

# Assessment of Immunohistochemical Expression of SOX2 and its Prognostic Significance in Prostatic Adenocarcinoma

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## Abstract

**Background:** SOX2 is a transcription factor involved in the self-renewal and pluripotency of embryonic stem cells (ESCs). It is involved in various cancer types and affects cancer cell physiology via involvement in complicated cell signaling and protein-protein interactions. **im:** Our work aimed to assess the expression patterns of SOX2 in prostatic adenocarcinoma in correlation with the histopathological findings to evaluate its role as a prognostic marker or possible therapeutic target. **Materials and Methods:** forty-eight specimens of prostatic adenocarcinoma were stained first H&E then immunohistochemically for SOX2 and scored by two histopathologists. **Results:** thirty-eight specimens showed positive nuclear expression of SOX2; with variable degrees. SOX2 expression showed a statistically significant association with different grade groups; with p-value = 0.025. There was no statistically significant association between its expression and the patient's demographic data. **Conclusion:** Overexpression of SOX2 in Prostatic Adenocarcinoma could be an indicator of higher tumor grade and poor prognosis. It could be used as a prognostic biomarker for tumor aggressiveness.

**Keywords:** SOX2, transcription factor, immunohistochemistry, prostatic adenocarcinoma.

## Introduction

Prostate cancer is a disease of increasing significance worldwide. In many industrialized nations such as the United States, it is one of the most common cancers and among the leading causes of cancer deaths<sup>(1)</sup>. In developing countries it may be less common, however its incidence and mortality has been on the rise<sup>(2)</sup>. According to the new theory of stem cell origin of cancer, most tumors originate from cancer stem cells. The factors that cause inhibition of the pro-

cess of differentiation and uncontrolled proliferation of tissue stem cells are the most important factors in the carcinogenesis process<sup>(3)</sup>. Stem cells are characterized by the capacity of continuous self-renewal and the potential to differentiate into one or more mature cellular lineages. They serve to form tissues and organs during mammalian development, and they maintain ongoing cellular turnover and provide regenerative capacity in certain adult tissues<sup>(4)</sup>. The self-renewal and differentiation of stem cells are intrinsically controlled by the inter-

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play of cell type-specific transcription factors and chromatin regulators. Although several such molecules have been implicated in stem cell biology over the last few years, the mechanistic modes of action of these molecules remain incompletely understood<sup>(4)</sup>. SRY (sex determining region Y)-box 2, also known as SOX2, is a transcription factor that is essential for maintaining self-renewal, or pluripotency, of embryonic stem cells. Sox2 has a critical role in maintenance of embryonic and neural stem cells<sup>(5)</sup>. Sox2 is a member of the Sox family of transcription factors, which has been shown to play key roles in many stages of mammalian development. Several studies revealed that this protein family shares highly conserved DNA binding domains known as HMG (High-mobility group) box domains containing approximately 80 amino acids<sup>(5)</sup>. The role of SOX2 in cancer pathogenesis has become of interest in the field. To date, studies have shown SOX2 to be amplified in various cancer types and affect cancer cell physiology *via* involvement in complicated cell signalling and protein-protein interactions<sup>(6)</sup>. The SOX2 is gaining a renewed interest as a key regulator of self-renewal and maintenance of Cancer Stem Cells (CSCs) in a variety of tumors including Prostate cancer<sup>(7-12)</sup>. In prostate cancer, SOX2 has been shown to increase cellular proliferation and survival, to stimulate epithelial-mesenchymal transition (EMT)<sup>(13,14)</sup> and to promote castration-resistant disease<sup>(15)</sup>. It was found that the EMT transcription factor SNAI2/Slug upregulates SOX2 in prostate cancer cells and that these genes are co-expressed at the invasion front and in Neuron Endocrine Differentiation (NED) areas of high-grade prostate cancer<sup>(16)</sup>. The aim of our work was to assess the

expression patterns of SOX2 in prostatic adenocarcinoma in correlation with the histopathological findings in order to evaluate its role as prognostic marker or possible therapeutic target.

