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ALDH1A1 expression in benign and malignant prostate tissues

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Summary

Background: Prostate cancer has few prognostic biomarkers, and differentiating between relapsing and non-relapsing tumour can be challenging clinically.

Aims: This study aims to investigate the hypothesis that ALDH1A1 might be a potential biomarker for prostate tumors and could distinguish between aggressive tumours requiring radical intervention and those that have a good prognosis. It is thought to be a potential biomarker that can predict the clinical progression and prognosis of different kinds of tumours. However, its role in prostate cancer remains unclear.

Materials and methods: ALDH1A1 expression has been evaluated by immunohistochemistry using 96 prostate tissue samples including normal, adjacent normal and cancer tissues.

Results: This study showed that ALDH1A1 was expressed in glandular and stromal regions of both benign and malignant prostate tissues. Nuclear and cytoplasmic ALDH1A1 was increased significantly in glandular regions of prostate cancer and was positively associated with increased tumor size. However, stromal ALDH1A1 staining was not associated with prostate cancer and other parameters such as primary Gleason grade and stage.

Conclusion: This preliminary data suggests that ALDH1A1 may have a role in cancer development and/or aggressiveness and warrants further investigation to understand its function and establish if it could be a potential diagnostic biomarker for prostate cancer.

Keywords: **Prostate cancer, ALDH1A1, Immunohistochemistry**

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Introduction

Prostate cancer (PCa) is an abnormal growth that usually begins in the prostate gland. It is a heterogeneous disease and represents the second most frequent malignancy and is the second highest cause of death in males after lung cancer (1,2). Adenocarcinoma is the most common type of PCa which is found in more than 90% of Pca patients, and it originates from the glandular regions of the prostate gland (3,4). A few diagnostic and prognostic biomarkers have been identified for PCa, including prostate-specific antigen (PSA) (5). However, there is a need for more specific and/or sensitive biomarkers in PCa diagnosis and especially for measuring PCa prognosis. The major goal of this study was to identify proteins that are differentially expressed between normal and malignant prostate tissues and/or between different Gleason grades and clinical stages. This is important to improve our understanding of the molecular basis of PCa formation and progression and potentially help in the development of future biomarkers.

Aldehyde hydrogenase 1 family member A1 (ALDH1A1) represents a marker of CSCs (6,7). This protein is localized in the cytosol of different kinds of human tissues, including the prostate (8). It has been reported that ALDH1A1 plays a role to catalyse the oxidation of retinal to retinoic acid that represents a regulator of cell differentiation during the development process (8,9), and is thought to have an essential role in cancer progression (7). Of particular importance for this study, altered expression levels and localisation of ALDH1A1 has been suggested to play a role in PCa formation and progression.

Materials and methods

This retrospective study was covered by the National Health Service (NHS) ethical and research approval (REC reference: 13/WS/0153; IRAS project ID: 112241). In this study, a TMA cohort (PR1921) consists of 96 cases, 80 of them were PCa, whereas, the rest were normal or normal tissues that were adjacent to the PCa, termed adjacent normal (8 cases for each). Each case was represented with two core tissue biopsies to form a total of 192 cores. The clinical data of the patients are shown in Table 1.

Table 1: Clinical data of prostate sample in TMA cohort.

Clinical data		TMA cohort %
Number of samples	Normal	16
	Malignant	80
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%)
T category	T1-T2	51 (63.8%)
	T3-T4	28 (35%)
	N/A	1 (1.2%)
N category	N0	65 (81.2%)
	N1	14 (17.5%)
	ND	1(1.3%)
M category	M0	64 (80%)
	M1	15 (18.7%)
	ND	1 (1.3%)

Immunohistochemistry

Immunohistochemistry (IHC) staining was carried out using anti-ALDH1A1 rabbit monoclonal (Abcam, catalogue number Ab52492). 5um thick sections of prostate tissues were baked overnight at 37°C. Prior to IHC, deparaffinization and rehydration through graded ethanol series of decreasing ethanol concentration (100%, 95% & 70% respectively) for a minute each concentration was necessary to remove the paraffin from tissues and to rehydrate tissue samples, respectively. Tissues were then permeabilized with 0.5% Triton X-100 in phosphate buffer saline (PBS), subjected to heat-induced epitope retrieval in a citrate buffer, pH 6 with 0.05% Tween 20 for 30 minutes at 90°C, and allowed to cool to room temperature for 20 minutes. Subsequently, the sections were incubated in 3% H_2O_2 (Dako peroxidase) at room temperature for 10 minutes, followed by rinsing gently three times with Phosphate buffer saline (PBS) for 5 minutes each. After blocking for 30 minutes in 10% normal goat serum and 0.5% BSA in PBS, samples were treated with anti-ALDH1A1 antibody, dilution1:100 (Dako, Ely, UK) overnight at 4°C.

