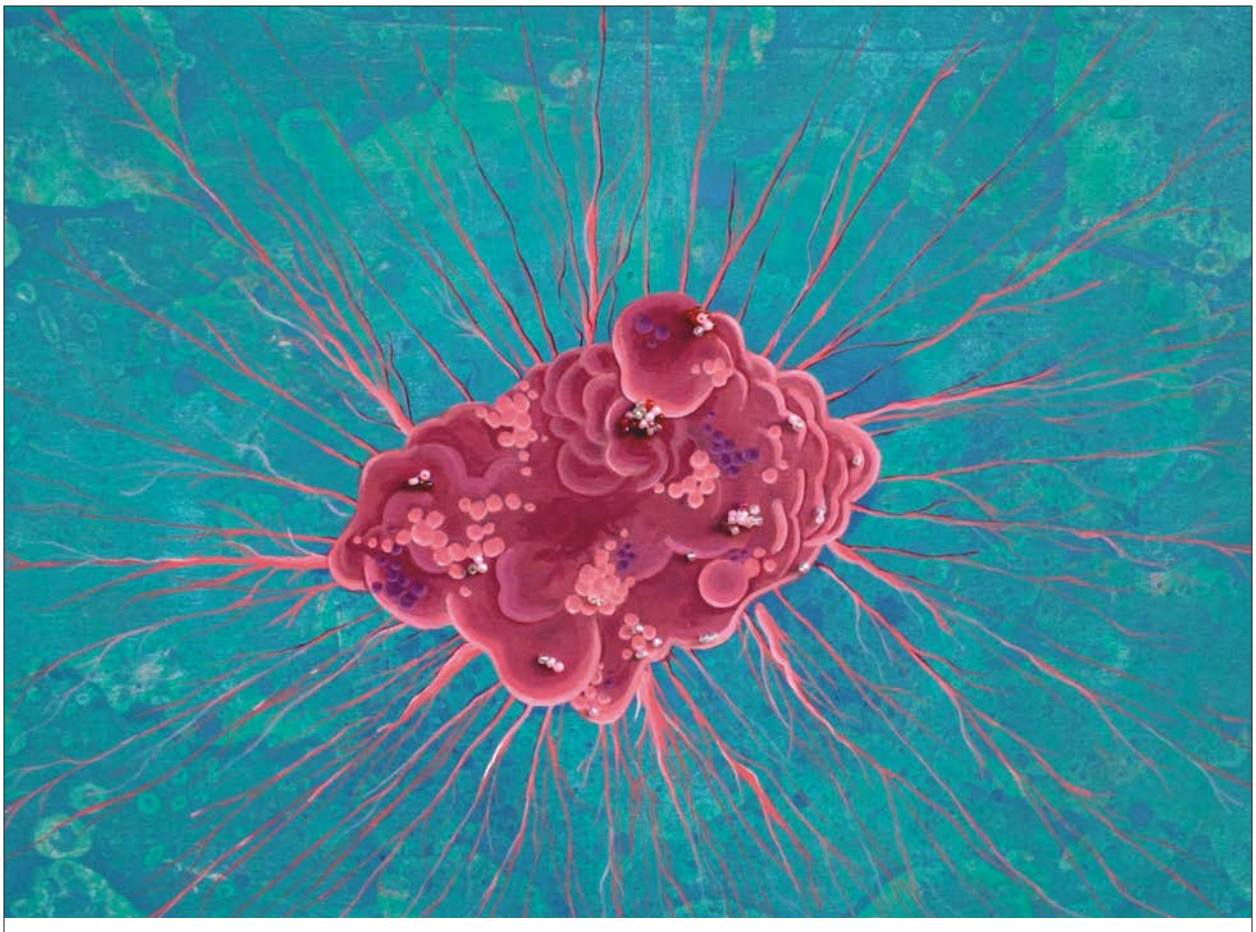

International Journal of Cancer Research

ISSN 1811-9727

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Cancer Research

ISSN 1811-9727
DOI: 10.3923/ijcr.2018.39.51

Research Article
Clinical Significance and Potential Utility of Cancer Stem Cell Markers: ALDH1A1 and CD133 in Prostate Tumors

[1]Hassan Fouad Huwait, [2]Anmar Mohammed Nassir, [1,3]Hanan Mohamed Abd Elmoneim, [4,5]Hala Fawzy Mohamed Kamel, [3]Nisreen Dahi Toni, [6]Nada Adel Babtain, [6]Alaa Sami Barhamain, [6]Abdul Basit Sadiq Malibari, [6]Shrouk Fares Munassar, [6]Raneem Sami Rawa and [7]Ashwak Zahed Kufiah

[1]Department of Pathology, Faculty of Medicine, Umm AL-Qura University, Makkah, Saudi Arabia
[2]Department of Surgery, Faculty of Medicine, Umm AL-Qura University, Makkah, Saudi Arabia
[3]Department of Pathology, Faculty of Medicine, Minyia University, Minyia, Egypt
[4]Department of Biochemistry, Faculty of Medicine, Umm AL-Qura University, Makkah, Saudi Arabia
[5]Department of Medical Biochemistry, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[6]Faculty of Medicine, Umm AL-Qura University, Makkah, Saudi Arabia
[7]Faculty of Pharmacy, Umm AL-Qura University, Makkah, Saudi Arabia

Abstract

Background and Objectives: Cancer stem cells (CSCs) have been shown to be associated with initiation of some prostate tumors with redirection towards cancer progression, metastasis and resistance to treatment. Therefore, evaluation of such markers for CSCs as aldehyde dehydrogenase (ALDH1A1) and CD133 may help in improving treatment modalities and better survival rates. Current study aimed to explore the clinical significance of expression levels of CSCs related markers: ALDH1A1 and CD133 in relation to other markers as androgen receptor (AR), prostate specific antigen and clinicopathological parameters in series of prostate tumors. **Materials and Methods:** Eighty-four male patients with prostate tumors recruited in this study, they included [n = 35] benign prostatic hyperplasia (BPH), [n = 17] prostatic intraepithelial neoplasia (PIN) and [n= 32] prostate cancer (PCa). Pre-operative blood samples examined for PSA by enzyme immunoassay and formalin-fixed paraffin-embedded archival blocks were assessed by immunohisto-chemical for expression of ALDH1A1, CD133 and AR, using avidin biotin-peroxidase complex method. Markers expression assessed microscopically and the data were analyzed and correlated with clinicopathological parameters. In addition, Kaplan Meier survival analysis was used to estimate overall survival of the patients. **Results:** Expression of ALDH1A1 levels were significantly higher in prostate cancer in comparison to both prostatic intraepithelial neoplasia and benign prostatic hyperplasia ($p<0.001$) while, CD133 expression showed statistically significant difference in between the three studied groups ($p = 0.001$). None of benign prostatic hyperplasia cases showed a high level of CD133 expression and (85.7%) of them showed negative expression of ALDH1A1. Expression of ALDH1A1 and CD133 showed positive significant correlation with prostate specific antigen and most of the studied clinicopathological parameters of prostate cancer, however, no correlation was found between CD133 expression and Gleason score. Joint expression of ALDH1A1 and CD133 had a significantly worse prognosis than the other groups ($p<0.001$) and is predictive of short survival duration. **Conclusion:** Joint detection of ALDH1A1 and CD133 helps in early diagnosis, prevention and improve predictions of prognosis for prostate tumors with better discrimination.

Key words: CSCs markers, ALDH1A1, CD133, androgen receptor, prostate tumors, prognosis, survival rate

Citation: Hassan Fouad Huwait, Anmar Mohammed Nassir, Hanan Mohamed Abd Elmoneim, Hala Fawzy Mohamed Kamel, Nisreen Dahi Toni, Nada Adel Babtain, Alaa Sami Barhamain, Abdul Basit Sadiq Malibari, Shrouk Fares Munassar, Raneem Sami Rawa and Ashwak Zahed Kufiah, 2018. Clinical significance and potential utility of cancer stem cell markers: ALDH1A1 and CD133 in prostate tumors. Int. J. Cancer Res., 14: 39-51.

