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ALDH1A1 as a Cancer Stem Cell Marker: Value of Immunohistochemical Expression in Benign Prostatic Hyperplasia, Prostatic Intraepithelial Neoplasia, and Prostatic Adenocarcinoma

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***Abstract*—**Introduction: Prostate cancer is one of the most common causes of morbidity and mortality in men in developed countries. Cancer Stem Cells (CSCs) could be responsible for the progression and relapse of cancer. Therefore, CSCs markers could provide a prognostic strategy for human malignancies. Aldehyde dehydrogenase 1A1 (ALDH1A1) activity has been shown to be associated with tumorigenesis and proposed to represent a functional marker for tumor initiating cells in various tumor types including prostate cancer. Material & Methods: We analyzed the immunohistochemical expression of ALDH1A1 in benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN) and prostatic adenocarcinoma and assessed their significant correlations in 50 TURP sections. They were microscopically interpreted and the results were correlated with histopathological types and tumor grade. Results: In different prostatic histopathological lesions we found that ALDH1A1 expression was low in BPH (13.3%) and PIN (6.7%) and then its expression increased with prostatic adenocarcinoma (40%), and this was statistically highly significant (P value = 0.02). However, in different grades of prostatic adenocarcinoma we found that the higher the Gleason grade the higher the expression for ALDH1A1 and this was statistically significant (P value = 0.02). We compared the expression of ALDH1A1 in PIN and prostatic adenocarcinoma. ALDH1A1 expression was decreased in PIN and highly expressed in prostatic adenocarcinoma and this was statistically significant (P value = 0.04). Conclusion: Increasing ALDH1A1 expression is correlated with aggressive behavior of the tumor. Immunohistochemical expression of ALDH1A1 might provide a potential approach to study tumorigenesis and progression of primary prostate carcinoma.

***Keywords*—**ALDH1A1, BPH, PIN, prostatic adenocarcinoma.

I. Background

PROSTATE cancer is considered the most prevalent cancer, as well as the second leading cause of cancer- related death among men. Mortality among prostate cancer patients is directly related to advanced disease and distant metastatic dissemination. Bone metastases contribute to significant proportions of prostate cancer-related deaths [1]. Patients with metastatic diseases are incurable and are generally candidates only for palliative hormonal therapy. However, early detection and diagnosis of prostate cancer can improve outcomes and provide more treatment options [1], [2]. Most cases of prostate tumors are treated through the traditional strategies of surgical resection and/or radiotherapy. In patients with metastatic disease, androgen targeted hormonal therapy is essential. However, a significant proportion of these patients have tumors that are unresponsive to hormonal therapy and will have limited treatment options [2].

Understanding the genetic and biochemical mechanisms of tumorigenesis of prostate cancer can provide us with an insight to key molecules that target prominent pathways involved in the development and progression of the disease. Several pathways involving proliferation, apoptosis, angiogenesis, androgen signaling and growth factor signaling have been shown to be altered in prostate cancer [3].

The cancer stem cell (CSC) hypothesis describes a unique subpopulation of stem-like cancer cells that possess the ability of self-renewal through altering developmental signaling pathways. These cells give rise to more aggressive tumors that have high metastatic potential, high risk of recurrence and are resistant to conventional therapy [4]. This hypothesis was first introduced in acute myeloid leukemia in 1997. Recently, CSCs have been found in several solid tumors, including breast, liver, lung, pancreatic, colorectal and brain cancers [5], [6].

Recent studies revealed that a small percentage of prostate tumors are compromised CSCs and are more tumorigenic compared to their progeny cells. They also possess the ability of self-renewal and high risk of metastasis characteristic of cancers of CSC. Indeed, initiation and progression of some prostate tumors are controlled by population of cancer stem cells (CSCs), which contribute to the high risk of metastasis, relapse and resistance to therapeutic interventions [7]. Therefore, special markers for CSCs may lead to early identification of these tumors and improvement in treatment strategies [8].

Aldehyde dehydrogenase (ALDH) enzymes are crucial for the detoxification of various toxins. They are responsible for the oxidization of intracellular aldehydes and the production of retinoic acid. Furthermore, many studies have shown that ALDH are involved in stem cell protection and differentiation [6], [9]. Increased ALDH activity has been linked to excessive proliferation of stem cell population [6]. ALDH has been considered as a CSC marker in certain solid tumors, such as breast, lung and colorectal cancers [9].