## Materials and methods

This cross-sectional analytic study included formalin fixed, paraffin embedded blocks of prostatectomy, TURP (Transurethral resection of prostate) and TRUS (Transrectal ultrasound) specimens diagnosed as prostatic adenocarcinoma archived in pathology laboratory, Suez Canal University Hospital during the period from January 2011 to December 2019. The required clinicopathological data was obtained from medical records. One slide was re-cut from each block and stained by Haematoxylin and Eosin (H&E) and re-examined for confirming the diagnosis. Sections from each block were cut at 5- $\mu$ m-thickness and prepared for immunohistochemical staining for SOX2. Sections were placed onto positive charged slides, heat-induced epitope retrieval will be done in a microwave, the prepared primary antibody of SOX2 from Novus biomedical was used according to the steps mentioned in the company data sheet. By using light microscopy, immunohistochemically stained tissue sections were examined at high power magnification, and the nuclear staining percentage for the marker was calculated semi-quantitatively and compared to the positive and negative controls. Immunohistochemical expression of SOX2 was classified into 3 categories according to the nuclear staining percentage of cells (defined as the cells with moderate or intense nuclear brownish staining)<sup>(17)</sup>: Score zero : negative or weak staining. Score 1: positive

staining in 1 % to 10% of cells. Score 2: positive staining in >10 % to 50% of cells. Score 3: positive staining in >50 % of cells. The immunohistochemical findings were correlated with the H&E findings.

### Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups. While Pearson coefficient was used to correlate between two normally dis-

tributed quantitative variables.

## Results

### Demographic data of patients:

The study included 48 specimens with age ranged from 50 to 90 years. 39.6% at age group (60-69), 29.2% at age group (70-79), 16.7% at age group (50-59) and 14.6% had age more than 80 years old.

### Histopathological assessment:

Total Gleason score of studied specimens ranged from 6 to 9, with Mean (7.29). According to grade group distribution, 9 specimens were found at grade group I (18.8%), 6 specimens were at grade group II (12.5%), 13 specimens were at grade group III (27.1%), 17 specimens were at grade group IV (35.4%) and 3 specimens were at grade group V (6.3%). So the majority of specimens were found at grade group IV representing 35.4% of all specimens.

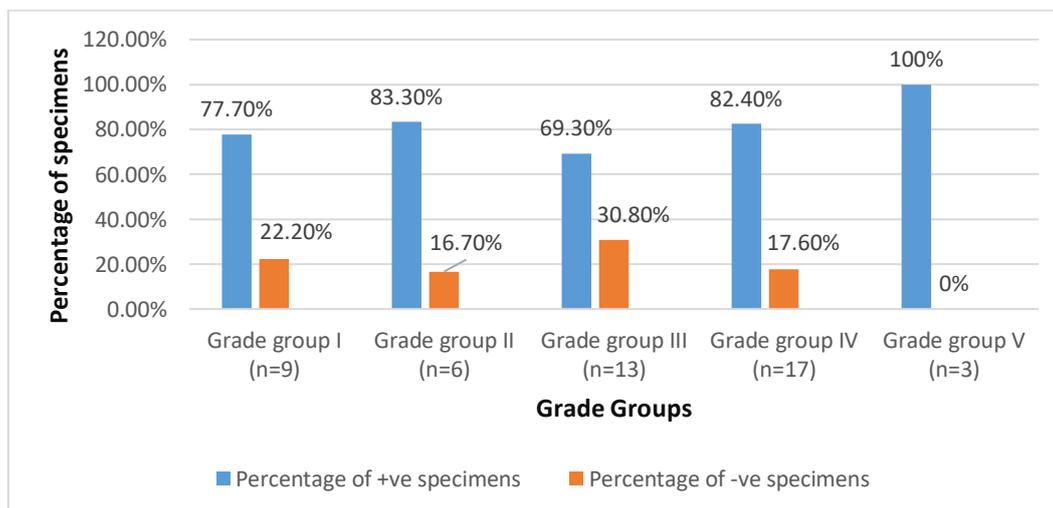


Figure 1: Correlation between SOX2 scoring and different grade groups.