On the next day, immuno-detection was performed using the EnVision+ Kit (K400611-2 and K401011-2, Dako, Ely, UK) following the manufacturer's instructions with DAB exposure for 5 minutes. The sections were counterstained with Vector Hematoxylin solution (H3401, Vector Laboratories, Peterborough, UK) at room temperature for a minute to stain the nucleus of cells. Slides then were rinsed thoroughly with the running tap water for 3 minutes. To differentiate the hematoxylin stain, the slides were then soaked three times in 70% ethanol with 1% HCl. The slides were also immersed for a minute in an alkaline solution that was prepared by adding 1% ammonium hydroxide to 70% ethanol to restore the bluing stain of Haematoxylin. At this point, the staining steps were finished. After that, the slides were washed with two changes of different ethanol concentrations 95% and 100% for a minute. Slides were then washed twice with Histoclear for 2 minutes each. The next day, the slides were ready to examine under a light microscope (Nikon Eclipse E800) equipped with a Nikon digital camera (DS-U1 CCD). The procedure of IHC was carried out according to (15).

For assessment of IHC staining, the whole sections were examined under a 20x objective to determine the glandular (nuclear and cytoplasmic) and stromal (proportion and intensity) expression of ALDH1A1 staining in prostate tissues. The nuclear and cytoplasmic ALDH1A1 staining was scored using a semi-quantitative scoring system as the following: the percentage of positive cells was scored as: (0: negative, 1: 1- 33%, 2: 34-65 % and 3: 66-100 %.) and the intensity was graded as (0: negative, 1: weak, 2: moderate; and 3: strong). The final score represents the sum of the proportion and intensity scores, which ranged from 0 to 7 (10). In addition, The proportion of stromal stained cells was scored as follows: (0: negative, 1: 1-33%, 2: 34-65 % and 3: 66-100 %.) (10). The intensity of stromal staining was scored as follows: (0: negative, 1: weak, 2: moderate and 3: strong) (10).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com, including mean, standard error and standard deviation values as well as the other statistical analysis such as a frequency distribution test and histogram. Statistical analysis was carried out either using unpaired T-test or one-way A-NOVA with Tukey's multiple comparisons tests. Results were considered significant if the P. value was ≤ 0.05.

Results

A) Immunohistochemical staining of ALDH1A1 in normal and malignant prostate samples

ALDH1A1 staining was observed in the glandular and stromal regions of the TMA prostate tissues. Nuclear ALDH1A1 staining was expressed in the glandular regions of the normal and malignant prostate tissues with different levels of staining patterns, ranging from strong (Figure 1 B&E arrows) to weak (Figure 1 D, arrow) or negative (Figure 1 A& G, arrows). Cytoplasmic ALDH1A1 staining was also shown in the glandular regions of normal and malignant prostate tissues, ranging

from strong (Figure 1 F, arrowhead) to weak (Figure 1 E, arrowhead) or negative (Figure 1 G). Interestingly, nuclear and cytoplasmic ALDH1A1 staining was found to be restricted in the basal cells of NP, whereas, it was found to be expressed in all PCa epithelial cells, which are mainly luminal, with only a few residual basal cells.

In addition to the glandular ALDH1A1 (nuclear and cytoplasmic) staining, the normal and malignant tissue samples had stromal ALDH1A1 staining, with different staining levels, ranging from strong (Figure 1 A & C, arrowheads) to weak (Figure 1 G, arrowheads). The negative control was free from background staining on prostate samples (Figure 1 H, arrow).

B) Association between ALDH1A1 immunostaining with histopathological parameters of prostate cancer.

Having carried out IHC staining, nuclear and cytoplasmic ALDH1A1 staining was then quantified using the proportion and intensity 3 scores, whereas stromal proportion and intensity ALDH1A1 staining were quantified using the proportion 1 and intensity 1 respectively, as carried out for the Bath cohort samples. The range of nuclear and cytoplasmic and proportion and intensity stromal ALDH1A1 staining varied between 0-6, 0-6, 0-3, and 0-3 respectively (Figure 2). The potential association between ALDH1A1 IHC and histopathological parameters of PCa was then analysed and is described below.

