several pretreatment steps were used before IHC staining. Firstly, the tissue sections were deparaffinized using histoclear, and rehydrated with graded alcohols (100%, 95%, 70%, respectively). After permeabilization with 0.5% triton X-100 in PBS (phosphate buffer saline), the tissue sections were subjected to heat-induced epitope retrieval in a citrate buffer, pH 6.0 for 30 minutes at 90°C, followed by a 20-minute cool down. To block the endogenous peroxidase activity, drops of H2O2 were then added on these sections and incubated in a humid chamber for 10 minutes at a room temperature. Finally, PBS was mixed with 10% normal goat serum and 0.05 bovine serum albumin, and drops of this solution were applied to tissue sections for 30 minutes at a room temperature. The tissue sections were then incubated overnight at 4°C with the primary antibody. Next day, the tissue sections were washed three times with PBS for 10 minutes each time. These sections were then incubated in a humid chamber with a secondary antibody for 30 minutes at room temperature. As a chromogen, diaminobenzidine tetrahydrochloride was used to view the reaction products. Finally, hematoxylin was used to counterstain the sections.

Five random pictures were captured and evaluated using a semi-quantitative scoring system for nuclear and cytoplasmic Sox2 staining to measure the expression level of Sox2 malignant and benign prostate tissues. The proportion and intensity score were then used for Sox2 immunoreactivity as follow: 0 (0%), 1 (1-30%), 2 (31-50-%), and 3 (>50%). Negative (0), mild (+1), moderate (+2), or high (+3) cytoplasmic Sox2 intensity was assigned. The total score was ranged from 0 to 6 [18].

2.2 Statistical analysis

GraphPad Prism version 8.00 for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com, was used to detect the mean, standard error, and standard deviation values. The unpaired t-test & one-way ANOVA with Tukey's multiple comparisons tests were used. P<0.05 was considered significant.

3. RESULTS

IHC was carried out to evaluate the expression level of Sox2 on prostate tissue sample. IHC result showed that both malignant and benign prostate tissues had nuclear and cytoplasmic Sox2 staining with varied level of signal intensity, ranging from strong (Figure 1 C&D), Moderate (Figure 1 A&E), and weak (Figure 1 B&F). The negative control (NC) group, which did not use a primary antibody, had no significant background staining in prostate tissues.

Quantification of the IHC staining revealed that nuclear and cytoplasmic Sox2 staining was significantly increased in PCa tissues compared to BP tissues (p=0.0029 & 0.0246, respectively) (Figure 2 &3, A and Table 2). In addition, nuclear Sox2 staining was significantly associated with increasing Gleason score (p=0.006) (Figure 2 E AND Table 2), whereas, the cytoplasmic staining was not associated with it (Table

2), using an ANOVA test. Further analysis using multi-comparison (Tukey) testing indicated that nuclear Sox2 staining was increased significantly in patients with poorly differentiated Gleason score when comparing to those with well and moderate differentiated Gleason scores ($p=0.0152$ & 0.045 , respectively). Both nuclear and cytoplasmic staining of Sox2 was significantly increased increasing tumor size (T3-4 vs T1-2) ($p=0.0497$ & 0.038 , respectively). In addition, the nuclear Sox2 results, but not cytoplasmic results, showed a negative association with other clinical stage parameters, including lymph node involved (N0 vs N1-2) (0.0339) and metastasis (M0 vs M1) (0.0466) (Figure 3 C&D; Table 2).