### Immunohistochemical assessment

According to SOX2 scoring, 38 specimens (representing 79.2% of all specimens) showed positive SOX2 nuclear staining while 10 specimens (20.8%) showed negative staining. Of the posi-

tive specimens, 17 specimens (about 35.4% of all specimens) were scored (+1) while 11 specimens (about 22.9%) were scored (+2) and 10 specimens (about 20.8%) were scored (+3). SOX2 scoring was correlated with patients' demo-

graphic data and different histopathological parameters. Correlation between SOX2 scoring and different grade groups showed that 9 specimens were at grade group I, 2 of them (22.2%) were SOX2 negative and 7 (77.7%) were SOX2 positive (divided as 44.4% score 3 and 33.3% score 1). 6 specimens were at grade group II, 1 of them (16.7%) was SOX2 negative and 5 (83.3%) were SOX2 positive (divided as 50% score 3 and 33.3% score 1). 13 specimens were at grade group III, 4 of them (30.8%) were SOX2 negative and 9 (69.3%) were SOX2 posi-

tive (divided as 38.5% score 1 and 30.8% score 2). 17 specimens were at grade group IV, 3 of them (17.6%) were SOX2 negative and 14 (82.4%) were SOX2 positive (divided as 41.2% score 1, 29.4% score 2 and 11.8% score 3). And 3 specimens were at grade group V, all of them (100%) were SOX2 positive (divided as 66.7% score 2 and 33.3% score 3). Based on the above-mentioned results, there is statistical significant difference between different grade groups regarding SOX2 scoring (P value = 0.025) (Figure 1).

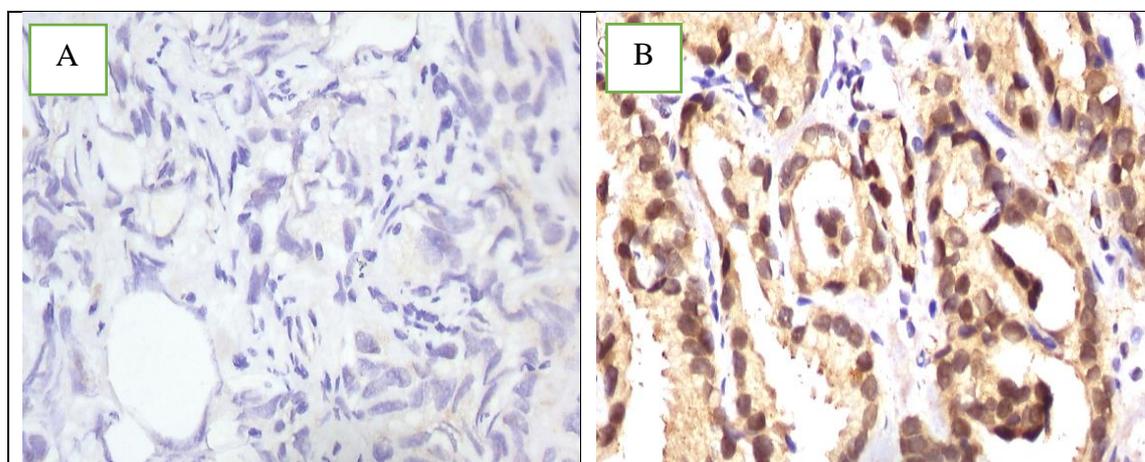


Figure 2: Representative photomicrographs of SOX2 immunostaining, A) Negative SOX2, B) Positive SOX2, (400X)

There was no statistically significant correlation between SOX2 scoring and demographic data of patients or other histopathological parameters including total Gleason scoring, Tumor burden in tissues, multi-centricity, benign changes and perineural invasion.