The statistical analysis looked at the ALDH1A1 staining in normal vs. malignant prostate tissues from the TMA cohort. Nuclear ALDH1A1 staining was expressed in 60% of NP and 41% of PCa tissues and the IHC analysis showed nuclear ALDH1A1 significantly increased significantly in PCa compared to NP tissues (p= 0.0451) (Figure 2 A & Table 2). Cytoplasmic ALDH1A1 staining was also observed in 25% of normal and 41% of malignant prostate tissues. Statistically, there was a significant increase in cytoplasmic ALDH1A1 scores in PCa compared to NP tissues (p= 0.0198) (Figure 2 B & Table 2). There was no significant association between stromal proportion and intensity of ALDH1A1 staining in normal vs. malignant prostate tissues (p= 0.6832 & 0.4775 respectively). Both glandular and stromal ALDH1A1 staining was not associated significantly with primary Gleason grade (Tables 2).

The statistical analysis showed nuclear and cytoplasmic ALDH1A1 staining significantly increased in advanced stage T (T3-4) compared to localised PCa stage T (T1-2) (P= 0.0011&0.0201 respectively) (Figure 2 C&D & Table 2). In contrast, nuclear and cytoplasmic ALDH1A1 showed no significant difference between N (N0 vs N1) (p= 0.2337 & 0.9157 respectively) and M (M0 vs M1) (p= 0.0566 & 0.4646 respectively) (Table 2). There was also no significant association between Stromal ALDH1A1 staining and clinical stage TNM.

In summary, nuclear and cytoplasmic ALDH1A1 staining was increased significantly in PCa compared to NP tissues and was positively associated with advanced PCa stages T (T3-T4 vs T1-T2), but not with primary Gleason grade or other clinical stages (M&N). Stromal ALDH1A1 staining (proportion and intensity) was also not associated with PCa or other parameters such as primary Gleason grade and stage.