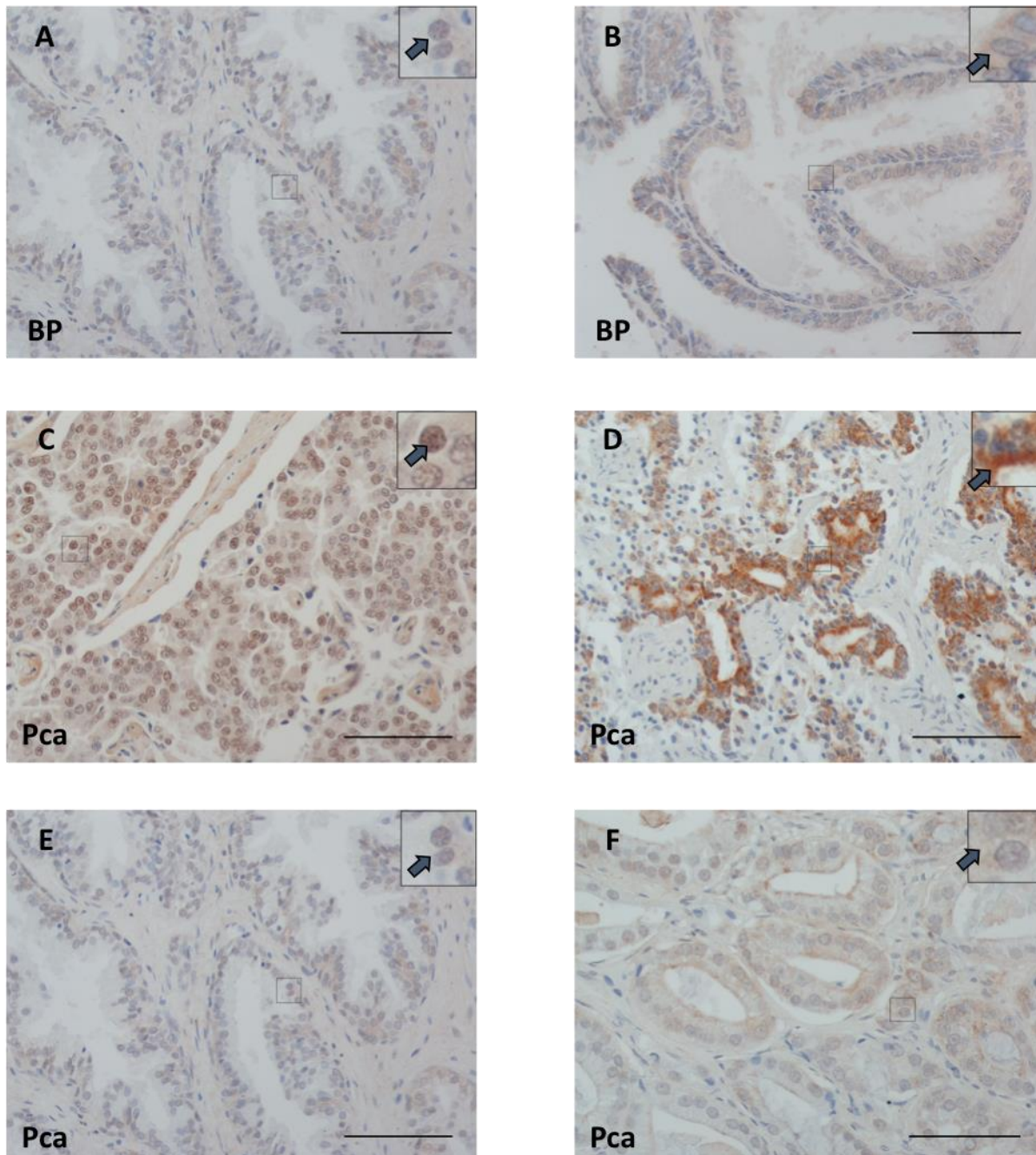


Figure 1: IHC results of Sox2 in PCa and BP tissues: A) Moderate nuclear Sox2 staining (arrow) in BP tissues. B) Weak cytoplasmic Sox2 staining (arrow) in BP tissues. C) Strong nuclear Sox2 staining (arrow) in PCa tissues. D) Strong cytoplasmic Sox2 staining (arrow) in PCa tissues. E) Weak nuclear Sox2 staining (arrow) in PCa tissues. F) Weak nuclear and cytoplasmic (arrow) Sox2 staining in PCa tissues. Scale

bars=100µm.