Corresponding Author: Hanan Mohamed Abd Elmoneim, Department of Pathology, Faculty of Medicine, Umm AL-Qura University, Makkah, Saudi Arabia
Tel: 00966594455902

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Prostate cancer (PCa) is one of the most prevalent cancers among men. It is considered the second leading cause of cancer related death worldwide[1]. Early detection and diagnosis of PCa is the best measure to improve outcomes and decrease mortality rate among PCa patients[1-3]. A considerable group of patients with hormonal resistant PCa will have limited treatment options and may have faster deterioration[3]. The presence of unique cancer stem cells (CSCs) gives rise to more resistant, aggressiveness and metastasis with higher risk of recurrence and less cure rate with traditional therapy[4,5]. Recently, CSCs have been identified in many tumors, including breast, liver, lung, pancreatic, colorectal and brain tumors[6-9]. Many studies claim that a percentage of PCa are in fact accumulated CSCs and had a greater power of tumorigenesis compared to their progeny cells[10].

Aldehyde dehydrogenase (ALDH) is a family of isoenzymes involved mainly in detoxification of intracellular aldehydes generated during metabolism by oxidizing them to their corresponding carboxylic acids including retinoic acid, detoxified reactive oxygen species and reactive aldehydes. Furthermore, recent studies have shown that ALDH is involved in protecting stem cells and in enhancing differentiation[11-13]. Increased ALDH activity has been linked to profound proliferation of stem cell population in a variety of diseases, including cancer progression. Therefore, the application of pharmacological antagonists or activators of ALDH isoenzymes may provide a rational approach for treatment of cancer[12,14-16]. ALDH1A1 has been identified as a CSCs marker in many solid tumors, such as breast, lung, colorectal cancers and lymphoma[6,16-19].

CD133 is a glycoprotein with five transmembrane domains expressed in various stem cells and is a highly reliable CSC marker for many malignant tumors including lung cancers, colorectal cancers and brain tumors[20-23]. Accumulating evidence show that increased CD133 expression in PCa is an important CSC marker led to metastasis and poor prognosis[24]. In PCa cells that express CD133 are more likely, to be in the G2-phase of the cell cycle[25].

Androgen receptor (AR) is a transcription factor and member of the nuclear steroid receptor family. AR signaling has a great role in cancer growth with critical point in the development and progression of benign and malignant prostatic lesions. The use of AR targeted therapy remains crucial with evidence as an integral part of PCa[26]. AR was activated by androgen hormones through transcription factor entrance into the nucleus and interacts with several genes. The main action of AR drug antagonists is to block this interaction[27].

Cancer CSCs may have a pivotal role in tumor relapse and metastasis due to their abilities to self-renew, differentiate and give rise to a new tumor in local or distant organs. Previous reports have studied implications of stem cell related markers as ALDH1A1 in prostate cancer outcome[15] or therapeutic resistance and bone metastasis[28].

This study will have a unique point of view over the previous studies[15,29,30], first of all it was studied either ALDH1A1 or CD133 but never both of them, secondly it was studied any of them in other distinctive tissue (lung cancer, pancreatic cancer and PCa cell lines). Finally to the far of current knowledge the early diagnostic, prognostic and predictive impact of both markers were not studied before. The aim is to evaluate the clinical implication of concomitant assessment for ALDH1A1 and CD133 in three groups of prostate tumors: BPH, PIN and PCa patients, in order to identify the values of both markers in early pathological changes of PIN, which could be of great benefit in early detection and decision making especially inconclusive cases of PIN or BPH.

Upon this evidence, this study investigated the immunohisto-chemical expression levels of CSC markers: ALDH1A1 and CD133 in relation to other markers as androgen receptor AR, PSA and clinicopathological parameters in prostate tumors emphasizing their potential value for prediction of outcome in prostate cancer.

MATERIALS AND METHODS

Patient characteristics: This study comprised of 84 male patients with prostate tumors who had a prostatic biopsy collected from transurethral resection of prostate or from open prostatectomy. Pre-operative blood samples and formalin-fixed paraffin-embedded archival blocks of tissue samples were obtained. The archival blocks presented to Pathology Department, Faculty of Medicine, Minya University, Egypt between 2011 and 2016. Hematoxylin and Eosin slides were examined in order to classify the patients into benign prostatic hyperplasia (BPH) [n = 35], prostatic intraepithelial neoplasia (PIN)[n = 17] and prostate cancer (PCa) [n= 32]. Patients' clinical data were compared with histopathological diagnosis. Grading of PCa was classified using Gleason score. Patients with total Gleason score $\leq$7 were considered as low Gleason grade. While patients with total Gleason score 8-10 were considered as high Gleason grade[31]. According to pre-operative PSA serum levels, the included patients

were classified into three groups: group one (serum PSA <4.0 ng mL^{-1}), group two (serum PSA 4.0-10.0 ng mL^{-1}) and group three (serum PSA >10.0 ng mL^{-1}). Patients who received hormonal therapy or chemotherapy were excluded.

Immunohisto-chemical staining: Immunostaining was performed on 5 μm sections of formalin-fixed, paraffin-embedded blocks using avidin biotin-peroxidase complex method. Tissue sections were deparaffinized in xylene and hydrated. After a rinse in PBS (137 mM NaCl, 2.7 mM KCl, 1 mM KH_2PO_4 and 10 mM Na_2HPO_4; pH 7.4), endogenous peroxidase activity was quenched by incubating the sections for 15 min with 0.3% H_2O_2 in absolute methanol. After 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), anti-CD133 (clone NCH-38, dilution 1 200; Dako) and anti-AR (clone AR441, dilution 1:100; Dako). The sections were treated with biotinylated goat anti-mouse antibody for 15 min, washed with PBS and incubated according to the manufacturer's instructions for the LASB kit. Sections were incubated with diaminobenzidine and counterstained for microscopic evaluation.

Immunohisto-chemical scoring: Expression scored with blind knowledge of clinicopathological data to obtain a consensus agreement. Immunohisto-chemical staining of ALDH1A1 was mainly localized to the cytoplasm of tumor cells and for CD133 expression was cytoplasmic membrane with regard to the intensity of staining and percentage of stained tumor cells. An overall score was assigned by multiplying the intensity score by the percentage of stained cells. A final score of ALDH1A1 and CD133 expression was at three levels; negative (tumor cells without any expression), low level (tumor cells with faint staining, demonstrating <10% of positive cells) and a high level (tumor cells with more than 10% overall score)[15,32]. For nuclear AR expression, the cutoff was ≥1% AR positive tumor cells. Brown staining intensity representing the level of AR expression was classified as follows; negative (cells without any expression of AR), low level (cells with faint staining), moderate level (cells with moderate staining) and a high level (tumor cells with strong brown staining) of AR[27].

PSA assay: Assessment of PSA levels were carried according to standard manufacture procedure as previously described[33]. PSA assay was performed using Commercial kit from (Teco Diagnostic Laboratory, USA). Enzyme immunoassay was based on the principle that PSA molecule was sandwiched between two solid phases: rabbit anti-PSA antibody and enzyme linked antibodies (monoclonal anti-PSA conjugated to Horse raddish peroxidise). About 5 mL of blood were drawn from each patient pre-operatively in a plain tube and samples were left to stand for about 30 min. Then, centrifugation was carried on at 2500 rpm for 5 min. Serum was separated and stored at -20°C until analysis.

Statistical analysis: Statistical analyses were performed using SPSS-software 16 (Chicago, IL, USA). The analysis of these results were assessed by using the Chi-square "x^2" test and z-test. The survival time analysis was performed by Kaplan–Meier analysis and differences were tested for statistical significance with the log-rank test. The correlation of various parameters was analyzed by Spearman's correlation analysis while influence of various parameters was analyzed with multivariate Cox proportional hazards regression analyses. All $p \leq 0.05$ were considered significant.