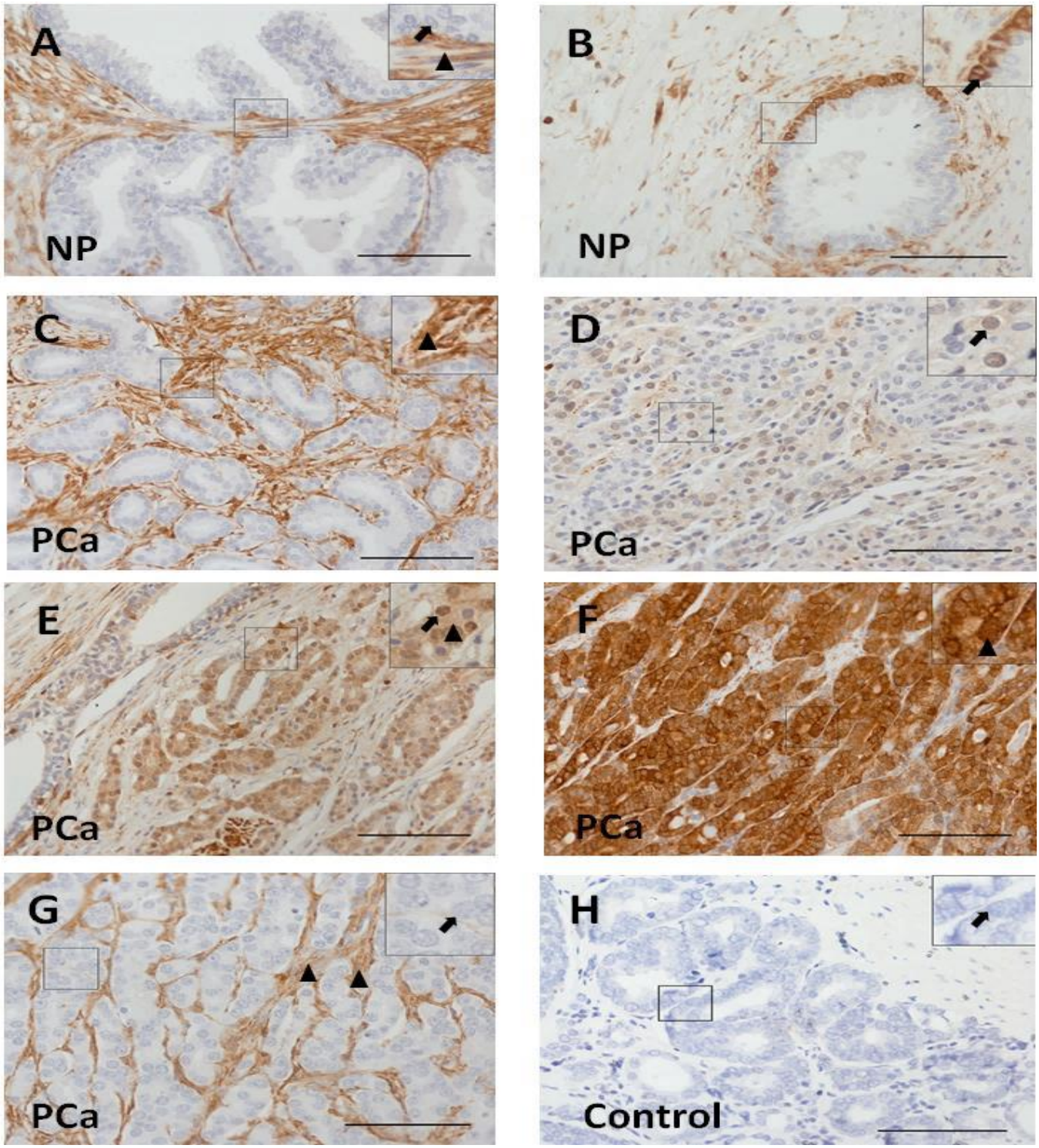

Figure 1: ALDH1A1 was stained heterogeneously in both normal and malignant tissues of the prostate. (A) ALDH1A1 was stained in the stromal region (arrowhead), but not in the glandular region (arrow) of NP. (B) Nuclear AlDH1A1 staining (Black arrow) in the basal cells of NP. (C) Strong stromal (Black arrowhead) with negative glandular ALDH1A1staining in PCa. (D) Weak nuclear ALDHIA1 staining (Black arrow) in the Glandular region of PCa. (E) Strong nuclear (Black arrow) with weak cytoplasmic (Black arrowhead) ALDH1A1 staining in the glandular region of PCa. (F) Strong cytoplasmic ALDH1A1 staining (Black arrowhead) in the glandular region of PCa. (G) Negative ALDH1A1 staining (Black arrow) with weak stromal staining in PCa. (H) The negative control (no primary antibody) showed no staining (Black arrow) in PCa tissue. Scale bars=100µm.