Given this evidence, we investigated whether the expression and activity of Aldehyde dehydrogenase 1A1 (ALDH1A1), an

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isoenzyme of the aldehyde dehydrogenase (ALDH) superfamily, is associated with tumorigenesis of prostate neoplasms.

II. Material and Methods

A. Patient Characteristics

This analysis is based on tissue samples obtained from male patients aged 50-85 years subjected to transurethral prostate resection biopsy and presented to El-Minia University Hospital between 2002 and 2004. These patients were diagnosed as one of: prostatic adenocarcinoma (prostate cancer), prostatic intraepithelial neoplasia (PIN) or benign prostatic hyperplasia (BPH). Patients' clinical records were compared with histopathological diagnosis. Patients who previously received hormonal therapy or chemotherapy were excluded from this study.

B. Tissue Sampling

A total of 50 formalin fixed paraffin embedded archival blocks were obtained from the department of pathology, faculty of medicine, El-Minia University. Sections were diagnosed as one of the following: prostate cancer, prostatic intraepithelial neoplasia (PIN) and benign prostatic hyperplasia (BPH).

C. Immunohistochemical Assay

The paraffin embedded blocks for each specimen were sectioned and subjected to routine haematoxylin and eosin staining and immunohistochemical study of ALDH1A1 antibodies, utilizing avidin biotin-peroxidase complex method. Routine haematoxylin and eosin stain was performed to confirm the original diagnosis and revise the grade according to Gleason grading system into well differentiated prostatic adenocarcinoma (Gleason score 2-4), moderately differentiated prostatic adenocarcinoma (Gleason score 5-7) and poorly differentiated prostatic adenocarcinoma (Gleason score 8-10) [10].

Immunostaining for ALDH1A1 was performed on 5 µm sections of formalin-fixed, paraffin-embedded blocks. Tissue sections were incubated at 60°C for 1 h, deparaffinized in xylene, and hydrated in a graded series of ethanol solutions. After a rinse in PBS (137 mMNaCl, 2.7 mMKCl, 1 mM KH2PO4, and 10 mM Na2HPO4; pH 7.4), endogenous peroxidase activity was quenched by incubating the sections for 15 min with 0.3% H2O2 in absolute methanol. After a 10-min rehydration in PBS, the sections were heated in a microwave oven for 3 min in 10 mM citrate buffer for antigen retrieval. Following incubation with blocking serum (4% normal horse serum) for 30 min at room temperature, sections were incubated overnight at 4°C with monoclonal antibodies, anti-ALDH1A1 (44/ALDH, 1:400, BD Biosciences). Small intestine was used as positive control. Negative controls were analyzed on adjacent sections incubated without antibody. After a PBS rinse, the sections were treated with biotinylated goat anti-mouse antibody for 15 min. The sections were washed with PBS and incubated with avidin-biotinylated horseradish peroxidase macromolecular complex for 10 min according to the manufacturer's instructions for the LASB kit. Sections were incubated with diaminobenzidine for 10 min and visualization of peroxidase was carried out. All were counterstained with hematoxylin, dehydrated in a series of ethanol solutions, cleared with xylene and coverslips were placed over mounting medium for microscopic evaluation.

D. Scoring of Positive Slides

Immunoreactive staining intensity of ALDH1A1 positive cells per 5 fields of view were rated according to the following scale: no visible staining = 0, faint staining = 1, moderate staining = 2, and strong staining = 3. The following percentages: 0 <10, 10-25, 25-50, 50-75%, and >75% were used to grade cells with positive staining. Final score was obtained by multiplying both scores. ALDH1A1 expression was then stratified at three levels; negative (a specimen without any expression of ALDH1A1), low level (a specimen with faint staining, demonstrating <10% of positive cells for ALDH1A1), and a high level (a specimen with more than 10% overall score) of ALDH1A1 expression [8].

E. Statistical Analysis

The significance of the results was assessed by determination of the probability factor "P" value using the Chi-square "x^2" test and Z test. The statistical analyses were performed with SPSS-software 11 (SPSS ASC GmbH, Erkrath, Germany). P value $\leq$ 0.05 was regarded as statistically significant.