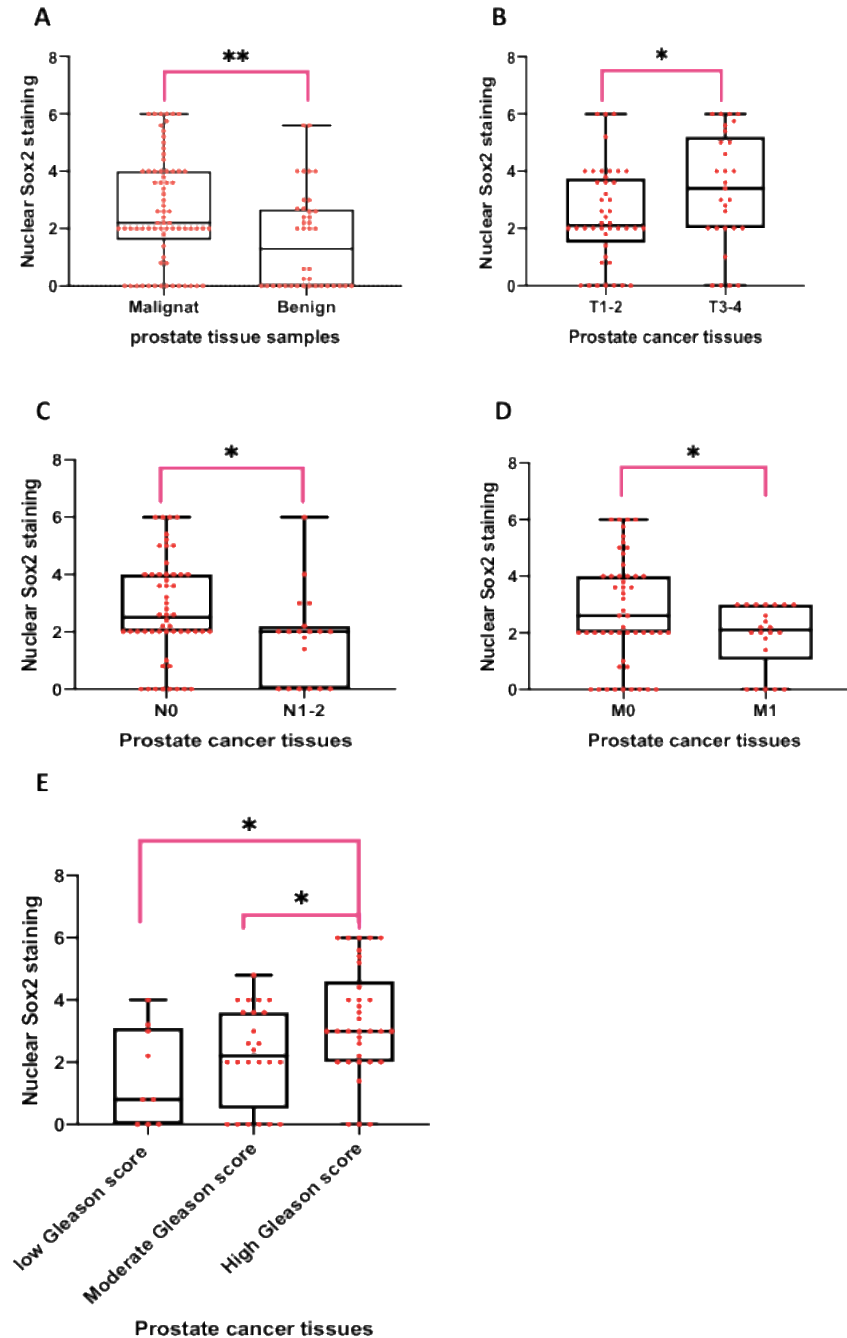


Figure 2: Nuclear Sox2 staining in BP and PCa quantified. (A) Nuclear Sox2 staining was significantly increased in PCa tissues compared to BP tissues ($p=0.0029$). (B) A significant positive association was observed between nuclear Sox2 staining and prostate tumor size ($P=0.0497$). (C) Decreased Sox2 staining significantly in patients with lymph node involved (0.0339). (D) Metastatic PCa patients has a lower Sox2 staining compared to those with non-metastatic PCa. ($p=0.0466$). (E) Nuclear Sox2 staining showed a significant difference among different Gleason scores ($p=0.006$). There was a significant increasing in the level of Sox2 staining when comparing PCa patients with a high Gleason score to those with a low ($p=0.0152$) or intermediate (0.045), using multiple comparison (Tukey) tests. PCa (76) and BP (40), low Gleason score 3 ($n=9$), moderate Gleason score (24) and high Gleason score (43), T1-2 ($n=44$) and T3-4 ($n=32$). N0 (54), N1(22), M0 (51), M1(25).

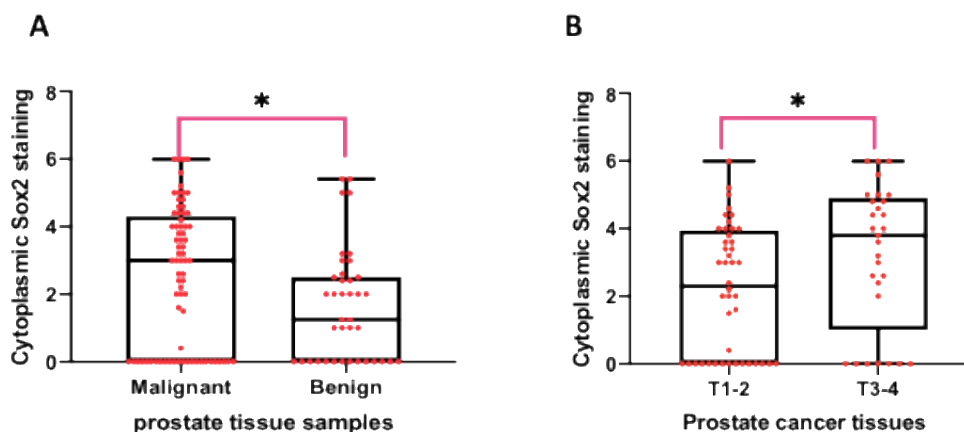


Figure 3: Cytoplasmic Sox2 staining in BP and PCa tissues quantified. (A) Cytoplasmic Sox2 staining was significantly increased in PCa tissues compared to BP tissues ($p=0.0246$). (B) A significant positive association was observed between nuclear Sox2 staining and prostate tumor size ($P=0.038$). PCa (76) and BP (40), T1-2 (44), T3-4(32).

