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Reduced Sox7 Expression in Prostate Cancer and Associated with Poorly Differentiated Gleason Grade

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Abstract
Diagnostic and prognostic biomarkers for prostate cancer are few. It has been discovered that it is clinically challenging to discriminate between aggressive and non-aggressive prostate cancer. The aim of this study is to examine the hypothesis that Sox7 may be used as a potential biomarker for prostate cancer diagnosis and prognosis. Sox7 is a transcription factor that is found to play a role in controlling different biological processes throughout embryonic development, such as hemopoiesis, vasculogenesis, and cardiogenesis. It is also believed to be a tumor suppressor in a number of malignancies. Its function in prostate cancer, however, is not well understood. Nuclear and cytoplasmic Sox7 expression has been evaluated by immunohistochemistry using normal (16) and malignant (80) prostate tissues. The immunohistochemical study showed reduced nuclear and cytoplasmic Sox7 expression in prostate cancer compared to normal prostate tissues. This reduction was also associated significantly with increased primary Gleason grade. This data suggests the possibility that Sox7 may play a key role in prostate tumor formation or aggressiveness, highlighting the need for further study to clarify its role and establish whether it might be used as a prognostic biomarker for prostate cancer.

Key words: Sox7, IHC, Gleason grade, Prostate cancer.

Introduction

One of the most important health issues in the world is increasing prostate cancer (PCa) mortality rate [1, 2]. The PCa, which has an incidence of 300,000 cases and 41,000 fatalities each year in the USA after skin cancer, represents a second-leading cause of cancer-related death [3]. About 95% of PCa cases are acinar adenocarcinomas, which originate in the glandular zone of the prostate gland [4,5]. In contrast, only 5% of PCa cases are histopathologically classified as ductal adenocarcinomas, which begin in the cells lining the tubes that convey the prostate gland[6].

PCa can be diagnosed using a variety of histopathological techniques. The Gleason grade system is the most popular histopathological grading system for evaluating PCa development [7,8]. It is divided into five different grades (1–5) based on an analysis of the histopathological architecture of the prostate, which shows how much of the prostate tissue is normal or aberrant [8]. For example, less aggressive PCa is more likely to resemble healthy tissue, whereas more aggressive PCa does not [8]. PCa is a heterogeneous disease with different histological characteristics in the same sample [8]. For this reason, a Gleason score which represents a summation of the two most common, primary and secondary, Gleason grades, is currently used [8]. For example, the Gleason sore 3+4=7 means that the primary, predominate, grade is 3 and the secondary, the second predominant, is grade 4 and the two are added to provide a Gleason score of 7. Furthermore, a Gleason score of 7 (4+3) indicates that grade 4 represents the most common malignancy and a little section is grade 3. The Gleason score is 3+3=6 [8] if all cancer areas are graded at the same level, such as grade 3. This technique can't always tell an aggressive tumor from a non-aggressive tumor [9].

The second system named the tumor-node-metastasis (TNM) is also used for PCa diagnosis and prognosis based on the size and the extent of tumor dissemination[2, 10]. This system is able to assess PCa prognosis and predict the disease course [11], as well as help as a manual for patients' treatment planning. In contrast, it is unable to discriminate between patients who will remain in remission after receiving initial therapy and those who will experience a recurrence.

The presence of basal cell loss is a need for a precise PCa diagnosis [12]. Identified a new potential biomarker that may validate the existence of prostate gland basal cells in is crucial because H&E staining may not be able to reliably detect these cells in PCa [13]. Clinical challenges exist in differentiating between prostate gland disorders like normal vs. malignant and localized vs. metastasized PCa, and only a few biomarkers have been identified for PCa diagnosis/prognosis. Therefore, finding new PCa biomarkers has taken on greater importance.

One of the potential biomarkers identified in this study for analysis is Sex-determining region Y box 7 (Sox7). It is a transcription factor that is found to play a role in controlling different biological processes throughout embryonic development, such as hemopoiesis, vasculogenesis, and cardiogenesis [14]. It is also believed to be a tumor suppressor in a number of tumors [15]. Reduced Sox7 was observed in a variety of malignant tissues compared to non- malignant tissues, including breast ovarian, liver, pancreas and prostate compared to the normal tissues of these malignancies [16,17,18,19,20, 21]. In addition, other studies on the breast and pancreatic cancer, but not PCa, were found a negative correlation between Sox7 expression and grade of these tumors [19,22]. Finally, another IHC investigation revealed increased Sox7 mRNA level

in PCa patients with low serum PSA levels and no metastasis compared to those with high serum PSA levels and metastasis [21]. The earlier discovery suggested that Sox7 may contribute to tumor development and may be associated with a bad prognosis in a number of malignancies; however, the prognostic significance of Sox7 in PCa is yet unknown. Therefore, this study objectives include determining if Sox7 expression is correlated with PCa clinical data, such as Gleason grade and stage, and assessing Sox7 expression levels in normal and malignant prostate tissues.

Materials and methods

Patients and Ethics statement.

The study was approved by the National Health Service's (NHS) ethical in Bath city, UK (REC reference: 13/WS/0153; IRAS project ID: 112241). This study used a tissue microarray slides (TMA) obtained from US Biomax (PR1921). In this TMA cohort, there are 96 samples of prostate tissue, of which 80 were from PCa and 16 were from normal prostate tissue. Two core tissue biopsies from each case were used to create a total of 192 cores. In this study, normal liver tissue was used as a positive control. The clinical information for prostate tissue samples is reported in Table 1.

Table 1 shows the clinical data of the prostate tissue sample.