RESULTS

Characteristics of cases: This study included 84 cases of them; 35 cases belong to BPH, 17 to PIN and 32 to PCa. Among the PCa cases; 18 had a Gleason score ≤7 and 14 had a Gleason score 8-10. Patients had a mean age of 66.83 years at the diagnosis, with a minimum age of 53 years and a maximum age of 79 years. Serum PSA levels; group one (<4.0 ng mL^{-1}) included 35 patients (41.7%), group two (4.0-10.0 ng mL^{-1}) included31 (36.9%) and group three (≥10.0 ng mL^{-1}) included 18 (21.4%).

Immunoreactivity of ALDH1A1: About 23 cases (27.4%) of total samples (n = 84) showed a high level of ALDH1A1 expression. In BPH 30/35 (85.7%) of cases were completely negative for ALDH1A1 expression. Few ALDH1A1 cells displayed low level of positive cytoplasmic staining, localized to the basal cell layers of BPH cases 5/35 (14.3%), however, no case of BPH shows a high level of ALDH1A1 expression. Meanwhile 7/17 of PIN cases (41.2%) revealed negative expression of ALDH1A1 and 4/17 (23.5%) showed high ALDH1A1 expression. A high level of expression of ALDH1A1 was observed in 19/32 (59.4%) of cases of prostatic carcinoma and only 6/32 (18.8%) were negative (Fig. 1). Thus, a statistically significant association in different levels of ALDH1A1 expression between BPH, PIN and PCa ($p<0.001$) was found. A positive trend could be observed in ALDH1A1

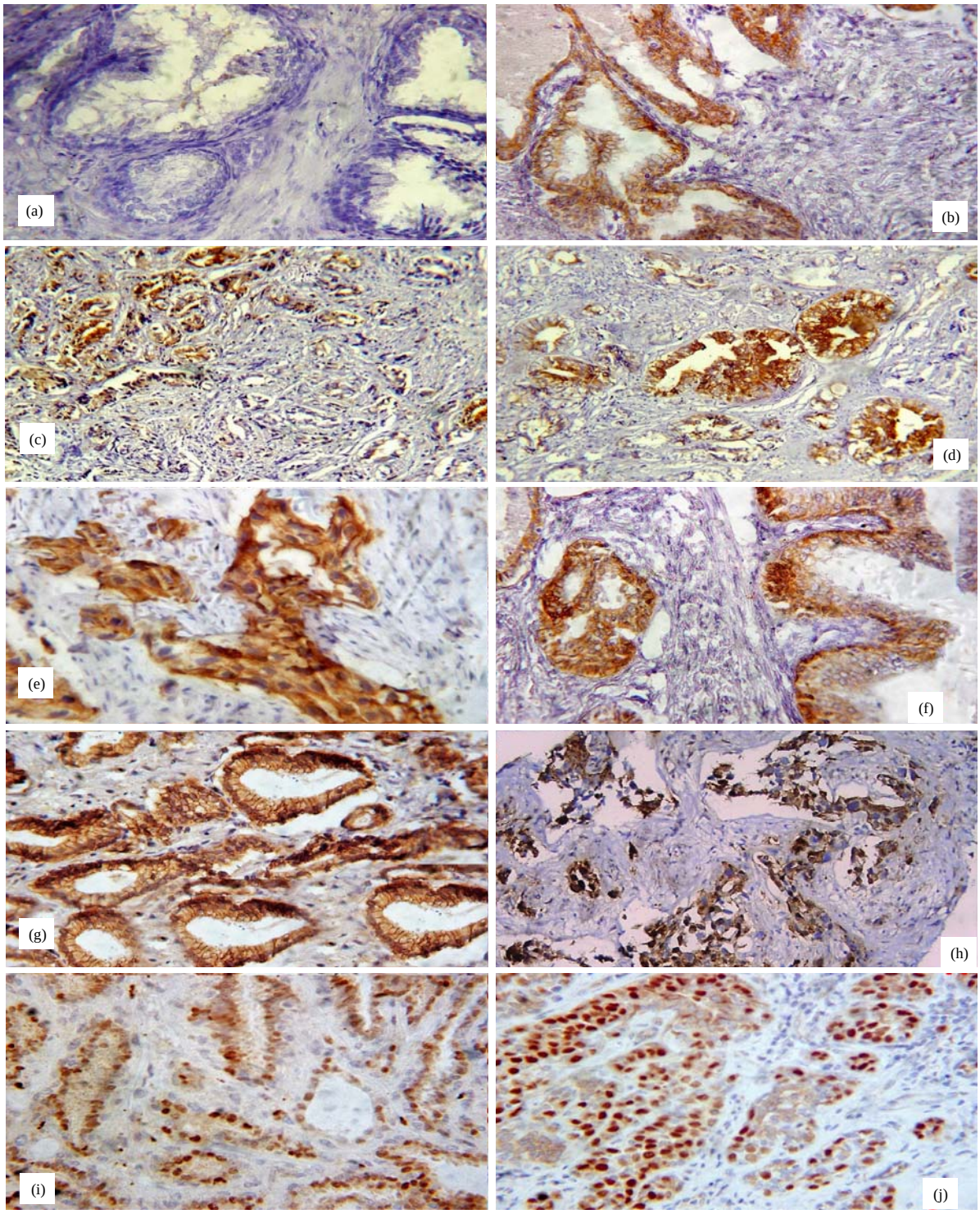

Fig. 1(a-j): Immunohisto-chemical expression of aldehyde dehydrogenase 1A1 (ALDH1A1) expression, (a) Benign prostate hyperplasia (BPH) show negative expression, (b) Prostatic intraepithelial neoplasia (PIN) exhibit high level expression of ALDH1A1, (c-d) High level with heterogeneous expression in low Gleason score, (e) Prostate cancer (PCa) with high gleason score showed high level of ALDH1A1 expression, (f) CD133 expression in PIN, (g) Low gleason score of PCa, (h) High Gleason score, (i) Androgen receptor (AR) expression in low Gleason score of PCa and (j) High gleason score of PCa. All figures are with a magnification of 200X

expression from low to high Gleason pattern (p = 0.01). Among PCa, 85.7% of high Gleason score cases showed high expression of ALDH1A1. A significant association between ALDH1A1 expression and Gleason score was found (p = 0.02). Correlation between ALDH1A1 expression of cells and pre-operative serum PSA level was statistically significant (p<0.001). However, there was no significant association between ALDH1A1 expression and patient's age. Among PCa cases, (90.9%) of them had high level of ALDH1A1 among the group of positive local nodal invasion (p = 0.03). Among cases that were positive for distant metastasis, all of them (100%) had a high level of ALDH1A1 (p = 0.01). A low level of expression of ALDH1A1 was observed in 3/4 (75%) cases of prostatic carcinoma stage I with no case of this group showed high expression of ALDH1A1. However, PCa stage IV cases showed high expression of ALDH1A1 in 87.5% of cases and only 12.5% were of low ALDH1A1 expression with statistically significant difference in different stages of PCa (p = 0.008) (Table 1).