Table 2: Nuclear and cytoplasmic Sox2 results were compared to prostate tissue clinical data.

Comparison	Nuclear Sox2 staining			Cytoplasmic Sox2 staining		
	Results	p. value		Results	p. value	
Normal vs malignant	Higher in malignant	0.0029		Higher in malignant	0.0246	
Gleason scores	Higher in high Gleason scores	Anova test	0.006	No significant difference	Anova test	0.6767
		low Gleason score vs. Moderate Gleason score	0.5262		low Gleason score vs. Moderate Gleason score	0.7754
		low Gleason score vs. High Gleason score	0.0152		low Gleason score vs. High Gleason score	0.9392
		Moderate Gleason score vs. High Gleason score	0.0454		Moderate Gleason score vs. High Gleason score	0.9330
T	Higher in T3-4		0.0497	Higher in T3-4		0.0246
N	Higher in N0		0.0339	No significant difference		0.7406
M	Higher in M0		0.0466	No significant difference		0.6105

4. DISCUSSION

PCa is the most common cancer in men, and currently its irremediable. The discovery of novel molecular markers that could improve tumor classification and prognosis is a major issue in current fundamental and clinical research [19]. There is currently no reliable clinical approach for predicting the response to PCa therapeutic therapy. As a result, finding a more sensitive and specific PCa biomarker that can offer significant information for disease diagnosis and therapy is critical.

In this research, nuclear and cytoplasmic Sox2 expression was increased significantly in Pca compared to BP. This study is consistent with the previous study [18]. This may suggest that Sox2 may play a vital role in PCa formation. In addition, IHC was utilized to explore the expression of Sox2 in prostate tissues and shown that increased nuclear Sox2 expression was associated with Increasing Gleason score and prostate cancer advanced stages. However, cytoplasmic Sox2 expression was only associated with tumor size. Because strong expression of Sox2 could only be detected in most tumor tissues in correlation with increased Gleason score, the current study suggests that Sox2 could be developed as a pathological indicator to distinguish tumor from non-tumor prostate tissues and to anticipate the prognosis of prostate tumor. This result is consistent with another study done by [18] which identified the Sox2 expression in the cytosol and nuclei of prostate cancer cells including the PC3 and DU145 using immunofluorescent technique. Several studies have also revealed that Sox2 expression is associated with tumor progression and high score Gleason score [18], [20], [21]. In addition, amplification of the Sox2 gene locus and enhanced Sox2 expression has been seen in a number of studies, which has implications for cancer progression [18], [20], [22]. Taken together, these findings may confirm that nuclear localisation of Sox2 may have a role in PCa proliferation and differentiation. In addition, Sox is a transcription factor and is expected to be expressed in the nuclei of cells. This might be explained the differences between the nuclear and cytoplasmic Sox2 expression in this study.

Sox2 has many functions including tumor initiation, cancer stem cell development, proliferation, sphere and colony formation, invasion, migration, metastasis, epithelial-to-mesenchymal transition (EMT), and resistance to apoptosis and therapy [16], [17]. It has been found that, Sox2 target gene is involved in neuroendocrine differentiation in adenocarcinoma and PCa dissemination and postulated that Sox2 as a functional biomarker of PCa lymph node metastasis [23]. Over expression of Sox2 in cell lines of PCa whether androgen dependent or not, greatly increases the expression of a number of neuroendocrine differentiation genes including S100, ENO2, CHGA and SYP [23], [24].

It has been shown that Sox2 may play a role in shaping the PC metastasis phenotype. As its established ability to boost a network of pro-metastasis genes such as those coding for neural tissue associated molecules L1CAM and CHL1 which as physiological signaling transducers of neuronal migration. In addition, to its great leading role in tumor cell invasion and motility [24- 26].

5. CONCLUSION

This study supports the concept that Sox2 could be a valuable biomarker for assessing prostate cancer progression and a target for prostate cancer therapy. However, further study is needed to evaluate the expression level of Sox2 a large cohort sample size using a secondary independent antibody.

Conflict of Interests

The authors state that the publishing of this work does not include any conflicts of interest.

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