The Prostate Clinical Data		Number	%
Number Of Samples	Normal	16 (100%)	
	Malignant	80 (100%)	
Age Range	Normal	21-68	
	Malignant	20-85	
Primary Gleason Grade	3	13	16.25
	4	46	57.5
	5	18	23. 75
	ND	3	2.5
Tumor Size T Category	T1-2	51	63.8
	T3-4	28	35
	ND	1	1.2
Lymph Node Metastasis (N) Category	(Absent)N0	65	81.2
	(Present)N1	14	17.5
	ND	1	1.3
Metastasis M Category	(Absent) M0	64	80
	(Present) M1	15	18.7
	ND	1	1.3

Immunohistochemistry (IHC)

Two distinct anti-Sox7 antibodies (Anti-Sox7 Rabbit polyclonal, dilution 1:200, Abcam, cat. number Ab8033, named as anti-Sox7 A and anti-Sox7 Rabbit polyclonal, dilution 1:200; Sigma, cat. number A40200, named as anti-Sox7 B) were used in this study to stain the normal and malignant prostate tissue using IHC. Several pretreatment steps prior to IHC were utilized. Graded ethanol (100%, 95%, and 70%, respectively) were used to rehydrate paraffin-embedded tissue sections. Following permeabilization with 0.5% triton X-100 in phosphate buffer saline (PBS), these sections were then heated to 90°C for 30 minutes to induce epitope retrieval, followed by 20-minutes cooling period. To inhibit endogenous peroxidase activity, 3% H_2O_2 drops were applied on these sections in a humid chamber for 10 minutes at room temperature and then washed twice with PBS for 5 minutes each. Additionally, drops of a 10% normal goat serum solution with 0.05 bovine serum albumin solution were applied to the prostate sections for 30 minutes, followed by washing with normal saline for a minute.

Drops of the primary anti-Sox7 antibody were added to these sections, incubated overnight at 4°C and then washed three times with PBS for 10 minutes each. The secondary antibody was then applied to these sections and allowed to sit for 30 minutes at room temperature before being washed three more times for 5 minutes each with PBS. Following the manufacturer's recommendations, the EnVision+Kit (K400611-2 and K401011-2, Dako, Ely, UK) was used to observe the reaction products using diaminobenzidine tetrahydrochloride as a chromogen. The sections were then counterstained with hematoxylin (H-3401, Vector Laboratories, Peterborough, UK). The prostate sections were then mounted using DPX (Sigma-Aldrich, Gillingham, UK).

Immunohistochemical analysis

Five randomly chosen images for each case were used and scored using a semi-quantitative scoring system for nuclear and cytoplasmic Sox7 expression in order to evaluate the Sox7 Immunostaining in prostate tissue samples. Nuclear and cytoplasmic Sox7 immunoreactive proportion scores were as follows: (0: negative, 1: 1-10%, 2: 11-50 % and 3: 51-100 %.). whereas, the intensity scores of Sox7 were as follows: negative (0), weak (+1), moderate (+2), or strong (+3). The final score for each case represents the sum of the proportion and intensity scores, which ranged from 0 to 6 [21].

Statistical analysis

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

Results

1) Sox7 IHC result in normal and malignant prostate tissues, using anti Sox7 A

A) Sox7 expression in normal and malignant prostate tissues, using anti Sox7 A

This study examined the Sox7 expression in cancerous and non-cancerous prostate tissues, using Anti Sox7 A. The IHC data found that all normal prostate tissues (16/16, 100%) had nuclear Sox7 expression in prostate tissues, with varying degrees of staining ranging from strong (Figure 1 A, arrow) to faint staining (Figure 2 B, arrow). Nuclear Sox7 staining was found in PCa tissues with varying degrees of signal strength, ranging from a strong (Figure 1 C&D, arrows), weak (Figure 1 E, arrow) or negative (Figure 1 F, arrow).

Cytoplasmic staining of Sox7 was shown in both normal and malignant tissues with varying degrees of signal staining, ranging from strong (Figure 1 A&C, arrowheads), weak (Figure 1, D arrowhead), and negative (Figure 1, E, arrowhead). Liver tissue used as Sox7 positive control in this study based of the previous data (Wang et al., 2017). As expected, cytoplasmic Sox7 staining was expressed in normal liver tissue sections (Figure 1 G, arrowhead). A negative control showed no background staining (Figure 5.4 H, arrow) in prostate tissue samples.

B) Decreased Sox7 expression in PCa and negatively associated with increasing primary Gleason grade, using anti Sox7 A.

IHC result showed that nuclear and cytoplasmic Sox7 expression was decreased signficanlty in PCa tissues compared to normal tissue of prostate ($p = < 0.0001$) (Figure 2 A&B & Table 2). This Sox7 reduction was also found to be associated with increasing primary Gleason grade groups ($p = 0.0084$ & 0.0488, respectively) (Figure 2 C&D, Table 2). Additionally, when comparing patients with grade 3 to those with grade 4, Sox7 nuclear and cytoplasmic staining was significantly reduced, using multi-comparison Tukey's tests ($p=0.0061$ & 0.0429, respectively) (Figure 2 C&D & Table 2). In contrast, there was no significant correlation between the expression of Sox7 in the nucleus or cytoplasm and the clinical stage of PCa (Table2).

2) Validation Sox7 results on prostate and liver tissues, using two different Sox7 antibodies

IHC was used to confirm that the two independent Sox7 antibodies (Anti-Sox7 A&B) produced a staining pattern that was similar on tissue sections taken from the same regions in both prostate and liver tissues. This data showed that staining pattern of these two independent antibodies were similar in both prostate and liver tissues.

In prostate tissues, Sox7 staining was observed in the prostate glandular (Figure 3 A&B arrows) and stromal regions(Figure 3 A&B arrowheads) of prostate tissues, using both antobodies. Nuclear Sox7 expression was also detected in prostate tissue samples, using anti- Sox7 A & B (Figure 3 C&D arrows, respectively). The postitive control, Liver tissue, had cytoplasmic Sox7 staining when using both antibodies (Figure 3 E & F arrowheads). Since the two Sox7 antibodies (Anti- Sox7 A&B) showed the same staining pattern in the prostate and liver tissues, it was decided to carry out IHC staining of the prostate tissue samples using the second independent anti-Sox7 B antibody.