Table 1: Immunohisto-chemical expression of ALDH1A1 in 84 prostate cases with clinicopathological characteristics

		ALDH1A1		
Characteristics	Total (100%)	Negative (%)	Low (%)	High (%)
Diagnosis				
BPH	35	30(85.7)	5(14.3)	0(0.0)
PIN	17	7(41.2)	6(35.3)	4(23.5)
PCa	32	6(18.7)	7(21.9)	19(59.4)
Total	84	43(51.2)	18(21.4)	23(27.4)
p-Value	0.000			
Gleason				
1	18	5(27.8)	6(33.3)	7(38.9)
2	14	1(7.1)	1(7.1)	12(85.7)
Total	32	6(18.7)	7 (21.9)	19 (59.4)
p-value	0.028			
PSA				
1	35	32(91.4)	3(8.6)	0 (0.0)
2	31	7(22.6)	9(29.0)	15(48.4)
3	18	4(22.2)	6(33.3)	8(44.4)
Total	84	43(51.2)	18(21.4)	23(27.4)
p-value	0.000			
Nodal				
0	21	6(28.6)	6(28.6)	9(42.9)
1	11	0 (0.0)	1(9.1)	10(90.9)
Total	32	6(18.7)	7 (21.9)	19 (59.4)
p-value	0.027			
Metastasis				
0	23	6(26.1)	7(30.4)	10(43.5)
1	9	0 (0.0)	0 (0.0)	9(100.0)
Total	32	6(18.7)	7 (21.9)	19 (59.4)
p-value	0.014			
Staging				
1	4	1(25.0)	3(75.0)	0(0.0)
2	14	5(35.7)	3(21.4)	6(42.9)
3	6	0 (0.0)	0 (0.0)	6(100.0)
4	8	0 (0.0)	1(12.5)	7(87.5)
Total	32	6(18.7)	7 (21.9)	19 (59.4)
p-value	0.008			

ALDH1A1: Aldehyde dehydrogenase 1 A1, BPH: Benign prostate hyperplasia, PIN: Prostatic intraepithelial neoplasia, PCa: Prostate cancer. Chi-squared and Fischer's exact tests p-value<0.05 is considered significant

Kaplan-Meier estimation performed on PCa patients indicated that the patients with high ALDH1A1 expression have shorter overall survival time than the patients with negative and low ALDH1A1 expression who have nearly similar curves (p = 0.001). Both groups of cases with low or negative ALDH1A1have longer overall survival than the cases with high expression of ALDH1A1. High ALDH1A1 expression is predictive of poor prognosis in PCa patients (Fig. 2). In addition, ALDH1A1 showed negative significant correlation with AR positivity (r = -0.363*, p = 0.041) (Table 2).

Immunoreactivity of CD133: Thirty-seven (44%) of total samples (n = 84) revealed completely negative CD133 expression. In BPH 23/35 (65.7%) were completely negative for CD133 expression. Meanwhile, 6/17 of PIN cases (35.3%) of them revealed low expression of CD133 and similar percentage revealed high expression. A high level of expression of CD133 was observed in 12/32 (37.5%) cases of prostatic carcinoma and 11/32 (34.4%) were of low expression (Fig. 1). A statistically significant association appear in different

Table 2: Spearman's correlation analysis table

	ALDH1A1		CD133		AR	
Characteristics	r	P	r	P	r	P
Staging	0.593**	0.000	0.762**	0.000	-0.220	0.227
Tumor	0.628**	0.000	0.626**	0.000	-0.294	0.103
Nodal	0.474**	0.006	0.670**	0.000	-0.154	0.400
Metastasis	0.500**	0.004	0.376*	0.034	-0.146	0.424
PSA	0.416*	0.018	0.421*	0.016	-0.232	0.201
Gleason	0.454**	0.009	0.225	0.216	0.147	0.421
ALDH1A1	1.00	-	0.331	0.064	-0.363*	0.041
CD133	0.331	0.064	1.00	-	-0.200	0.271
AR	-0.363*	0.041	-0.200	0.271	1.00	-

Spearman's correlation analysis of ALDH1A1, CD133 and AR (androgen receptor) with prognostic markers among prostate cancer patients (Total = 32). *p<0.05 is considered significant, **p<0.001 is considered highly significant

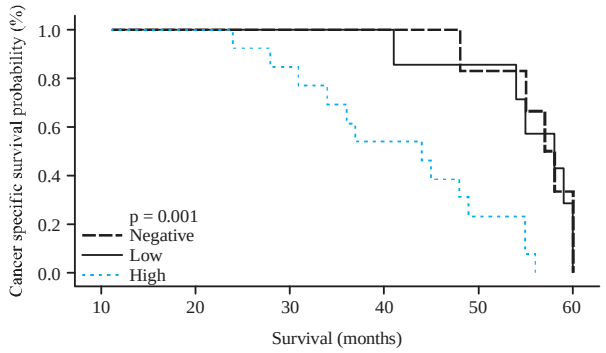

Fig. 2: ALDH1A1 high expression is predictive of poor prognosis in prostate cancer patients. Kaplan-Meier curves of patient 5 years disease free survival according to ALDH1A1 protein expression. Log-rank (Mantel-Cox) tests were used to compare ALDH1A1 negative, low and high expression in prostate cancer patients. Cases that showed high ALDH1A1 expression had lower disease free survival durations than those in other groups. No difference in survival was found between negative and low ALDH1A1 expression (p = 0.001)

expression of CD133 between BPH, PIN and PCa (p = 0.001). The high expression of CD133 was seen in 12/32 (37.5%) of cases belong to high Gleason. Among PCa, 8/14 (57.1%) cases of high Gleason score showed high expression of CD133, while only 4/18 (22.2%) of low Gleason score cases had a high level of CD133. No significant association between CD133 expression and Gleason score was found (p = 0.06). Correlation between CD133 expression and pre-operative serum PSA was statistically significant (p<0.001). In positive local nodal invasion group (90.9%) of the cases showed high level of CD133 expression (p<0.001). In (77.78%) of positive distant metastasis cases a high level of CD133 expression was detected (p = 0.007). A statistically significant difference in CD133 expression between different stages of PCa was confirmed (p<0.001) (Table 3).

Kaplan-Meier estimation indicated that the patients with CD133 high expression have shorter overall survival time than the patients with CD133 negative and low expression (p = 0.000). So high CD133 expression is predictive of shorter survival duration (Fig. 3). Nearly significant positive correlation was found between CD133 and ALDH1A1 expression r = 0.331, p = 0.06) (Table 2).

Immunoreactivity of AR: Sixteen cases (88.9%) of total samples (n = 18) that showed a high level of AR expression were PCa. In BPH (85.7%) of them displayed low level of

Table 3: Immunohistochemical expression of CD133 in 84 prostate cases with clinicopathological characteristics

		CD133		
Characteristics	Total (100%)	Negative (%)	Low (%)	High (%)
Diagnosis				
BPH	35	23(65.7)	12(35.3)	0(0.0)
PIN	17	5(29.4)	6 (35.3)	6 (35.3)
PCa	32	9(28.1)	11(34.4)	12(37.5)
Total	84	37(44.1)	29(34.5)	18(21.4)
p-value	0.001			
Gleason				
1	18	5(27.8)	9(50.0)	4(22.2)
2	14	4(28.6)	2(14.3)	8(57.1)
Total	32	9(28.1)	11(34.4)	12(37.5)
p-value	0.064			
PSA				
1	35	23(65.7)	12(34.3)	0(0.0)
2	31	10(32.3)	8(25.8)	13(41.9)
3	18	4(22.2)	9(50.0)	5(27.8)
Total	84	37(44.1)	29(34.5)	18(21.4)
p-value	0.000			
Nodal				
0	21	8(38.1)	11(52.4)	2(9.5)
1	11	1(9.1)	0 (0.0)	10(90.9)
Total	32	9(28.1)	11(34.4)	12(37.5)
p-value	0.000			
Metastasis				
0	23	7(30.4)	11(47.8)	5(21.7)
1	9	2(22.2)	0 (0.0)	7(77.8)
Total	32	9(28.1)	11(34.4)	12(37.5)
p-value	0.007			
Staging				
1	4	2(50.0)	2(50.0)	0(0.0)
2	14	7(50.0)	6(42.9)	1(7.1)
3	6	0(0.0)	3(50.0)	3(50.0)
4	8	0(0.0)	0(0.0)	8(100.0)
Total	32	9(28.1)	11(34.4)	12(37.5)
p-value	0.000			

BPH: Benign prostate hyperplasia, PIN: Prostatic intraepithelial neoplasia, PCa: Prostate cancer. Chi-squared and Fischer's exact tests p<0.05 is considered significant

positive nuclear staining of AR expression. Meanwhile 7/17 of PIN cases (41.2%) revealed moderate expression of AR and only 2/17 (11.8%) of cases showed high AR expression (Fig. 1). A statistically significant difference in AR expression noticed between BPH, PIN and PCa (p<0.001). No significant difference was found between AR expression and Gleason score. The group of highest PSA level had high expression of AR in 33.3% of cases. Difference between AR expression of cells and pre-operative serum PSA was statistically significant (p = 0.002). No significant correlation was found between AR expression and nodal status, metastatic status or tumor staging (Table 4). In contrast to ALDH1A1, AR expression does not show any significant correlation with any of the prognostic parameters. Although negative significant correlation was found between AR