III. Results

A. Histopathological Types and Grades

Of the total 50 cases, 15/50 cases were benign prostatic hyperplasia, 15/50 cases were prostatic intraepithelial neoplasia and 20/50 cases were diagnosed as prostatic adenocarcinoma. Of the total 15 cases of prostatic intraepithelial neoplasia, 4/15 (26.6%) of cases were graded as low grade PIN, whereas 11/15 (73%) of cases were graded as high grade PIN. Upon its microscopic appearance, Prostatic adenocarcinoma was graded according to Gleason grading system into well differentiated prostatic adenocarcinoma 4/20 (20%) of cases (Gleason score of 2-4). Moderately differentiated prostatic adenocarcinoma were 9/20 (45%) of cases (Gleason score of 5-7). Poorly differentiated prostatic adenocarcinoma 7/20 (35%) of cases (Gleason score of 8-10).

B. Age Distribution

The mean age for BPH patients was (45±3) years. For PIN, this was (63±3). Prostatic adenocarcinoma was found in older age group, with a mean age of (67±5) years.

C. The Immunoreactivity of ALDH1A1 in BPH

In BPH 10 out of 15 cases (66.8%) were ALDH1 negative (Fig. 1) and 3/15 (20%) of cases showed low level of expression of ALDH1A1. Only 2/15 (13%) cases of BPH showed high level of expression of ALDH1A1.

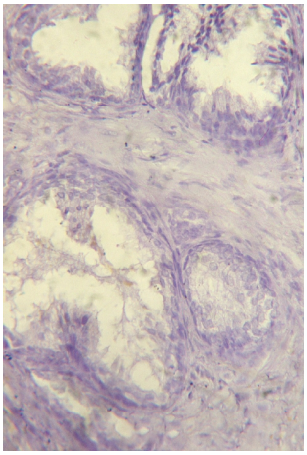
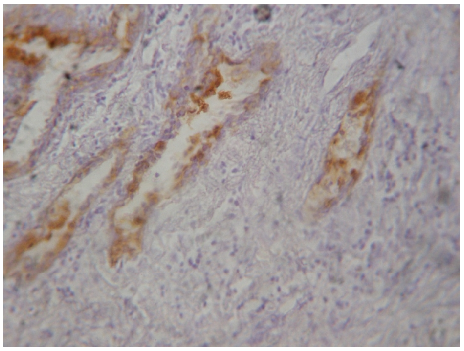


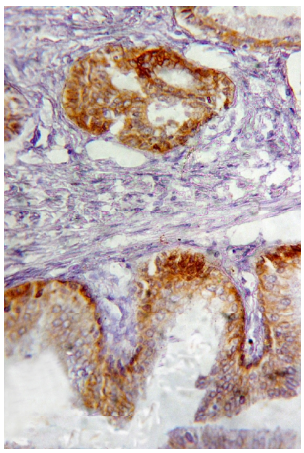
Fig. 1 Immunoreactivity patterns of ALDH1A1 in BPH, with negative cytoplasmic staining for ALDH1A1 (x400)

D. The Immunoreactivity of ALDH1A1 in PIN

Most cases of PIN showed low level of expression of ALDH1A1 but did not reach a significant level (P value = 0.2). In 9/15 (60%) of cases showed a low level of expression of ALDHA1, 5/15 (33.3%) cases were ALDH1A1 negative and only 1/15 (6.7%) showed a high level of expression (Table I). Furthermore, we also compared the level of ALDH1A1 expression in different grades of PIN. In low grade PIN, low level of expression of ALDH1A1was seen in 3/4 (75%) of cases (Fig. 2 (A)). Only 1/4 (25%) was ALDH1A1 negative. In high grade PIN, high level of expression of ALDH1A1 was demonstrated in 1/11 (9%) of cases (Fig. 2 (B)), low level of expression in 6/11 (55%) and the remaining 4/11 (36%) cases were ALDH1A1 negative (Table I).



(A)



(B)

Fig. 2 Immunoreactivity patterns of ALDH1A1 in PIN. (A) LGPIN shows low level of expression of ALDH1A1 with faint cytoplasmic staining (x200). (B) HGPIN shows high level of expression of ALDH1A1 with intense staining (x400)

TABLE I
THE IMMUNOREACTIVITY OF ALDH1A1 IN PIN AND PROSTATIC ADENOCARCINOMA

ALDHA1 expressions	Low grade PIN		High grad PIN		Total No.	P value*
	No. (4)	%	No. (11)	%	(15)	
High level expression	0	0%	1	9%	1	0.7 (>0.05)
Low level expression	3	75%	6	55%	9	
Negative	1	25%	4	36%	5	

ALDHA1 expression	WD		MD		PD		Total No.	P value*
	No. (4)	%	No. (9)	%	No. (7)	%	(20)	
High level expression	0	0%	2	22.2%	6	85.7%	8	0.02 (<0.05)
Low level expression	1	25%	4	44.5%	1	14.3%	6	
Negative	3	75%	3	33.3%	0	0%	6	

From the previous results, we concluded that most cases of both low grade and high grade PIN showed low level of expression of ALDH1A1 and statistical analysis failed to demonstrate a significant correlation between ALDH1A1 expression and PIN grades (P value = 0.7).