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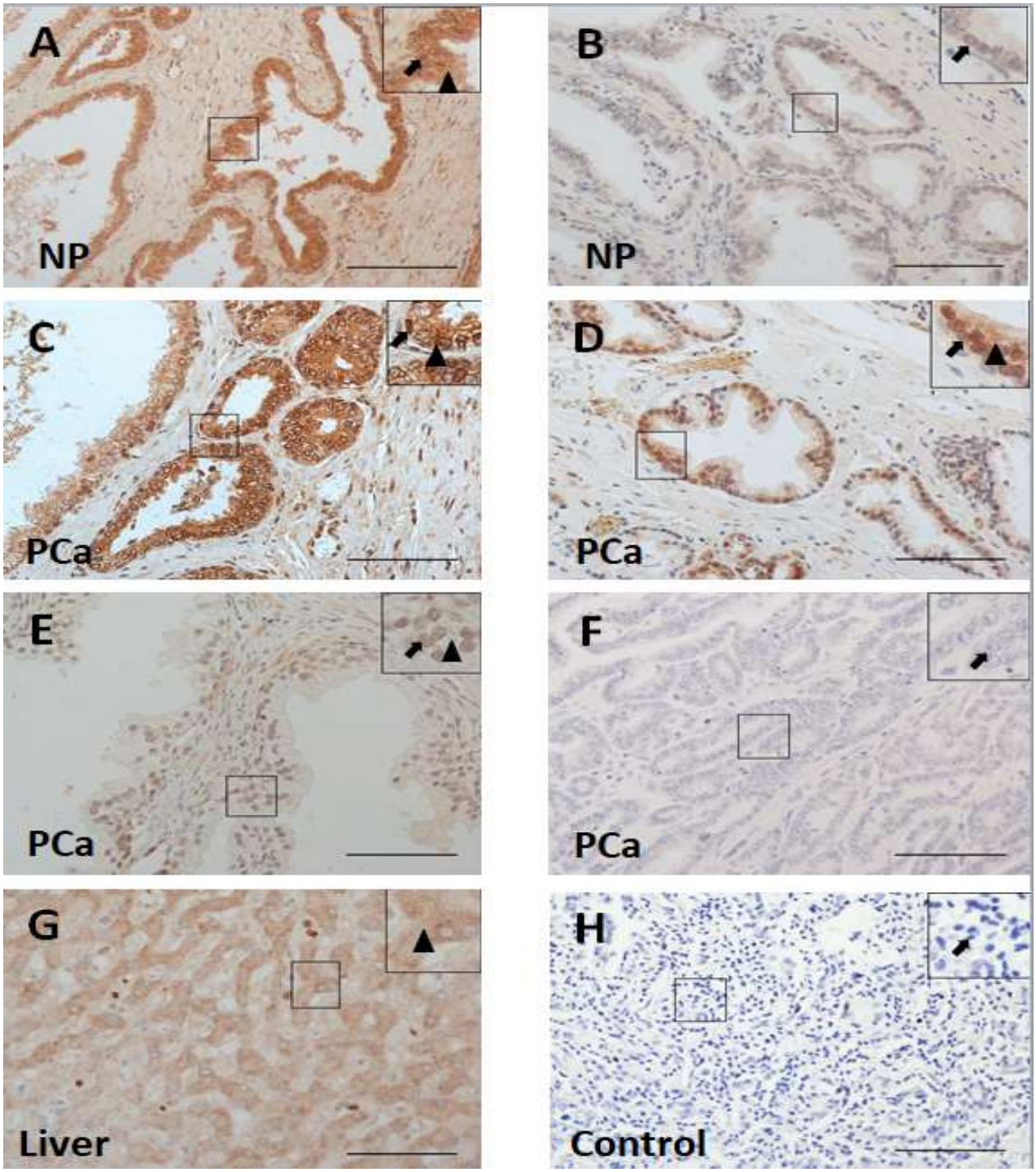

Figure 1: Shows Sox7 expression in prostate tissues samples, using anti Sox7 A. A) Strong nuclear (arrow) and cytoplasmic (arrowhead) Sox7 expression was observed in the normal prostate tissue. B) Weak nuclear (arrow) Sox7 expression was observed in the normal tissue of prostate. C) Strong nuclear (arrow) and cytoplasmic(arrowhead) Sox7 expression was shown in PCa tissue. D) Strong nuclear (arrow) and weak cytoplasmic (arrowhead) Sox7 expression was observed in PCa tissue. E) Weak nuclear (arrow) Sox7 expression was observed in PCa tissue. F) There was no Sox7 expression (arrow) shown in PCa tissue. G) Cytoplasmic Sox7 expression (arrowhead) was shown in the normal liver tissue, as appositive control. H) Negative control

showed no background staining in PCa (arrow). PCa: Prostate cancer; NP: Normal prostate. Scale bars=100µm.