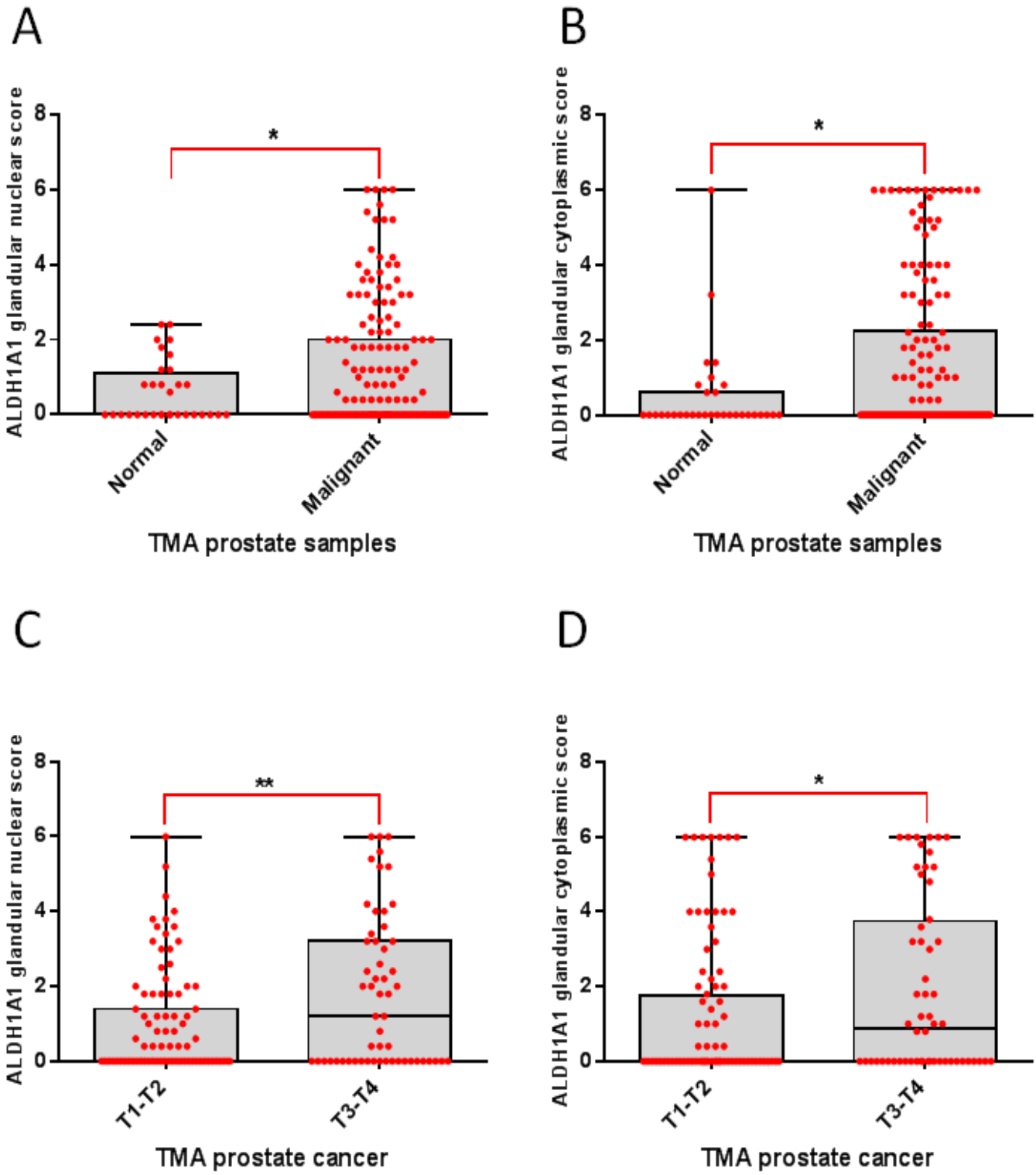

Figure 2 Quantification of nuclear and cytoplasmic ALDH1A1 staining in the glandular region of normal and malignant TMA prostate tissues. IHC staining of ALDH1A1 was quantified in the TMA group using the proportion and intensity 3 scores for nuclear and cytoplasmic IHC staining. (A) Nuclear ALDH1A1 staining was significantly increased in PCa compared with NP tissues (P= 0.0451). (B) Cytoplasmic ALDH1A1 staining was significantly increased in PCa compared with NP tissues (p= 0.0198). (C) Nuclear ALDH1A1 staining was significantly increased in advanced prostate cancer stages T (T3-4) compared to localised PCa T (T1-2) (p= 0.0011). (B) Cytoplasmic ALDH1A1 staining was also significantly increased in advanced PCa stages T (T3-4) compared to localised PCa T (T1-2) (p= 0.0201). Unpaired t-tests were conducted to determine the statistical difference for each set of conditions. NP (n=16), PCa (n=80), T1-2 (n=51) and T3-4 (n=28).

Table 2 Nuclear and cytoplasmic ALDH1A1 staining results with clinical data

Comparison	Nuclear ALDH1A1 staining			Cytoplasmic ALDH1A1 staining		
	Result		P. value			Result
Normal vs malignant	Higher in malignant		0.0451	Higher in malignant		0.0198
Primary Gleason grades (3, 4 &5)	No statistically significant difference	Anova test	0.1224	No statistically significant difference	Anova test	0.5533
		Grade 4vs. Grade 3	0.9373		Grade 4vs. Grade 3	0.9598
		Grade 5 vs. Grade 3	0.9995		Grade 5 vs. Grade 3	0.6093
		Grade 5 vs. Grade 4	0.9024		Grade 5 vs. Grade 4	0.608
Stage (T)	Higher in PCa with high stage (T3-T4)		0.001	lightly higher in PCa with high stage (T3-T4)		0.0201
Stage (M)	No statistically significant difference		0.0566	No statistically significant difference		0.4646
Stage (N)	No statistically significant difference		0.2337	No statistically significant difference		0.9157

Discussion:

IHC demonstrated glandular and stromal staining for ALDH1A1 in both normal and malignant prostate tissues. This was consistent with a previous finding (10,11). ALDH1A1 staining was found to be restricted to basal cells of NP tissues but was expressed in all epithelial cells of PCa tissues.