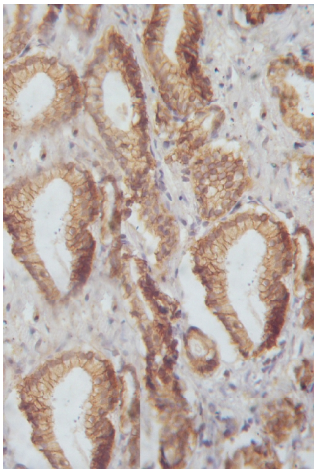
E. The Immunoreactivity of ALDH1A1 in Prostatic Adenocarcinoma

A high level of expression of ALDH1A1 was observed in 8/20 (40%) cases of prostatic adenocarcinoma but 6/20 (30%) of cases showed a low level of expression, also 6/20 (30%) of cases were ALDH1A1 negative. Low level of expression and negative expression was seen in equal percentage of cases, though this level was not statistically significant (P value = 0.7) (Table I).

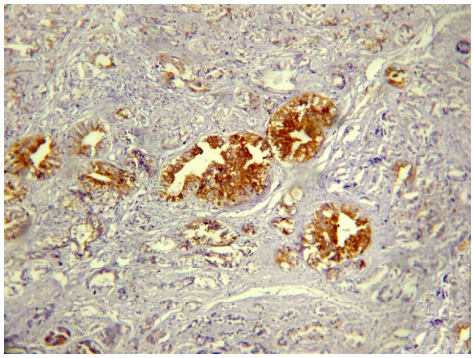
F. ALDH1A1 Expression and Correlation with Gleason Grade

Correlation was found between the expression of the ALDH1A1 and the grade of prostatic adenocarcinoma, and

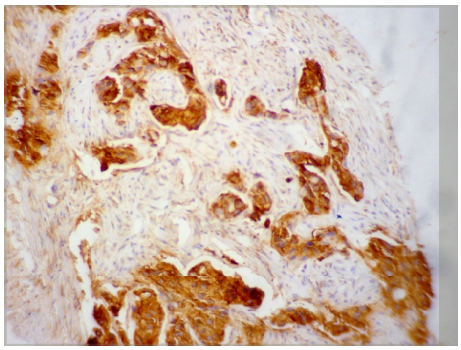
over-expression of ALDH1A1 directly correlates with higher Gleason grade.



(A)



(B)



(C)

Fig. 3 Immunoreactivity patterns of ALDH1A1 in prostatic adenocarcinoma. All samples demonstrate intense cytoplasmic staining and high level of expression of ALDH1A1 (A) Well differentiated prostatic adenocarcinoma (Gleason score of 4, grade 2+2) (x400). (B) Moderately differentiated adenocarcinoma (Gleason score of 6, grade 3+3) (x200). (C) Poorly differentiated adenocarcinoma (Gleason score 9, grade 4+5) (x400)

Well differentiated prostatic adenocarcinoma (WD) showed a low level of expression of ALDH1A1 in 1/4 (25%) of cases. No ALDH1A1 was expressed in the majority and 3/4 (75%) of cases were ALDH1A1 negative. Moderately differentiated prostatic adenocarcinoma (MD) showed a high level of expression of ALDH1A1 in 2/9 (22%) of cases, a low level of expression in 4/9 (44.5%) of cases and 3/9 (33.3%) cases were ALDH1A1 negative. Poorly differentiated prostatic adenocarcinoma (PD) showed a high level of expression of ALDH1A1 in 6/7 (85.7%) cases and low level of expression in 1/7 (14.3%). None of these tumors were ALDH1A1 negative (Table I) (Figs. 3 (A)-(C)). This direct relationship between Gleason grade and the level of expression of ALDH1A1 was statistically significant (P value = 0.02).