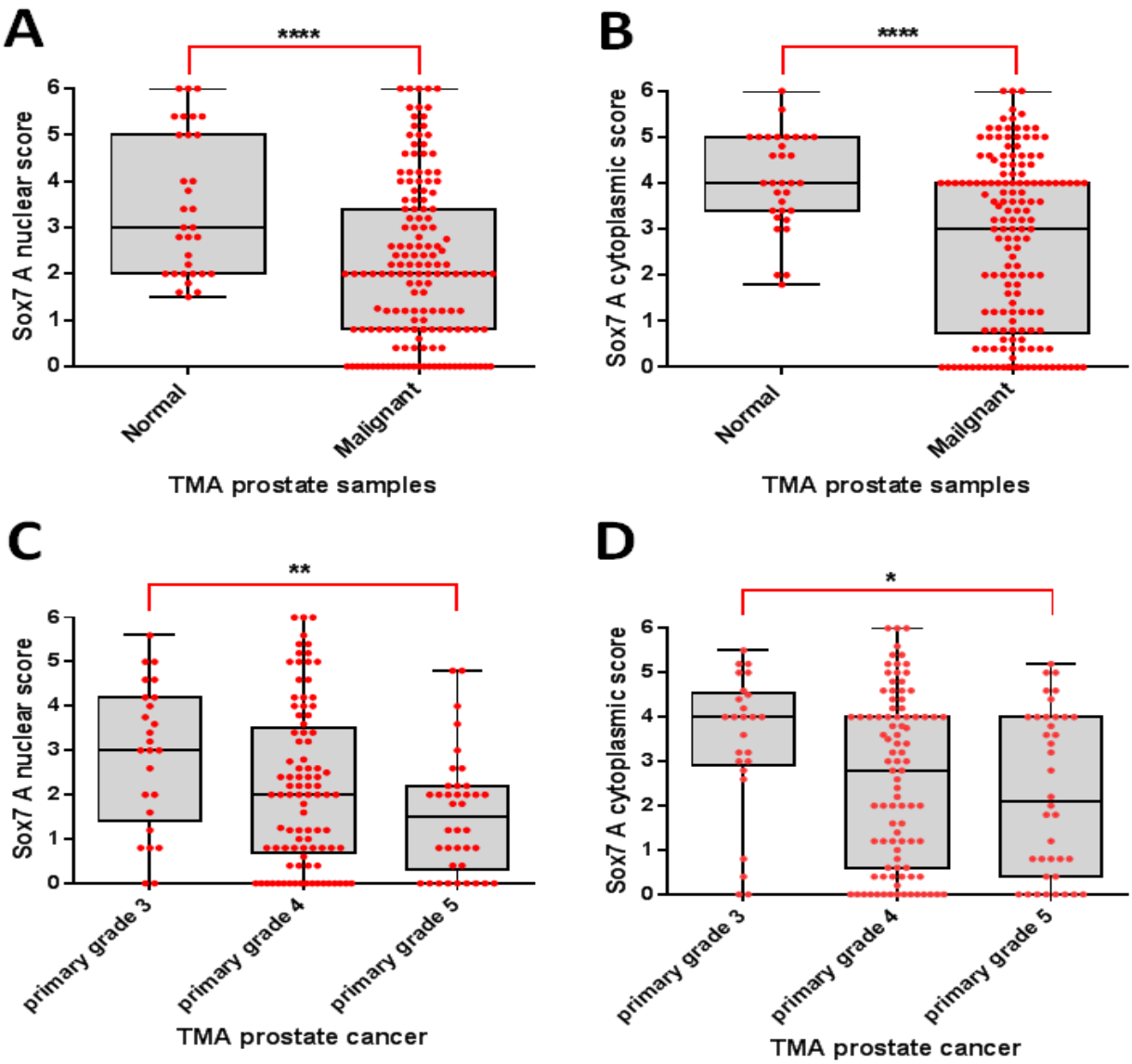

Figure 2: Quantification of nuclear and cytoplasmic Sox7 staining in both normal and malignant prostate tissues, susing Anti Sox7 A antibody. IHC Sox7 staining was quantified in the prostate tissues using the proportion and intensity scores for nuclear and cytoplasmic expression. A) A significant reduction of nuclear Sox7 expression was observed in PCa compared to normal tissues of prostate (p= < 0.0001). B) A significant reduction of cytoplasmic Sox7 expression was observed in PCa compared to normal tissues of prostate (p= < 0.0001). (C) Nuclear Sox7 expression showed a significant difference among different primary Gleason grade (p= 0.0084) and multiple comparison tests (Tukey) showed a negative association with increasing primary Gleason grades. This reduction was only statistically significant between primary Gleason grade 5 & 3 (p= 0.0061). (D) Cytoplasmic Sox7 expression showed a significant difference among different primary Gleason scores (P= 0.0088). Cytoplasmic anti-SOX7 staining was negatively associated with increasing grade, using multiple comparison tests (Tukey). This reduction of cytoplasmic Sox7 was found between primary Gleason grade 5 and 3

(p= 0.0429). Unpaired or one-way ANOVA tests were conducted to determine the statistical difference for each set of conditions. Normal prostate (n=16), Prostate cancer (n=80), primary grade 3 (n=13), primary grade 4 (n=46) and primary grade 5 (n=18). Y axis: Final score (proportion and intensity) of Nuclear and cytoplasmic Sox7 expression in each case.

Table 2: Summary of nuclear and cytoplasmic anti- Sox7 A staining results with clinical data in the TMA cohort.

Comparison	Nuclear Anti-Sox7 A Staining			Cytoplasmic Anti-Sox7 A Staining		
	Results		P. Value	Results		P. Value
Normal Vs Malignant	Lower In Malignant		< 0.0001	Lower In Malignant		<0.0001
Primary Gleason Grade (3,4 &5)	Lower In High Gleason Grade	Anova Test	0.0084	Lower In High Gleason Grade	Anova Test	0.0488
		Grade 4 Vs. Grade 3	0.1454		Grade 4 Vs. Grade 3	0.1048
		Grade 5 Vs. Grade 3	0.0061		Grade 5 Vs. Grade 3	0.0429
		Grade 5 Vs. Grade 4	0.1247		Grade 5 Vs. Grade 4	0.67
Stage (T)	No Statistically Significant Difference		0.685	No Statistically Significant Difference		0.9196
Stage (M)	No Statistically Significant Difference		0.9364	No Statistically Significant Difference		0.1572
Stage (N)	No Statistically Significant Difference		0.5601	No Statistically Significant Difference		0.5595