Nuclear and cytoplasmic ALDH1A1 staining was increased significantly in PCa compared to NP and was positively associated with clinical stage T. The results largely agreed with (6,10,12), suggesting increased ALDH1A1 may play a role in PCa formation and progression. Nuclear and cytoplasmic ALDH1A1 staining was not associated significantly with primary Gleason grade and metastasis. These results are consistent with a previous gastric cancer study (7), but was inconsistent with other PCa studies (6,12). The cause of this difference is not known, but might be explained as the previous prostate studies (6,12) used different antibody concentrations and IHC staining protocols. It might also be that the difference is caused by differences in the patient populations. The current study shows for the first time that the stromal ALDH1A1 staining was not associated significantly with PCa and other histopathological parameters, including primary Gleason grade and clinical stage.

ALDHIA1 has been linked to the SC population and is thought to be a marker of SCs and CSCs (8). Other work reported that ALDH1A1 plays an important role in catalyzing the oxidation of retinal to retinoic acid (RA) that is linked to differentiation during the development of cells (8,12). Increased ALDH1A1 in cells could cause RA to accumulate in the nucleus and then either bind with RA receptor RAR and/ or retinoid X receptors (RXRS) to increase RARβ genes. These play a role in differentiation, apoptosis and growth inhibition and/ or increases c-Myc and Cyclin D1 transcription which can promote cell proliferation, anti-apoptosis and tumour growth in CSCs (8). Therefore, increased ALDH1A1 could lead to increased cell proliferation and tumour growth through its role in increasing RA levels. Further study is needed to confirm the staining patterns of ALDH1A1 in prostate tissues, using a second independent antibody in normal and malignant prostate tissues from both cohorts.

Future research is very important to determine the expression patterns of ALDH1A1 at protein and mRNA levels using IHC and RNAscope in a large TMA cohort from US Bio max (HproA180PGO5), which consists of 90 PCa and 90 adjacent NP tissues. The current array used in this work only had 16 NP cases, so the new array would allow confirmation of the link between the expression of these proteins and tumor formation. In addition, it will be important to validate the expression patterns of ALDH1A1 that was stained with a single antibody, using either a second independent antibody (IHC) and/ or mRNA probe (RNA scope).

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تعبير ALDH1A1 في أنسجة البروستاتا الحميدة والخبيثة

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الخلاصة

المقدمة: يحتوي سرطان البروستاتا على عدد قليل من المؤشرات الحيوية التنبؤية، ويمكن أن يكون التشخيص والتشخيص التفريقي للسرطان العدواني مقابل غير العدواني تحديًا سريريًا.

الهدف من الدراسة: تهدف هذه الدراسة إلى التحقق من الفرضية القائلة بأن ALDH1A1 قد يكون علامة بيولوجية محتملة لورم البروستاتا ويمكنه التمييز بين الأورام العدوانية التي تتطلب تدخلاً جذريًا وتلك التي لها تشخيص جيد. يُعتقد أنه علامة بيولوجية محتملة يمكنها التنبؤ بالتقدم السريري والتشخيص لأنواع مختلفة من الأورام. ومع ذلك ، لا يزال دوره في سرطان البروستاتا غير واضح.

المواد وطرق العمل: تم تقييم التعبير المناعي لـ NDRG1 بطريقة التصبيغ المناعي النسيجي الكيميائي باستخدام ٩٦عينه نسيجه طبيعية وسرطانيه.

النتائج: اظهرت هذه الدراسة أنه تم التعبير عن ALDH1A1 في المناطق الغدية والستروما في كل من أنسجة البروستاتا الحميدة والخبيثة. لوحظ زيادة التعبير النووية والسيتوبلازمي بشكل ملحوظ لمعلم ALDH1A1 في المناطق الغدية لسرطان البروستاتا وكان مرتبطًا بشكل إيجابي بزيادة حجم الورم. ومع ذلك، لم يكن تلطيخ الستروما لل ALDH1A1 مرتبطًا بسرطان البروستاتا ومعلمات أخرى مثل درجة السرطان ومرحلة جليسون الأولية..

الاستنتاج: تشير هذه البيانات الأولية إلى أن ALDH1A1 قد يكون له دور في تطور السرطان و / أو العدوانية ويتطلب مزيدًا من التحقيق لفهم وظيفته وتحديد ما إذا كان يمكن أن يكون علامة بيولوجية تشخيصية محتملة لسرطان البروستاتا.

الكلمات المفتاحية: سرطان البروستات، ALDH1A1 ، التصبيغ المناعي النسيجي الكيميائي