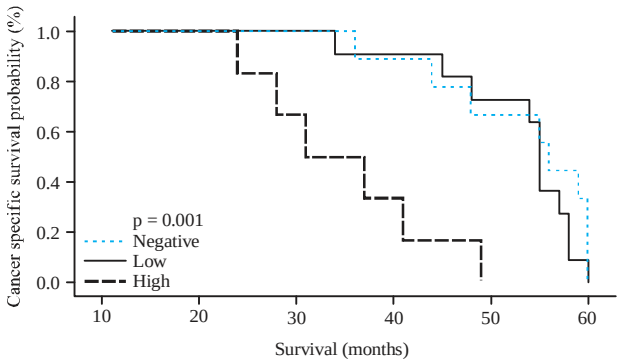

Fig. 3: CD133 high expression is predictive of poor prognosis and short survival duration in prostate cancer patients. Kaplan-Meier curves of patient 5 years disease free survival according to CD133 protein expression. Log-rank (Mantel-Cox) tests were used to compare CD133 negative, low and high expression in prostate cancer patients. Cases of high CD133 expression have lower disease free survival than that of the other groups. Nearly similar disease free survival I cases of low or no CD133 expression (p<0.001)

Table 4: Immunohisto-chemical expression of AR in 84 prostate cases with clinicopathological characteristics

		AR			
Characteristics	Total (100%)	Negative (%)	Low (%)	Moderate (%)	High (%)
Diagnosis					
BPH	35	0 (0.0)	30(85.7)	5(14.3)	0 (0.0)
PIN	17	2(11.8)	6(35.3)	7(41.2)	2(11.8)
PCa	32	4(12.5)	4(12.5)	8(25.0)	16(50.0)
Total	84	6(7.1)	40(47.6)	20(23.8)	18(21.4)
p-value	0.000				
Gleason					
1	18	2(11.1)	4(22.2)	4(22.2)	8(44.4)
2	14	2(14.3)	0 (0.0)	4(28.6)	8(57.1)
Total	32	4(12.5)	4(12.5)	8(25.0)	16(50.0)
p-value	0.314				
PSA					
1	35	0(0.0)	24(68.6)	4(11.4)	7(20.0)
2	31	5(16.1)	13(41.9)	8(25.8)	5(16.1)
3	18	1(5.6)	3(16.7)	8(44.4)	6(33.3)
Total	84	6(7.1)	40(47.6)	20(23.8)	18(21.4)
p-value	0.002				
Nodal					
0	21	1(4.8)	3(14.3)	6(28.5)	11(52.4)
1	11	3(27.3)	1(9.1)	2(18.2)	5(45.5)
Total	32	4(12.5)	4(12.5)	8(25.0)	16(50.0)
p-value	0.326				
Metastasis					
0	23	1(4.3)	4(17.4)	6(26.1)	12(52.2)
1	9	3(33.3)	0 (0.0)	2(22.2)	4(44.4)
Total	32	4(12.5)	4(12.5)	8(25.0)	16(50.0)
p-value	0.11				
Staging					
1	4	0(0.0)	0(0.0)	0(0.0)	4(100.0)
2	14	1(7.1)	3(21.4)	3(21.4)	7(50.0)
3	6	2(33.3)	0(0.0)	4(66.7)	0(0.0)
4	8	1(12.5)	1(12.5)	1(12.5)	5(62.5)
Total	32	4(12.5)	4(12.5)	8(25.0)	16(50.0)
p-value	0.066				

AR: Androgen receptor, BPH: Benign prostate hyperplasia, PIN: Prostatic intraepithelial neoplasia, PCa: Prostate cancer. Chi-squared and Fischer's exact tests p<0.05 is considered significant

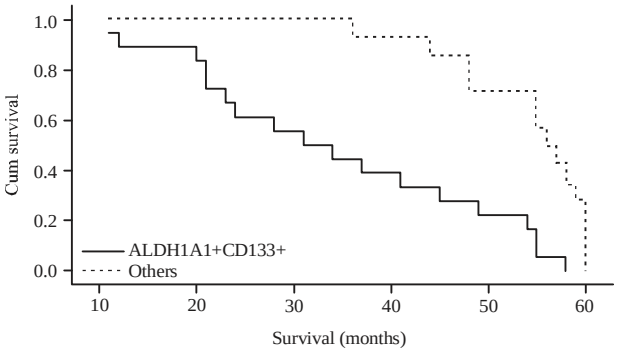

Fig. 4: Double positive ALDH1A1 and CD133 is predictive of poor prognosis and short survival duration in prostate cancer patients. Kaplan-Meier curves of patient 5 years disease free survival according to double positive ALDH1A1 and CD133 protein expression. Log-rank by (Mantel-Cox) tests, they had a significantly worse prognosis than the other groups ($p<0.001$)

expression and ALDH1A1 ($r = -0.363$, $p = 0.041$). No correlation was found between CD133 and AR expression (Table 2).

Joint analysis of the ALDH1A1/CD133 status and survival: PCa cases were divided into three categories based on their ALDH1A1 and CD133 expression levels: ALDH1A1-/CD133- (n = 1; double-negative category), ALDH1A1+/CD133- (n = 8) and ALDH1A1-/CD133+ (n = 5). Only one positive (n = 13: any-positive category) and ALDH1A1+/CD133+ (n = 18; double-positive category). The double-positive group had a significantly worse prognosis than the other groups ($p<0.001$), however all other groups show no significant difference with survival (Fig. 4).

DISCUSSION

High activity of ALDH1A1 plays an important role in the regulation of growth and differentiation of both normal and cancer cells and is associated with more aggressive PCa[34-36].

In this study, only 14.3% of BPH cases demonstrated a low level of expression of ALDH1A1, that could be a strong evidence for the stem cell character of ALDH1A1 positive cells in benign tissues and add verification of benign stromal ALDH1A1 cells[6]. In another study, BPH tissues displayed $\leq$10% population of ALDH1A1 positive cells with weak staining[37]. The low-level expression of ALDH1A1 in this study suggests the presence of stem like cells in BPH samples. BPH could eventually occur as a result of altered stem cell properties that could subsequently lead to a clonal expansion of prostatic cell populations[38]. Inhibition of stem cell proliferation may minimize the risk of benign and malignant changes in the prostate[39].

Increased risk of PIN and PCa was proposed on the supporting role of hyperplasia associated with inflammation and overgrowth[40]. In this study, it was found that 23.5% of PIN 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[37]. In a similar study, ALDH1A1 expression was found in 13% of samples of high-grade PIN. Although, ALDH1A1 expression is believed to be limited only to malignant glands, interestingly, it was detected and expressed in some cases of PIN[10,41].

Herein, 59.4% of cases of prostatic adenocarcinoma showed high levels of expression of ALDH1A1. The finding is consistent with other studies showing highly expressed ALDH1A1 in about 60% population among PCa cases[35,37,41]. Although ALDH1A1 has a critical role in normal and malignant cell proliferation and differentiation, it also promotes tumor cell survival through direct inactivation of DNA repair and enhancement of the oxidative stress resistance response[11,42,43]. Activation of ALDH1A1 had an ultimate role in tumor cells metastasis, aggressiveness as well as continuous cellular proliferation and plasticity of primary tumors[11,44]. Inhibition of ALDH1A1 could leads the way to reverse the radio-resistant phenotype of CSCs[45].

In current study, the level of expression of ALDH1A1 showed a statistically significant association with Gleason grade ($p = 0.02$). Expression of ALDH1A1 was up regulated in high-grade prostatic adenocarcinomas in comparison to low-grade adenocarcinomas[12]. Previous studies have demonstrated a significant association between the level of ALDH1A1 expression and tumor stage[15,35,37,41]. This indicated the potential role of CSCs in PCa tumorigenesis and aggressive behavior that could be a compatible promising candidate for targeted therapy of high Gleason score PCa[41].