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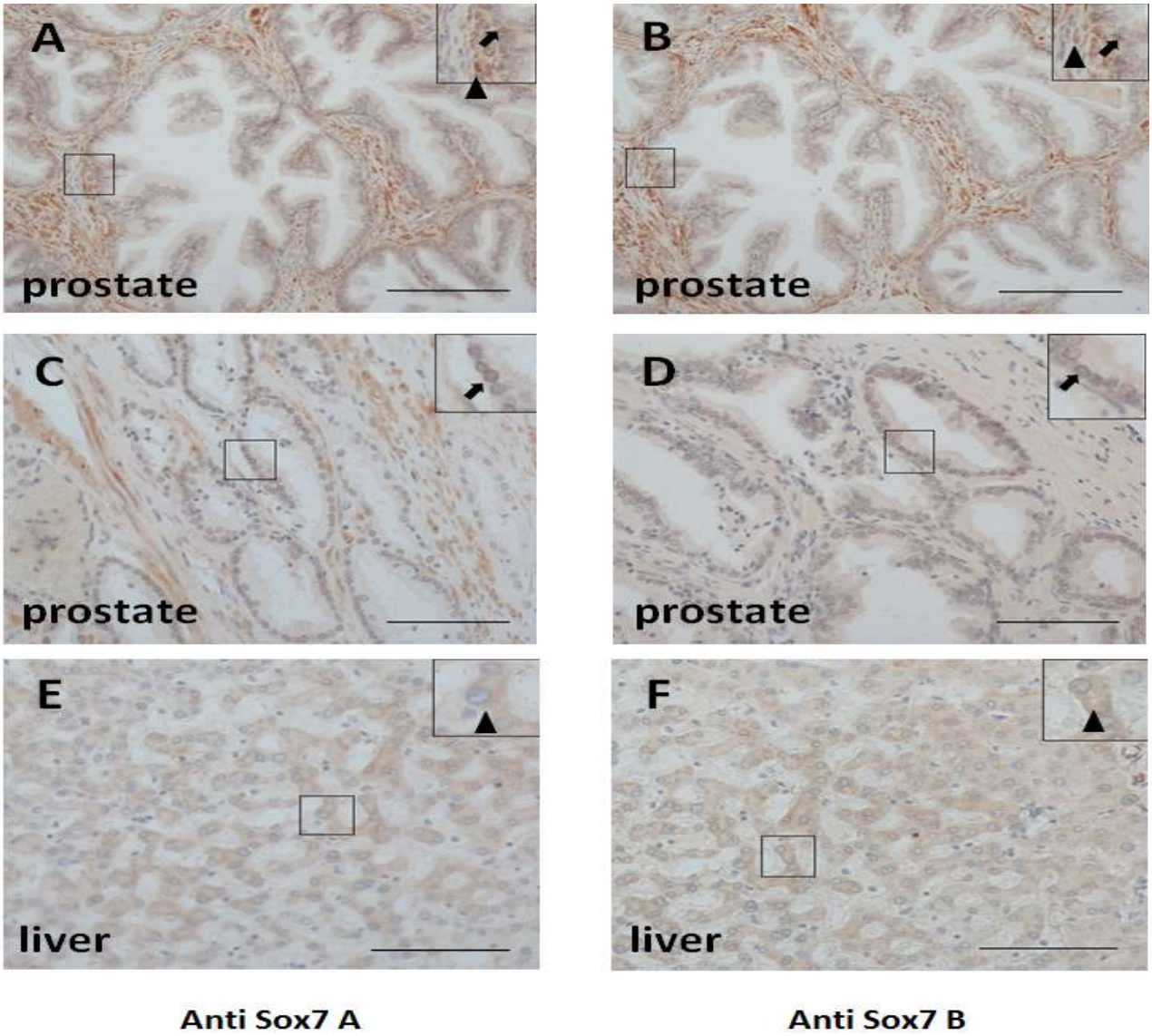

Figure 3: Two distinct Sox7 antibodies showed the expected Sox7 staining patterns in prostate and liver tissues. (A) Glandular (Black arrow) and stromal (Black arrowhead) Sox7 staining was observed in prostate tissue, using anti Sox7 A antibody (B) Glandular (Black arrow) and stromal (Black arrowhead) Sox7 expression was observed in prostate tissue, using anti Sox7 B antibody (C) Nuclear Sox7 staining was found in prostate tissues, using anti Sox7 A antibody (D) Nuclear Sox7 staining was seen in prostate tissues, using anti Sox7 B antibody (E) Cytoplasmic staining (Black arrowhead) Sox7 expression was shown in liver tissue, using anti Sox7 A antibody (F) Cytoplasmic staining (Black arrowhead) Sox7 staining was found in liver tissue, using anti Sox7 B antibody. Scale bars—100 μm with inserts at 2x zoom.

3) Sox7 IHC result in normal and malignant prostate tissues, using anti Sox7 B

A) Sox7 IHC staining in normal and malignant prostate tissues, using anti-Sox7 B

IHC result showed that Sox7 was found in expressed in cancerous and non-cancerous prostate tissues, using anti-Sox7 B. Nuclear Sox7 expression was found in all normal prostate tissues (16/16, 100%), with varying degrees of staining ranging from strong (Figure 4 A, arrow) to faint staining (Figure 4 B, arrow). Nuclear Sox7 expression was also detected in PCa tissues with

different level of staining, ranging from strong (Figure 4 C, arrow), weak and scattered (Figure 4 E, arrow) or negative (Figure 4 F).
Cytoplasmic expression of Sox7 was also seen in cancerous and non-cancerous prostate tissues with different degrees of signal staining, ranging from strong (Figure 4 D arrowhead), moderate (Figure 4 A, arrowhead), weak (Figure 4 B & F, arrowheads), and negative (Figure 4 G). A negative control showed negative staining in prostate tissue (Figure 4 H, arrow).

B) Decreased Sox7 expression in PCa and negatively associated with increasing primary Gleason grade and tumor size, using Anti Sox7 B.