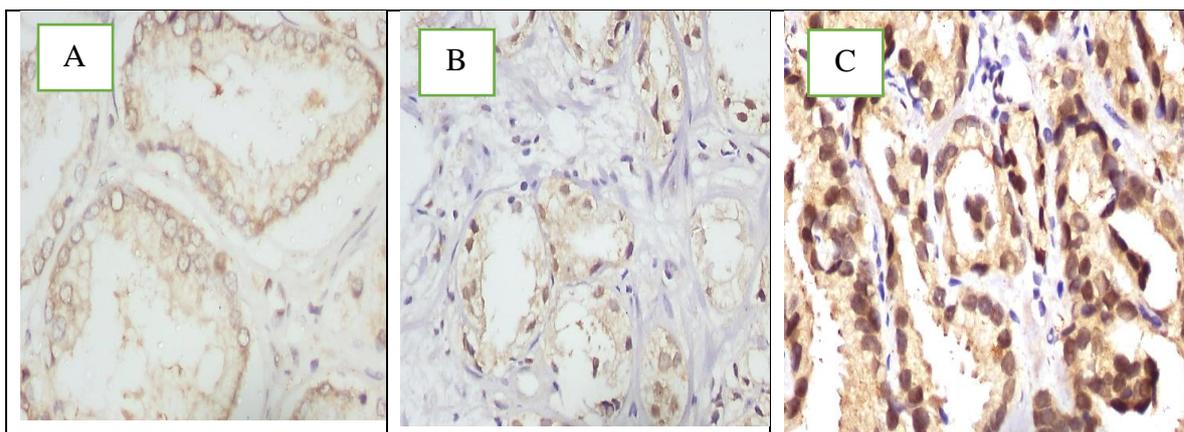
## Discussion

In prostate cancer SOX2 is overexpressed in CSCs where it mediates tumorigenesis and has been linked to poor prognosis<sup>(18)</sup>. Otsubo *et al.* study revealed that SOX2, regularly expressed in the basal cell layer of normal prostatic glands, is substantially downregulated,

most likely by gene promoter methylation, in prostate cancer epithelia and cell lines, as previously observed in gastric cancer<sup>(19)</sup>. Although the lack of basal cells is a typical histologic feature of prostate cancer, it has emerged that this type of tumor originates in basal cells and subsequently evolves to adenocarcinoma, which is maintained by more differentiated luminal-like cells<sup>(20)</sup>. It may thus be conceivable that the epigenetic mechanisms of pluripotency gene silencing accompanying differentiation in developing embryos, may aberrantly occur in prostate carcinogenesis<sup>(21)</sup>. We have investigated the immunohisto-

chemical expression of SOX2 and its correlation with different histopathological parameters in a cross-sectional analytic study of 48 specimens. Our study revealed that the percentage of SOX2 positive specimens is higher than negative ones, where 79.2% of specimens were

SOX2 positive and 20.8% were SOX2 negative. These results match with those of Aboushousha et al.,(2019) who reported a significant higher percentage of SOX2 positive cells in prostate cancer than negative ones (with percentage of positive cells 70.56%).<sup>(22)</sup>.



**Figure 3: Representative photomicrographs of SOX2 immunostaining scoring, A) Score 1, B) Score 2, C) Score 3, (400X)**

Similar results were obtained by Bae et al., (2010) and Jia et al., (2011), and this suggests an active role of SOX2 in the development and progression of prostate cancer<sup>(23)</sup>. An interesting finding documented by Jia et al., (2011) was the location of SOX2 in prostate cancer tissues and cell lines. The nuclei localized SOX2 in prostate cancer cells may function as a transcriptional regulator, however, the function of SOX2 in the cytosol of prostate cancer cells and the factors that may regulate the cellular location of SOX2 still need to be further investigated. The progress in revealing these underlying mechanisms will deepen our understanding of SOX2's function in cancer cells<sup>(24)</sup>. In our study there is statistical significant difference between different grade groups regarding SOX2 scoring and positivity (P value =0.025), where percentage of SOX2 positive specimens is greater than negative ones in all grade groups. Also our study re-