In line with the studies, correlation of PSA with ALDH1A1 expression has become an integral component in the management of patients with PCa in either monitoring treatment outcome or in predicting survival rate and watchful waiting[41]. This study showed a statistically significant positive correlation between ALDH1A1 expression and serum PSA levels ($p<0.001$). Even with limitation of a single PSA measurement, its high sensitivity and specificity in detecting PCa provides a more accurate analysis of PSA has need to be associated with other parameters[46,47]. Men with an elevated PSA level, low Gleason score biopsy and low clinical stage are

at a higher risk of worse outcomes when compared with men with only low-risk features without high level of PSA[48,49]. Additional associated markers are useful to increase PSA specificity for screening PCa without reducing the sensitivity[50]. PCa cells that possess low serum PSA express stem cell genes and can undergo asymmetrical cell division to generate more PSA positive cells. Eventually these generated cells with PSA can initiate active aggressive tumor development and potent resistance to androgen ablation in castrated hosts associated with highly tumorigenic resistant PCa cells that can be prospectively enriched using ALDH1A1 and other isoforms of ALDH[51].

Furthermore, this study identify that the high ALDH1A1 expression inversely associated with 5 year survival of the patients which in agreement with previous studies[10,15,44,52,53]. It is mandatory to suppress CSCs to reach a complete tumor eradication and prevent tumor recurrence[54]. Discriminating patients with low risk of progression from those with lethal PCa is imperative to avoid over treatment and improve survival rate[55].

It is important in prostate tumorigenesis to define specific markers for normal prostate stem cells[56]. The current study showed absence of high CD133 expression in BPH. Tumorigenic potential did not emerge from positive CD133 stem cells but could be seen in the negative CD133 population[57,58]. High level of CD133 expression in PIN and PCa revealed a significant difference, PCa cells with high level of CD133 are more proliferative and AR pathway activation within prostate cancer cells grown in vitro increase the percentage of CD133 positive cells[59].

Herein, a high significant positive correlation has been noticed in relation to tumor stage, positive local nodal invasion and positive distant metastasis and through survival analysis, a high CD133 expression affected patient survival times. CD133 is significantly associated with higher stage, worse prognosis, worse 5 year overall survival rate and disease free survival rate in colorectal cancer[60], gastric cancer[61], glioma patients[62] and lung cancer[30,63].

AR-signaling pathway can interact with a number of additional oncogenic signaling pathways, including those involved in promoting growth and resistance across PCa. Eventually, targeting and inhibition of this AR signaling pathway may have beneficial effects in PCa[26]. In this study, all the prostatic lesions demonstrated variable intensity of AR immunostaining. The staining intensity for AR was heterogeneous specifically in cases of PCa[27,64]. The intensity of AR staining in PCa reduces as the Gleason grade of the tumor increases but it did not reach a significant level. AR target genes are usually expressed even in men progressing on androgen deprivation therapy, with AR pathway alterations commonly observed in late stage[26]. AR could gain novel growth-promoting functions during PCa development and progression through multiple genetic and epigenetic mechanisms[65]. An association observed between AR expression and nuclear localization and cancer cell proliferation in patients with advanced PCa resistance and progression despite multiple AR targeted therapies[59]. AR expression showed significantly positive correlation with pre-operative serum PSA. Recognition of hormone-activated targets of the AR has been exaggerated by using useful markers of PCa progression and PSA is widely used for screening and tumor monitoring technique in diagnosis of cancer prostate[66].

Despite using ALDH1 and CD133 as markers of CSCs and being closely associated with the prognosis of cancer patients, however, controversies about using single CSC marker to identify CSCs is sufficient remains an important point[32,67]. A concomitant positive expression of both ALDH1A1+ CD133+ cells demonstrated potential and more rapid tumor generation than ALDH1A1+CD133-tumors, ALDH1A1+ CD133+ cells may identify a more aggressive tumor phenotype. Expression of CD133 combined with ALDH identifies a more aggressive CSC suggesting a potential hierarchy of cells with distinct cancer growth potential[68]. Although the ALDH1A1 score and the expression of CD133 were nearly significant in this analysis, the double-positive group had the significant worse prognosis based on the overall survival. Furthermore, a landmark study evaluates CD133 and ALDH1A1 in lung adenocarcinoma considered both as novel diagnostic and therapeutic markers for targeted lung adenocarcinoma therapy[30]. AR may behave differently within CD133 positive cells when compared to CD133 negative cells[25]. Although AR signals play important roles in PCa tumorigenesis, many cases of PCa may progress to resistant or metastasis. Detailed explanation remains controversial but cancer stem cells and their progenitor may be a key factor contribute to the metastasis development. Targeting cancer stem cells like CD133 or ALDH1A1 with androgen deprivation therapy has been suggested as a potential novel therapy to suppress the PCa metastasis[24]. The development of new target cancer treatment against CSC will require careful clinical data and clinical trials with definite criteria[69].

So far, there is very little data regarding the comparison of the expression of ALDH1A1 and CD133 in benign, precancerous and prostate cancer. There is no sufficient information regarding the role of these markers in the three groups of prostate tumors. ALDH1A1 and CD133 expression in early pathological changes of PIN could be of great benefit in

early detection. However, such comparative analysis might lead to better characterization of stem cell function in prostate diseases. Overall, the identification and functional characterization of prostate CSCs have paved the way for aiding PCa diagnosis and prognosis prediction. To date, PCa can be identified by several markers assisting in PCa patient stratification with the potential for personalized adjuvant therapy. Various combinations of known markers may be of value in identifying prostate CSCs since there is no single marker being exclusively expressed by CSCs in PCa. Also, androgen receptor expression status was included because the hormone dependency of CSCs in PCa is unclear and several findings suggest that at least a group of prostate cancers stem cells express AR.

CONCLUSION

In conclusion, this study showed a formalistic difference in cytoplasmic ALDH1A1 and CD133 expression between BPH as a benign lesion, precancerous PIN and PCa. With respect to previous studies, that could be of great benefit for decision making for prostate cancer. A novel aspect of findings in this study is that, joint detection of ALDH1A1 and CD133 expression is suggested to help in diagnosis and predictions for outcomes of PCa, which would be reflected upon the overall successful management for patients with PCa. Establishment and validation of the clinical utility of combined promising PCa immunohisto-chemical markers, including AR, ALDH1A1 and CD133 will lead to guidelines for cost-efficient strategies for detection and treatment. The optimum results from therapeutic modalities is to involve a combination of targeted CSCs therapy and established agents targeted androgen sensitive cells in PCa. Further clinical research is required to consider these markers as targets of new therapeutic strategies in PCa.

SIGNIFICANCE STATEMENT

This study reported detailed immunohisto-chemical expression levels of CSC markers: ALDH1A1 and CD133 in relation to other markers as androgen receptor AR, PSA and clinicopathological parameters in prostate tumors emphasizing their potential value for prediction of outcome in prostate cancer. The results indicated that joint detection of ALDH1A1 and CD133 expression help in diagnosis and predictions for outcomes of PCa, which would be reflected upon the overall successful management for patients with PCa. Establishment and validation of the clinical utility of combined promising PCa immunohisto-chemical markers, including AR, ALDH1A1 and CD133 will lead to guidelines for cost-efficient strategies for detection and treatment.

ACKNOWLEDGMENT

Thanks to Jomana Nasser a medical student at king Abdulaziz University for her contribution in revising some data of this paper.