Using Anti-Sox7 B antibody, Sox7 IHC staining was performed on normal and malignant tissues of prostate. The statistical analysis showed decreased nuclear and cytoplasmic Sox7 expression in PCa compared to normal prostate tissues (p= < 0.0001) (Figure 5 (A&B) & Table 3). There was a negative association between nuclear and cytoplasmic Sox7 expression and primary Gleason grade (p= <0.0001) (Figure 4 (C&D), Table 3). Using multi-comparison Tukey's tests showed that both nuclear and cytoplasmic expression of Sox7 was sigficanlty decreased in patients with grade 5 compared to those with grade 4 (p= 0.0061 & 0.0429, respectively), or grade 3 (p= <0.0001) (Figure 4 (C&D), Table 3). There was no asignficant assocaiton between expression of Sox7 and PCa clinical stage (Table 3), except the result of cytoplasmic Sox7 expression that showed a negtive linked with tumor size (P=0.0085) (Figure 4 F, Table 3). In summary, this data was consistent with the anti-Sox7 A IHC result. PCa was observed to have decreased nuclear and cytoplasmic Sox7 expression, which was negatively linked with Gleason grades but not stage (TNM).

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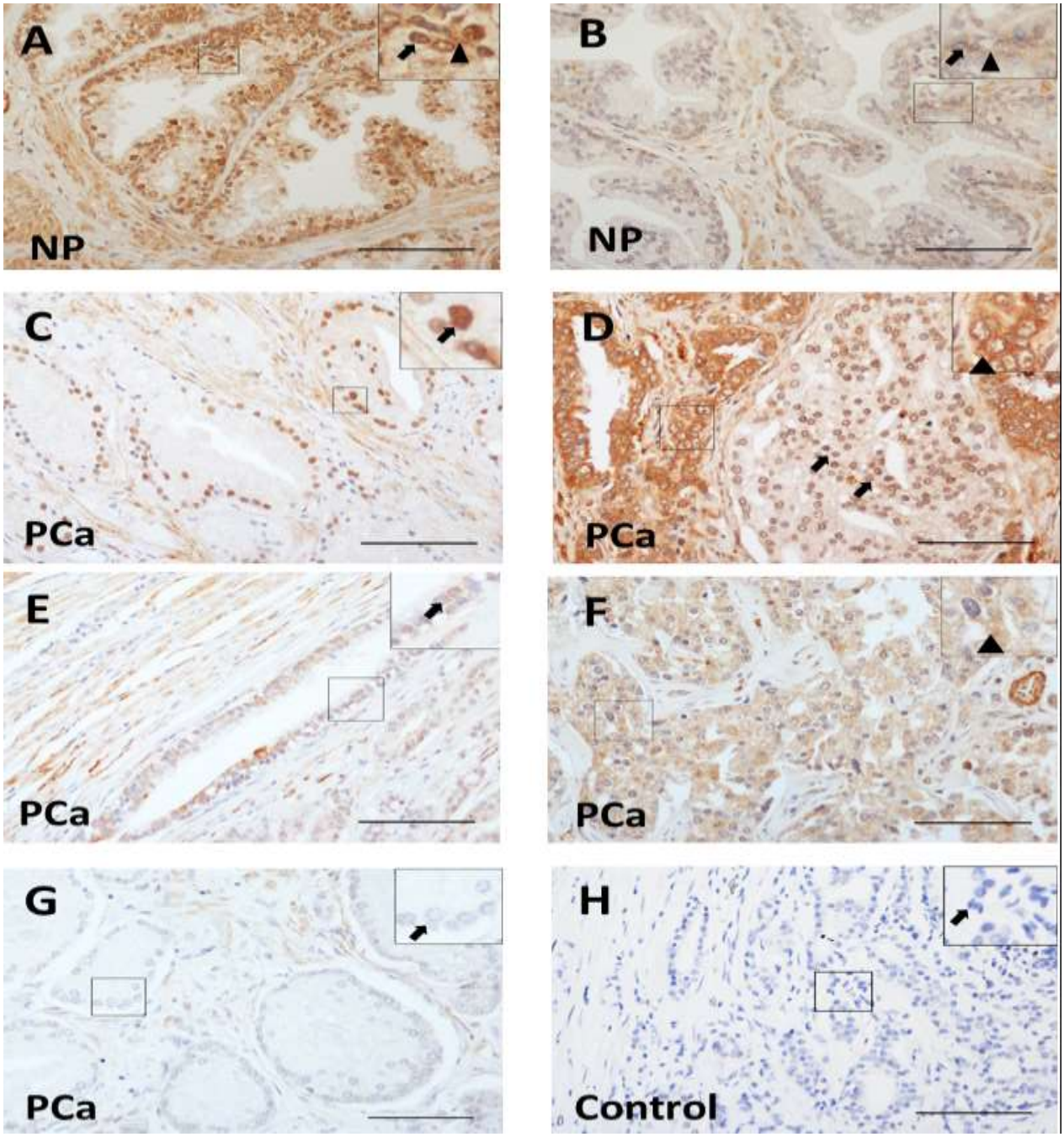

Figure 1: Shows Sox7 expression in prostate tissues samples. A) Strong nuclear (arrow) and moderate cytoplasmic (arrowhead) Sox7 expression was observed in the normal prostate tissue. B) Weak nuclear (arrow) and cytoplasmic (arrowhead) Sox7 expression was observed in the normal prostate tissue. C) Strong nuclear Sox7 expression was shown in Pca tissue. D) strong nuclear (arrows) and cytoplasmic (arrowhead) Sox7 expression was found in Pca tissue. E) Weak nuclear (arrow) Sox7 expression was observed in Pca tissue. F) Weak cytoplasmic (arrowhead) Sox7 expression was observed in Pca tissue. G) There was no Sox7 expression (arrow) shown in Pca tissue. H) Negative control was showed no background staining in PCa (arrow). PCa: Prostate cancer; NP: Normal prostate. Scale bars=100µm.