vealed that SOX2 positive specimens had higher total Gleason score than negative ones. The previous mentioned results are in accordance with previous studies which reported a correlation between SOX2 expression and both Gleason score and grade group<sup>(22-24)</sup> Aboushousha et al. (2019) found a statistical significant increase in percentage of SOX2 positive cells in higher Gleason scores and grade groups compared with lower ones. Since a higher Gleason grade indicates a worse prognosis, so it is suggested that SOX2 may contribute to the tumorigenesis of prostate cancer and may play an important role in the clinical progress of prostate cancer<sup>(22)</sup>. Bae et al., (2010) postulated that, the expression of SOX2 in prostate tumors has been thought to promote a less differentiated embryonic stem cell tumor phenotype; that confers a worse disease prognosis<sup>(23)</sup>. Kergel et al., (2013) concluded that Prostate tumors were either

SOX2-positive or SOX2-negative, with the percentage of SOX2-positive tumors increasing with Gleason Score and metastases<sup>(17)</sup>. Jia et al., (2011) reported that SOX2 could be potentially developed as a pathological criterion to distinguish tumor from non-tumor prostate tissues and to predicate the prognosis of prostate tumor, since strong expression of SOX2 could only be detected in most of the tumor tissues in correlation with increased histologic grade and Gleason score<sup>(24)</sup>. In the present study, we found a positive significant correlation between percentage of SOX2 positive cells and grade Group. Many previous studies reported an association between SOX2 expression and advanced stages in several human tumors. Kitamura et al., (2013) suggested that SOX2 expression was significantly associated with tumor grade, pathological T stage and pathological N stage and this supports the cancer stem cell theory for upper urinary tract urothelial cell carcinoma, which suggests that therapeutic targeting of cancer stem-like cells/tumor-initiating cells in upper urinary tract urothelial cell carcinoma is a future possibility<sup>(25)</sup>. Similar results were obtained by Bao Z, Zhan Y, He S et al., (2019) who found that SOX2 is an independent prognostic marker of poor disease free survival and cancer specific survival in upper tract urothelial carcinoma patients who have undergone radical nephroureterectomy. Moreover, these data suggest that SOX2 may be a promising therapeutic target in upper tract urothelial carcinoma<sup>(26)</sup>. Yang et al., (2013) reported that SOX2 expression was associated with clinical stage and lymph node status in patients with small cell lung cancer<sup>(27)</sup>. Tang et al., (2013) suggested that SOX2 expression was

significantly associated with clinical stage, lymph node metastasis and recurrence in laryngeal squamous cell carcinoma<sup>(28)</sup>. Zhang et al., (2010) reported that patients with strong SOX2 showed deeper invasion and advanced clinical stages compared to patients with low SOX2 expression in gastric cancer<sup>(29)</sup>. Wang X et al., (2014) suggested that the expression of SOX2 in primary ovarian tumors is much lower than that in the corresponding metastatic lesions and that SOX2 overexpression promotes proliferation, migration and invasion, while inhibiting adhesion abilities of serious ovarian carcinoma cells<sup>(30)</sup>. Neumann et al., (2011) reported that overexpression of SOX2 significantly correlated with lymph-node and distant metastases in right-sided colon cancers<sup>(31)</sup>.

## Conclusion

Based on the results of the current study we concluded that, SOX2 was up-regulated in prostate cancer; mainly in cancers with a worse prognosis which have higher Gleason Score and higher-grade group. So, we suggest a role played by SOX2 in the progression of prostate cancer that can be used as a predictive tool or therapeutic target.

## Recommendations

Further studies are required to determine if this marker has a role in triaging of prostate cancer in core biopsies and whether they can be future targets for treatment of aggressive prostate cancers. Larger sample size in future studies is recommended. Future studies will need to look at the practical utility of SOX2 in prostate core biopsies to predict tumor aggressiveness before radical treatment is rendered.

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