REFERENCES

1. Siegel, R.L., K.D. Miller and A. Jemal, 2016. Cancer statistics, 2016. CA: Cancer J. Clinicians, 66: 7-30.
2. Siegel, R.L., K.D. Miller and A. Jemal, 2015. Cancer statistics, 2015. CA: Cancer J. Clin., 65: 5-29.
3. Valdes-Mora, F. and S.J. Clark, 2015. Prostate cancer epigenetic biomarkers: Next-generation technologies. Oncogene, 34: 1609-1618.
4. Dimov, I., M. Visnjic and V. Stefanovic, 2010. Urothelial cancer stem cells. Scient. World J., 10: 1400-1415.
5. Gkountela, S. and N. Aceto, 2016. Stem-like features of cancer cells on their way to metastasis. Biol. Direct., Vol. 11. 10.1186/s13062-016-0135-4.
6. Isfoss, B.L., B. Holmqvist, P. Alm and H. Olsson, 2012. Distribution of aldehyde dehydrogenase 1-positive stem cells in benign mammary tissue from women with and without breast cancer. Histopathology, 60: 617-633.
7. Xu, S.L., S. Liu, W. Cui, Y. Shi and Q. Liu *et al*., 2015. Aldehyde dehydrogenase 1A1 circumscribes high invasive glioma cells and predicts poor prognosis. Am. J. Cancer Res., 5: 1471-1483.
8. Alison, M.R., N.J. Guppy, S.M. Lim and L.J. Nicholson, 2010. Finding cancer stem cells: Are aldehyde dehydrogenases fit for purpose? J. Pathol., 222: 335-344.
9. Ma, I. and A.L. Allan, 2011. The role of human aldehyde dehydrogenase in normal and cancer stem cells. Stem Cell Rev., 7: 292-306.
10. Le Magnen, C., L. Bubendorf, C.A. Rentsch, C. Mengus and J. Gsponer *et al*., 2013. Characterization and clinical relevance of ALDH bright populations in prostate cancer. Clin. Cancer Res., 19: 5361-5371.
11. Rodriguez-Torres, M. and A.L. Allan, 2016. Aldehyde dehydrogenase as a marker and functional mediator of metastasis in solid tumors. Clin. Exp. Metastasis, 33: 97-113.
12. Yan, J., J. De Melo, J.C. Cutz, T. Aziz and D. Tang, 2014. Aldehyde dehydrogenase 3A1 associates with prostate tumorigenesis. Br. J. Cancer, 110: 2593-2603.
13. Van den Hoogen, C., G. van der Horst, H. Cheung, J.T. Buijs and J.M. Lippitt *et al*., 2010. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. Cancer Res., 70: 5163-5173.

14. Koppaka, V., D.C. Thompson, Y. Chen, M. Ellermann and K.C. Nicolaou *et al*., 2012. Aldehyde dehydrogenase inhibitors: A comprehensive review of the pharmacology, mechanism of action, substrate specificity and clinical application. Pharmacol. Rev., 64: 520-539.
15. Li, T., Y. Su, Y. Mei, Q. Leng and B. Leng *et al*., 2010. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patient's outcome. Lab. Invest.: J. Techn. Methods Pathol., 90: 234-244.
16. Lin, L., B. Hutzen, H.F. Lee, Z. Peng and W. Wang *et al*., 2013. Evaluation of STAT3 signaling in ALDH+ and ALDH+/CD44+/CD24-subpopulations of breast cancer cells. PloS One, Vol. 8, No. 12. 10.1371/journal.pone.0082821.
17. Sullivan, J.P., M. Spinola, M. Dodge, M.G. Raso and C. Behrens *et al*., 2010. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. Cancer Res., 70: 9937-9948.
18. Jiang, J., Y. Liu, Y. Tang, L. Li, R. Zeng, S. Zeng and M. Zhong, 2016. ALDH1A1 induces resistance to chop in diffuse large B-cell lymphoma through activation of the JAK2/Stat3 pathway. Onco Targets Ther., 9: 5349-5360.
19. Wang, L., D. Lin, Y. Fu and M. Lai, 2016. Nuclear aldehyde dehydrogenase 1A1 (ALDH1A1) expression is a favorable prognostic indicator in colorectal carcinoma. Pathol. Res. Pract., 212: 791-799.
20. Trerotola, M., S. Rathore, H.L. Goel, J. Li and S. Alberti *et al*., 2010. CD133, Trop-2 and α2β1 integrin surface receptors as markers of putative human prostate cancer stem cells. Am. J. Transl. Res., 2: 135-144.
21. Lugli, A., G. Iezzi, I. Hostettler, M.G. Muraro and V. Mele *et al*., 2010. Prognostic impact of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM and ALDH1 in colorectal cancer. Br. J. Cancer, 103: 382-390.
22. Bidlingmaier, S., X. Zhu and B. Liu, 2008. The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. J. Mol. Med. (Berl.), 86: 1025-1032.
23. Woo, T., K. Okudela, H. Mitsui, T. Yazawa and N. Ogawa *et al*., 2010. Prognostic value of CD133 expression in stage I lung adenocarcinomas. Int. J. Clin. Exp. Pathol., 4: 32-42.
24. Zhu, J., D.R. Yang, Y. Sun, X. Qiu and H.C. Chang *et al*., 2015. Tr4 nuclear receptor alters the prostate cancer CD133+ stem/progenitor cell invasion via modulating the EZH2-related metastasis gene expression. Mol. Cancer Ther., 14: 1445-1453.
25. Reyes, E.E., M. Gillard, R. Duggan, K. Wroblewski and S. Kregel *et al*., 2015. Molecular analysis of CD133-positive circulating tumor cells from patients with metastatic castration-resistant prostate cancer. J. Transl. Sci., Vol. 1, No. 1.
26. Schweizer, M.T. and E.Y. Yu, 2017. Ar-signaling in human malignancies: Prostate cancer and beyond. Cancers, Vol. 9, No. 1. 10.3390/cancers9010007.
27. Husain, I., S. Shukla, P. Soni and N. Husain, 2016. Role of androgen receptor in prostatic neoplasia versus hyperplasia. J. Cancer Res. Ther., 12: 112-116.
28. Harris, K.S. and B.A. Kerr, 2017. Prostate cancer stem cell markers drive progression, therapeutic resistance and bone metastasis. Stem Cells Int. 10.1155/2017/8629234.
29. Durko, L., W. Wlodarski, O. Stasikowska-Kanicka, M. Wagrowska-Danilewicz and M. Danilewicz *et al*., 2017. Expression and clinical significance of cancer stem cell markers CD24, CD44 and CD133 in pancreatic ductal adenocarcinoma and chronic pancreatitis. Dis. Markers. 10.1155/2017/3276806.
30. Miyata, T., T. Oyama, T. Yoshimatsu, H. Higa and D. Kawano *et al*., 2017. The clinical significance of cancer stem cell markers ALDH1A1 and CD133 in lung adenocarcinoma. Anticancer Res., 37: 2541-2547.
31. Egevad, L., B.J. Norlen and M. Norberg, 2001. The value of multiple core biopsies for predicting the gleason score of prostate cancer. BJU Int., 88: 716-721.
32. Zhou, F., Y.D. Mu, J. Liang, Z.X. Liu, H.S. Chen and J.F. Zhang, 2014. Expression and prognostic value of tumor stem cell markers ALDH1 and CD133 in colorectal carcinoma. Oncol. Lett., 7: 507-512.
33. Stowell, L.I., L.E. Sharman and K. Hamel, 1991. An enzyme-linked immunosorbent assay (ELISA) for prostate-specific antigen. Forensic Sci. Int., 50: 125-138.
34. Moreb, J.S., 2008. Aldehyde dehydrogenase as a marker for stem cells. Curr. Stem Cell Res. Ther., 3: 237-246.
35. Matsika, A., B. Srinivasan, C. Day, S.A. Mader and D.M. Kiernan *et al*., 2015. Cancer stem cell markers in prostate cancer: An immunohistochemical study of ALDH1, SOX2 and EZH2. Pathology, 47: 622-628.
36. Tomita, H., K. Tanaka, T. Tanaka and A. Hara, 2016. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget, 7: 11018-11032.
37. Ferronika, P., F.X.E. Triningsih, A. Ghozali, A. Moeljono and S. Rahmayanti *et al*., 2012. P63 cytoplasmic aberrance is associated with high prostate cancer stem cell expression. Asian Pac. J. Cancer Prev., 13: 1943-1948.
38. Prajapati, A., S. Gupta, B. Mistry and S. Gupta, 2013. Prostate stem cells in the development of benign prostate hyperplasia and prostate cancer: Emerging role and concepts. BioMed Res. Int. 10.1155/2013/107954.
39. Hassan, A.U., G. Hassan, Z. Rasool and Zubeida, 2013. Aims and objectives of histological studies of prostate. Univ. J. Clin. Med., 1: 13-21.
40. Sciarra, A., G. Mariotti, S. Salciccia, A.A. Gomez, S. Monti, V. Toscano and F. Di Silverio, 2008. Prostate growth and inflammation. J. Steroid Biochem. Mol. Biol., 108: 254-260.
41. Kalantari, E., F.H. Saadi, M. Asgari, A. Shariftabrizi, R. Roudi and Z. Madjd, 2016. Increased expression of ALDH1A1 in prostate cancer is correlated with tumor aggressiveness: A tissue microarray study of iranian patients. Applied Immunohistochem. Mol. Morphol., 25: 592-598.