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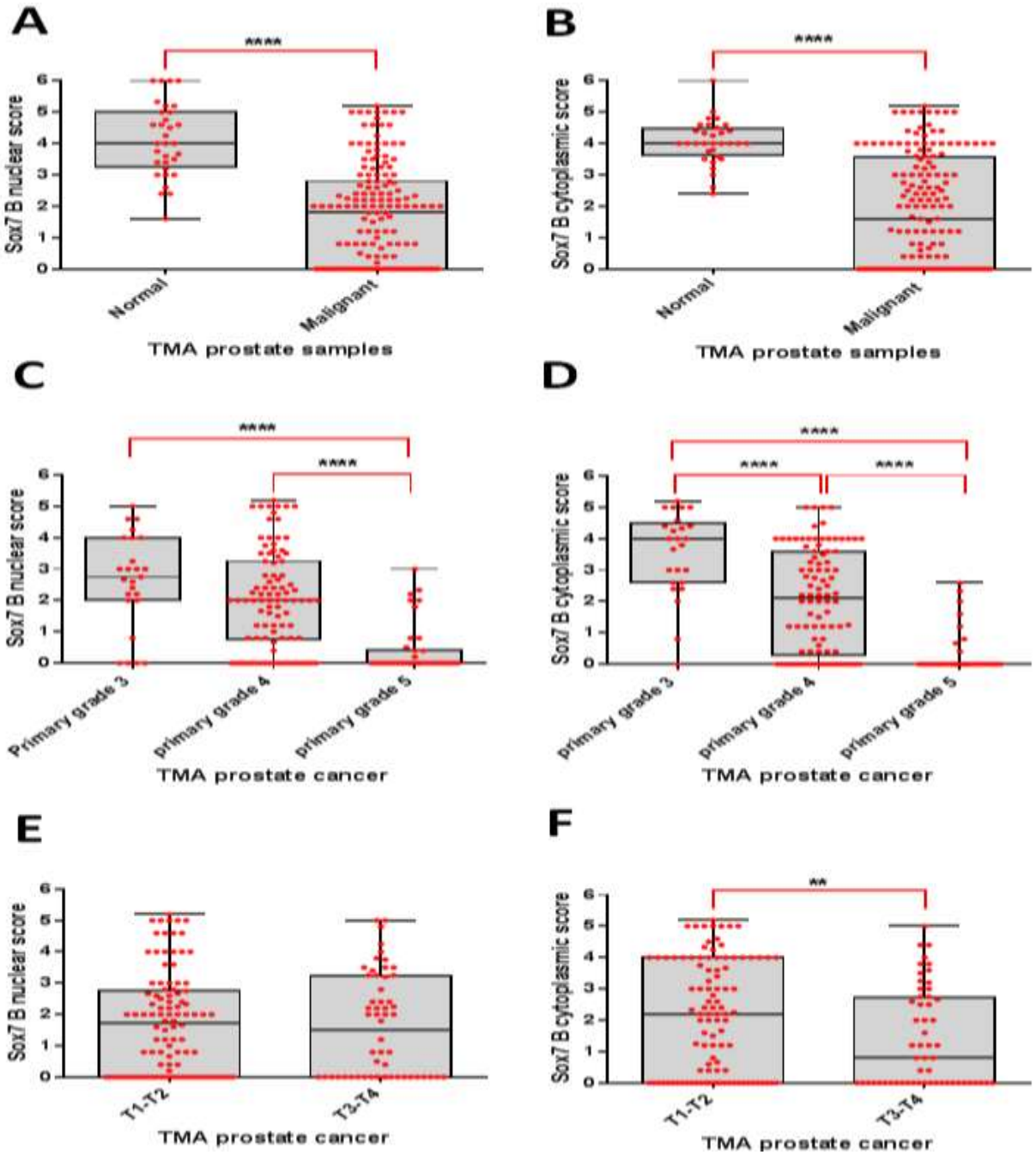

Figure 4: Nuclear and cytoplasmic Sox7 expression in normal and malignant prostate tissues quantified. The proportion and intensity scores for Sox7 nuclear and cytoplasmic IHC staining were used, using anti-Sox7 B antibody. (A) Decreased nuclear Sox7 expression significantly in PCa compared to NP tissues (p= < 0.0001). (B) Decreased cytoplasmic Sox7 staining significantly in PCa compared to NP tissues (p= < 0.0001). C) Nuclear Sox7 expression was negatively associated with increasing primary Gleason grade (p= <0.0001) and multiple comparison tests (Tukey) showed a significant reducing with increasing primary Gleason grades. When comparing PCa patient with a Gleason grade 5 to grade 3 (p= <0.0001) or grade 4

(p=<0.0001), the decreased was significant. D) Cytoplasmic Sox7 expression was observed to be negatively associated with primary Gleason grade (p= <0.0001). This reduction was significant when comparing PCa patients with a primary Gleason grade 5 to those with grade 3 (p= <0.0001) or grade 4 (p=<0.0001) and when comparing grade 4 tissues to those with grade 3 (p=<0.0001), using Multiple comparison tests (Tukey) test. E) There was no significant association between Nuclear Sox7 expression and tumor size T (p=0.6656). (F) There is a negative association between cytoplasmic Sox7 expression and Tumor size (p= 0.0085). Unpaired or one-way ANOVA tests were conducted to determine the statistical difference for each set of conditions. Prostate cancer (n=80), normal prostate (n=16), Primary grade 3 (n=13), Primary grade 4 (n=46) and Primary grade 5 (n=18), Tumor size T1-2 (n= 51) and Tumor size T3-4 (n= 28).Y axis: Final score (proportion and intensity) of Nuclear and cytoplasmic Sox7 expression in each case.