G. The Significance of the Level of Expression of ALDH1A1 in PIN and Prostatic Adenocarcinoma

A high level of expression of ALDH1A1 was demonstrated in only 1/15 (6.7%) of cases of PIN in contrast to 8/20 (40%) of cases of prostatic adenocarcinoma. A low level of expression of ALDH1A1 was demonstrated in 9/15 (60%) cases of PIN in opposition to 6/20 (30%) of prostatic adenocarcinoma. Absence of ALDH1A1 expression was demonstrated in 5/15 (33.3%) of PIN and 6/20 (30%) of prostatic adenocarcinoma (Table II). On that basis, the depth of invasion of prostatic neoplasia correlates with the expression of ALDH1A1. The low expression of ALDH1 in PIN in opposition to the high expression in prostatic adenocarcinoma was statically significant (P value = 0.04).

TABLE II
THE SIGNIFICANCE OF THE LEVEL OF EXPRESSION OF ALDH1A1 IN PIN AND PROSTATIC ADENOCARCINOMA

ALDHA1 expression	PIN		PC		P value*
	No. (15)	%	No. (20)	%	
High level expression	1	6.7%	8	40%	0.04 (<0.05)
Low level expression	9	60%	6	30%	
Negative	5	33.3%	6	30%	

* P value by Chi-square test. P value <0.05 denotes statistical significance. P value >0.05 denotes statistical insignificance.
PIN=Prostatic intraepithelial neoplasia, PC= Prostatic carcinoma, WD= Well differentiated, MD=Moderately differentiated, PD=Poorly differentiated.

IV. DISCUSSION

Several pathways that involve proliferation, apoptosis, angiogenesis, androgen signaling and growth factor signaling are altered in prostatic adenocarcinoma. Understanding the molecular biology and process of tumorigenesis of prostatic adenocarcinoma can facilitate the development of new treatment methods and optimize existing therapies [3], [11].

There is convincing evidence that several solid tumors, including breast, liver, lung, pancreatic, colorectal and brain cancers originate from CSC population. CSCs give rise to more aggressive tumors that have high metastatic potential, high risk of recurrence and are resistant to conventional therapy [4], [12]. Furthermore, recent studies revealed that a small percentage of prostate tumors are composed of CSC populations, which contribute to the severity and high risk of metastasis of some prostate cancers [7]. In vitro cultivation and identification of CSCs make the development of targeted

therapies possible [13], [14]. Such therapies can significantly decrease the risk of metastasis and recurrence [13].

Aldehyde dehydrogenase (ALDH) enzymes, a family of detoxification enzymes, are involved in CSC protection and differentiation. High activity of ALDH is associated with more aggressive forms of prostate cancers [6]. ALDH represents a functional marker of CSC activity in prostate cancer as well as other types of cancers [15]. ALDH may have a role in predicting aggressiveness of the diseases before treatment [16]. In addition, evidence suggests that ALDH enzymes play important roles in androgen receptor binding and retinoic acid metabolism, which are involved in the development of prostatic adenocarcinoma [17], [18].

In the present study, 30% of cases were diagnosed as BPH, 30% as PIN and 40% as prostatic adenocarcinoma. In a previously conducted study, 74.53% of samples were of BPH, 1.89% were PIN and 23.58% were prostatic adenocarcinoma. In cases of BPH, prostatitis was the most common associated lesion [19].

The mean age for BPH patients in our study was (45±3) years. For PIN, this was (63±3). For prostatic adenocarcinoma, it was (67±5) years. Prostatic adenocarcinoma tends to occur in older males compared to PIN and BPH, which occur in younger age group [13]. The incidence of PIN increases with advancing age [20]. PIN is first detected in the third decade of life. Size and grade of PIN lesions increase with advancing age [21]. The incidence of prostatic adenocarcinoma significantly increases after the age of 60 [8]. Advancing age is associated with higher rates of histological changes. The proliferation of any of the fibrous, muscular or glandular portions of the prostate can lead to hyperplasia. Glandular proliferation is highly associated with the development of malignancy [22].