42. Lohberger, B., B. Rinner, N. Stuendl, M. Absenger and B. Liegl-Atzwanger *et al*., 2012. Aldehyde dehydrogenase 1, a potential marker for cancer stem cells in human sarcoma. PLoS One. Vol. 7. 10.1371/journal.pone.0043664.
43. Webber, J.P., L.K. Spary, M.D. Mason, Z. Tabi, I.A. Brewis and A. Clayton, 2016. Prostate stromal cell proteomics analysis discriminates normal from tumour reactive stromal phenotypes. Oncotarget, 7: 20124-20139.
44. Gangavarapu, K.J., G. Azabdaftari, C.D. Morrison, A. Miller, B.A. Foster and W.J. Huss, 2013. Aldehyde dehydrogenase and atp binding cassette transporter G2 (ABCG2) functional assays isolate different populations of prostate stem cells where ABCG2 function selects for cells with increased stem cell activity. Stem Cell Res. Ther. Vol. 4, No. 5. 10.1186/scrt343.
45. Cojoc, M., C. Peitzsch, I. Kurth, F. Trautmann and L.A. Kunz-Schughart *et al*., 2015. Aldehyde dehydrogenase is regulated by β-catenin/TCF and promotes radioresistance in prostate cancer progenitor cells. Cancer Res., 75: 1482-1494.
46. Saini, S., 2016. PSA and beyond: Alternative prostate cancer biomarkers. Cell. Oncol., 39: 97-106.
47. Fitzpatrick, J.M., E. Banu and S. Oudard, 2009. Prostate-specific antigen kinetics in localized and advanced prostate cancer. BJU Int., 103: 578-587.
48. Faisal, F.A., D. Sundi, P.M. Pierorazio, M.W. Ball and E.B. Humphreys *et al*., 2014. Outcomes of men with an elevated Prostate-Specific Antigen (PSA) level as their sole preoperative intermediate-or high-risk feature. BJU Int., 114: E120-E129.
49. Bayat, A.A., R. Ghods, M. Shabani, A.R. Mahmoudi and O. Yeganeh *et al*., 2015. Production and characterization of monoclonal antibodies against human prostate specific antigen. Avicenna J. Med. Biotechnol., 7: 2-7.
50. Amayo, A. and W. Obara, 2004. Serum prostate specific antigen levels in men with benign prostatic hyperplasia and cancer of prostate. East Afr. Med. J., 81: 22-26.
51. Qin, J., X. Liu, B. Laffin, X. Chen and G. Choy *et al*., 2012. The PSA(-/lo) prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration. Cell Stem Cell, 10: 556-569.
52. Hellsten, R., M. Johansson, A. Dahlman, O. Sterner and A. Bjartell, 2011. Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. PloS One, Vol. 6, No. 7. 10.1371/journal.pone.0022118.
53. Deng, S., X. Yang, H. Lassus, S. Liang and S. Kaur *et al*., 2010. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PloS One, Vol. 5, No. 4. 10.1371/journal.pone.0010277.
54. Ma, S., K.W. Chan, T.K. Lee, K.H. Tang, J.Y. Wo, B.J. Zheng and X.Y. Guan, 2008. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. Mol. Cancer Res., 6: 1146-1153.
55. Spahn, M., S. Boxler, S. Joniau, M. Moschini, B. Tombal and R.J. Karnes, 2015. What is the need for prostatic biomarkers in prostate cancer management? Curr. Urol. Rep., Vol. 16, No. 10. 10.1007/s11934-015-0545-3.
56. Leong, K.G., B.E. Wang, L. Johnson and W.Q. Gao, 2008. Generation of a prostate from a single adult stem cell. Nature, 456: 804-808.
57. Missol-Kolka, E., J. Karbanova, P. Janich, M. Haase, C.A. Fargeas, W.B. Huttner and D. Corbeil, 2011. Prominin-1 (CD133) is not restricted to stem cells located in the basal compartment of murine and human prostate. Prostate, 71: 254-267.
58. Zhang, K., S. Zhou, L. Wang, J. Wang and Q. Zou *et al*., 2016. Current stem cell biomarkers and their functional mechanisms in prostate cancer. Int. J. Mol. Sci., Vol. 17, No. 7. 10.3390/ijms17071163.
59. Reyes, E.E., D.J. VanderWeele, M. Isikbay, R. Duggan and A. Campanile *et al*., 2014. Quantitative characterization of androgen receptor protein expression and cellular localization in circulating tumor cells from patients with metastatic castration-resistant prostate cancer. J. Transl. Med., Vol. 12. 10.1186/s12967-014-0313-z.
60. Chen, S., X. Song, Z. Chen, X. Li, M. Li, H. Liu and J. Li, 2013. CD133 expression and the prognosis of colorectal cancer: A systematic review and meta-analysis. PloS One, Vol. 8, No. 2. 10.1371/journal.pone.0056380.
61. Yiming, L., G. Yunshan, M. Bo, Z. Yu and W. Tao *et al*., 2015. CD133 overexpression correlates with clinicopathological features of gastric cancer patients and its impact on survival: A systematic review and meta-analysis. Oncotarget, 6: 42019-42027.
62. Wu, B., C. Sun, F. Feng, M. Ge and L. Xia, 2015. Do relevant markers of cancer stem cells CD133 and nestin indicate a poor prognosis in glioma patients? A systematic review and meta-analysis. J. Exp. Clin. Cancer Res., Vol. 34. 10.1186/s13046-015-0163-4.
63. Qu, H., R. Li, Z. Liu, J. Zhang and R. Luo, 2013. Prognostic value of cancer stem cell marker CD133 expression in non-small cell lung cancer: A systematic review. Int. J. Clin. Exp. Pathol., 6: 2644-2650.
64. Proverbs-Singh, T., J.L. Feldman, M.J. Morris, K.A. Autio and T.A. Traina, 2015. Targeting the androgen receptor in prostate and breast cancer: Several new agents in development. Endocr. Relat. Cancer, 22: R87-R106.
65. Yuan, X., C. Cai, S. Chen, S. Chen, Z. Yu and S.P. Balk, 2014. Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis. Oncogene, 33: 2815-2825.

66. Eisermann, K. and G. Fraizer, 2017. The androgen receptor and vegf: Mechanisms of androgen-regulated angiogenesis in prostate cancer. Cancers, Vol. 9, No. 4. 10.3390/cancers9040032.
67. Saricanbaz, I., E. Karahacioglu, O. Ekinci, H. Bora, D. Kilic and M. Akmansu, 2014. Prognostic significance of expression of CD133 and Ki-67 in gastric cancer. Asian Pac. J. Cancer Prev., 15: 8215-8219.
68. Silva, I.A., S. Bai, K. McLean, K. Yang and K. Griffith *et al*., 2011. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res., 71: 3991-4001.
69. Collins, A.T. and N.J. Maitland, 2009. Prostate cancer: Regeneration of interest in the prostate. Nat. Rev. Urol., 6: 184-186.