Table 3 Summary of nuclear and cytoplasmic anti- B staining results with clinical data in the TMA cohort.

Comparison	Nuclear Anti-Sox7 B Staining			Cytoplasmic Anti-Sox7 B Staining		
	Results		P. Value	Results		P. Value
Normal Vs Malignant	Lower In Malignant		< 0.0001	Lower In Malignant		<0.0001
Primary Gleason Grade (3,4 &5)	Lower In High Gleason Grade	Anova Test	< 0.0001	Lower In High Gleason Grade	Anova Test	< 0.0001
		Grade 4 Vs. Grade 3	0.1541		Grade 4 Vs. Grade 3	< 0.0001
		Grade 5 Vs. Grade 3	< 0.0001		Grade 5 Vs. Grade 3	< 0.0001
		Grade 5 Vs. Grade 4	< 0.0001		Grade 5 Vs. Grade 4	< 0.0001
Stage (T)	No Statistically Significant Difference		0.6656	Lower In T3-4		0.0085
Stage (M)	No Statistically Significant Difference		0.3205	No Statistically Significant Difference		0.136
Stage (N)	No Statistically Significant Difference		0.1808	No Statistically Significant Difference		0.0988

Discussion

Using two distinct anti-Sox7(A&B) antibodies, this study used IHC to look at Sox7 immunostaining in normal and malignant prostate tissues and see if there was a correlation between Sox7 immunostaining and PCa clinical and histopathological parameters, including primary Gleason grade, clinical stage. The current data revealed decreased nuclear and cytoplasmic Sox7 expresssion in malignant compared to non maligannt prostate tissues, using two different antibodies. This is consistent with other Sox7 data for breast, liver, pancreatic, ovarian, gastric and prostate cancers [16, 17, 19, 20,21]. In additon, Zhong, *et al* study in China found that 147 PCa tissues had downregulated Sox7 expression compared to 28 normal prostate tissues [21]. Taken together, these studies suggest that reduction of Sox7 level might represent a common phenomena in different kind of cancer, including PCa and this protein may have an essential role in tumor development.

In addition, this study showed a negative association between Sox7 expression and primary Gleason grade, using two different antibodies. This result agreed with the previous pancreatic and gastric cancer studies [16, 17]. According to previous studies, prostate and breast tumor cells may proliferate, migrate, invade, and form colonies less frequently when Sox7 is expressed [20]. This data showed no association between Sox7 expression and PCa clinical stage. Except, in patients with large tumor size T3-4 compared to those with smaller size T1-2, cytoplasmic Sox7 expression was significantly decreased. These results agreed with the previous pancreatic, breast and prostate carcinomas data [16, 19,20], suggesting that Sox7 expression may not be able to differentiate between PCa patients with different clinical stages, including invasion and metastasis. In contrast, this study was in consistent with the previous liver finding that showed a negative assocition between Sox7 expression and the clinical stage of liver cancer [19]. This difference could be due to a variety of factors, such as sample collection, antibodies used, as well as cancer type.

Functionally, the previous studies suggest that a β-catenin interaction site can be found on the C-terminal region of the Sox7 protein [14, 20, 23]. β-catenin/T cell factor (TCF) regulated transcription (CRT) has been shown to be inhibited by Sox7 [23]. It has been found that Sox7 may reduce β-catenin /T cell factor (TCF) regulated transcription (CRT) [23]. In addition, another study found that Sox7 interacts physically with the β-catenin via its β-catenin binding motif before inhibiting its activity [16,20,24], indicating that Sox7 negatively controls active β-catenin. Taken together, The loss of overall β-catenin expression observed in PCa may be partially offset by an increase in active β-catenin, which would aid in the development and spread of tumors. The Wnt/ β-catenin signaling pathway is interestingly controlled by Sox7 and Axin-2 (Axin inhibitory protein-2), with Sox7 expression being lower in breast cancer than in the healthy breast [16].

Different strategies have been proposed to reduce Sox7 expression in PCa, including promoter hypermethylation and allelic deletion [20]. The previous study showed that the promoter region of Sox7's often methylated CPG island has a significant role in controlling the expression of

Sox7 [14]. Using methylation-specific PCR (MSP), it was found that Sox7 level was significantly lower in PCa and PCa cell lines compared to normal prostate and cell line of normal prostate as a result of a tumour specific promoter hypermethylation that was seen in 48% of PCa and 44% of PCa cell lines [20]. Another study indicated that Sox7 promoter hypermethylation, which was increased in breast cancer cell lines compared to normal cell line of breast, was the cause of the reduced Sox7 mRNA and protein levels in breast cancer cell lines compared to normal breast cell lines [14]. Another data indicated that 18 PCa samples (61%) exhibited allelic loss, and that 64% of PCa (7/11) had both promoter methylation and allelic loss [20]. Sox7 gene deletion is another mechanism for Sox7 loss in malignancies, including PCa. The Sox7 gene is located on a short arm of chromosome 8 that is frequently lost in a variety of malignancies, including PCa, according to pervious data [14, 25].

Conclusion

Sox7 expression was observed to be decreased in PCa, and it was inversely correlated with the primary Gleason grade. Sox7 reduction could be a sign of aggressive PCa development and aggresivness. This data suggest that Sox7 may have a key role in PCa formation and progression and could be a potential biomarker for PCa. Therefore, the reduction of Sox7 is likely to be due to a combination of promoter hypermethylation and allelic loss. Further study is required to detect the mRNA level of Sox7 in normal and malignant prostate tissues using an RNAscope. It will be very interesting to exam the functional role of Sox7 in prostate cell lines using tissue culture.

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Conflict of Interests

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

Author Contributions

Dhafer wrote the first manuscript, did the experimental techniques, designed the entire study, and performed the statistical analysis. Collecting data was done by Dhafer .Writing - review & editing: Dhafer, Andrew and Paul. All authors read and approved the final manuscript.

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