BPH is one of the most common urological conditions affecting adult males [23]. While BPH is not life threatening, it can adversely affect quality of life due to lower urinary tract symptoms [24]. The pathogenesis of BPH is multifactorial. It involves abnormal signaling affecting cellular proliferation, hormone action and induction of inflammation [25]. In previous studies found that 66.8% of cases of BPH were ALDH1 negative and only 13% demonstrated a positive high level of expression [26]. In this study, 20% of cases of BPH demonstrated a low level of expression of ALDH1A1, an isoenzyme of ALDH superfamily, and 13% of cases showed high level of expression. The expression of ALDH suggests the presence of stem like cells in these samples. This indicates that these hyperplastic cells originate from highly proliferative stem cells and can ultimately lead to a clonal expansion of cell populations [27]. This can predict the risk of future development of prostate cancer in BPH patients. Detection of prostate stem cell antigen expression in BPH is an acceptable indication of the development of prostate cancer in these patients [28]. Identification of agents that target cell proliferation can reduce the risk of benign and malignant tumors of the prostate. As BPH and prostatic carcinoma represent the most common urological diseases and clinical burden in aging males, reduction in their incidence can have a positive impact on health services [29], [30]

Prostatic Intraepithelial neoplasia (PIN) is a unique precancerous lesion. High grade PIN is considered a precursor lesion for many intermediate and high grade prostatic adenocarcinomas [31]. PIN is considered as a transitional lesion from benign to malignant prostatic tumors [32], [33]. High grade PIN shares prominent genetic markers with prostatic adenocarcinoma. These include: loss of telomere length [34] and gain of chromosomes 7, 8, 9 and 10 [35]. More than 400 common genes were mutated in both HGPIN and prostatic adenocarcinoma [36]. In addition to the fact that incidence and severity of both diseases increase with advancing age, these genetic and cytological similar features can reinforce the evidence that PIN is a premalignant condition. HGPIN is the most established precursor of prostatic adenocarcinoma [37], [38]. ALDH1A1 expression was thought to be limited to cancerous glands, but interestingly, it was also expressed in PIN. In PIN samples contain both benign and neoplastic glands ALDH1A1 expression was restricted to PIN lesions, PIN can be considered as a precursor for prostatic adenocarcinoma [15].

In our study, it was found that none of the cases of low grade PIN showed high level of expression of ALDH1A1, whereas 75% of cases showed a low level of expression. In high grade PIN, 9% of cases showed a high level of expression. In another study, ALDH1A1 was detected in multiple samples of PIN and 11.1% of these samples showed a high level of expression of ALDH1A1 [26]. In the current study, 60% of cases of PIN showed a low level of expression of ALDH1A1 and 6.7% showed a high level of expression. However, statistical analysis did not demonstrate any significant correlation between ALDH1A1 expression and the grade of PIN grades (P value = 0.7).

Herein, 40% of cases of prostatic adenocarcinoma showed a high level of expression of ALDH1A1 and 30% of cases showed a low level of expression. High ALDH activity has been shown to be associated with tumorigenesis in various types of cancers, including prostatic adenocarcinoma [39]. In addition, previous studies have demonstrated a significant association between the level of ALDH1 expression and tumor stage [12]. In our current study, the level of expression ALDH1A1 showed a statistically significant association with Gleason grade as well (P value = 0.02). ALDH1A1 was significantly expressed at higher levels in high grade prostate cancer. About 85.7% of poorly differentiated prostatic adenocarcinoma showed a high level of expression of ALDH1A1. Expression of ALDH1A1 was upregulated in high grade prostatic adenocarcinomas compared to lower grade adenocarcinomas [39]. ALDH1A1 is suggested to have a correlation with Gleason score and an important role in prostate cancer progression. There is a significant association between high level of expression of ALDH1A1 with pathological diagnosis and Gleason score. Previous study had proved an existing association between the level of ALDH1A1 and histological grade [8], [10]. Therefore, analysis of ALDH activity may become a useful tool for risk stratification of

patients with possible distant metastasis [7]. In addition, identification and regulation of ALDH1A1/retinoic acid signaling pathway can have multiple clinical implications in managing patients [40].

V. Conclusions

Our study suggests that the expression of ALDH1A1 correlates with the aggressive behavior of prostate tumors and may act as a functional marker for prostate cancer. Immunohistochemical expression of ALDH1A1 can open new avenues for future research that can provide a better understanding of the cellular mechanisms of tumorigenesis and progression of primary prostate adenocarcinoma.

Conflict of Interest

The authors declare that there is no conflict of interests.

Ethical Approval

Collection of patient personal data was kept minimal. All data was stored in a secure database. Authors were the only individuals with access to this data.

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