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ORIGINAL ARTICLE

Co-Expression of Putative Cancer Stem Cell Markers CD44 and CD133 in Prostate Carcinomas

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Abstract Cancer stem cells (CSCs) are the main players of prostate tumorigenesis thus; characterization of CSCs can pave the way for understanding the early detection, drug resistance, metastasis and relapse. The current study was conducted to evaluate the expression level and clinical significance of the potential CSC markers CD44 and CD133 in a series of prostate tissues. One hundred and forty eight prostate tissues composed of prostate cancer (PCa), high-grade prostatic intraepithelial neoplasia (HGPIN), and benign prostate hyperplasia (BPH) were immunostained for the putative CSC markers CD44 and CD133. Subsequently, the correlation between the expression of these markers and the clinicopathological variables was examined. A higher level of CD44 expression was observed in 42% of PCa, 57% of HGPIN, and 42% BPH tissues. In the case of CD133 expression PCa, HGPIN, and BPH samples demonstrated high immunoreactivity in 46%, 43%, and 42% of cells, respectively. Statistical analysis showed an inverse significant correlation between CD44 expression with Gleason score of PCa ($P = 0.02$), while no significant correlation was observed between CD133 expression and clinicopathological parameters. A significant reciprocal correlation was observed between the expression of two putative CSC markers CD44 and CD133 in PCa specimens while not indicating clinical significance. Further clinical investigation is required to consider these markers as targets of new therapeutic strategies for PCa

Keywords Prostate cancer · Tissue microarray · Immunohistochemistry · CD44 · CD133

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed male malignancy in the world [1], however, despite early detection and treatment, PCa is the sixth cause of cancer mortality among males worldwide [2]. Although radiotherapy, surgery, hormone therapy, and chemotherapy are effective in the earlier phases of treatment, many prostate cancers ultimately develop into invasive and drug-resistant metastatic cancers [3–5].

For many years, studies have shown that the tumor initiation, cancer progression, relapse and resistance to therapy in many cancers are due to a population of rare mutated stem cells, known as cancer stem cells (CSC) [5]. These cells display various features including self renewal, multipotency and differentiation into a spectrum of specialized cell types [6]. Several studies suggested that both the mortality and heterogeneity of prostate cancer are related to the expansion of cancer stem cells [7]. It is widely accepted that the alignment of basic science with clinical science findings is an essential

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parameter in designing an effective and holistic strategy for targeted therapy of PCa. For the last decade, the identification and isolation of CSCs by specific markers in different tumors has been considered in several studies. CD44 and CD133 have been introduced as putative cancer stem cell markers in variety of solid tumors including prostate cancer [8–10]. CD44, known as an adhesion molecule with multiple signaling functions, has been suggested to play a role in tumor migration, progression, and metastasis [11]. Although CD44 expression has been detected in most basal cells of normal prostate tissues [12], the expression pattern of this CSC marker has been remained controversial in prostate carcinomas. A group of studies have suggested CD44 as a promising cancer stem cell marker in PCa [12–14], reporting that around 60% of primary PCa cells demonstrated a moderate to high expression level of CD44 [8, 15], however, another group of studies showed a decreased expression level of CD44 in PCa specimens [16, 17]. Another cancer stem cell marker, CD133, a cell surface glycoprotein with unknown function, has been identified in human solid tumors which has been correlated with highly aggressive behavior, metastasis, and shorter survival in PCa [18–22]. Previous studies showed e weak to moderate immunoreactivity of CD133 in only 1% of normal prostate basal cells and almost 6% of PCa tissues [8, 13, 23, 24].

Considering the limited human clinical studies and contradictory immuhistochemical analysis regarding expression of CD44 and CD133 as putative CSC markers in prostate cancer, this study was designed to investigate the expression patterns of these CSC markers in a set of prostate samples using the tissue microarray technique. In order to determine the clinical significance of CD44 and CD133, we also assessed the possible correlations of their expression with established clinicopathological parameters in prostate carcinomas.

Materials and Methods

Tissue Collection

This retrospective study consisted of 148 consecutive series of PCa ($n = 101$), HGPIN ($n = 21$), and BPH ($n = 26$) tissues with completely available histopathological data. Archived, formalin-fixed, paraffin-embedded tissues were collected from Hasheminejad hospital, a major university-based referral Urology–Nephrology hospital in Tehran, Iran, between 2006 and 2011. Hematoxylin and Eosin (H&E)-stained slides, pathological reports, and other medical records were reviewed to confirm the diagnosis while clinicopathologic parameters, including age, tumor type, tumor size, pTNM staging, Gleason score, serum PSA level, vascular invasion, perineural invasion, surgical margins, bladder neck involvement, seminal vesicles deferentia involvement, and regional lymph node involvement were all recorded. The consensus guidelines of International Society of Urological Pathology in 2005 were used to determine tumor grade with the Gleason score system [25]. The last version of the AJCC/UICC TNM staging system was used for the definition of tumor stage [26]. Radical prostatectomy specimens were obtained before systemic treatment including hormone or radiation therapy. Paraffin-embedding was completed within the frame-work of routine diagnostic procedures. Patient information was kept fully anonymous at all steps to maintain the highest level of patient confidentiality. This research study was approved by Iran University of Medical Sciences Research Ethics Committee.

TMA Construction

The tissue microarray blocks were constructed using a TMA instrument (Minicore; ALPHELYS, Plaisir, France) as described in our previous studies [21, 27–29]. Three representative tumor regions were selected and marked out from the H and E slides of each case. The recipient TMA blocks each contains 60 tissue cores with a 0.6 mm diameter from radical prostatectomy and normal prostate samples (adjacent to tumors), were punched out carefully from selected regions of each "donor" block and precisely arrayed into a new recipient paraffin block in three copies. The mean scoring of three cores was then calculated as the final score by an expert genitourinary pathologist who supervised all TMA construction steps. TMA sections were cut with a Histoline microtom (4 μm thick), and mounted on adhesive-coated slides (Superfrost plus, Thermo Scientific, Germany).

In the present study, three cores were evaluated from each tumor to increase the accuracy and validity of analysis and to overcome heterogeneity of antigen expression. Previous studies validated that despite the variability of antigen expression between cores as each core can represent more than 90% of the staining pattern of a whole tissue section, whereas analysis of the two readable cores accomplished greater than 95% accuracy [30].

Imunohistochemical Staining

Immunohistochemical staining was performed based on the standard chain polymer-conjugated (Envision) technique as described previously [27, 31, 32]. The TMA slides were deparaffinized by heating at 60 °C for 25 min and rehydrated by a series of washes with xylene and graded ethanol treatment. Antigen retrieval was performed by autoclaving samples for 10 min in sodium citrate buffer (pH: 6.0). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxidase for 20 min. The primary antibodies; mouse monoclonal anti-CD44 (Novocastra-UK) was diluted by 1:40, at 4° C overnight and anti-CD133 antibody (Gifted by Avicenna Research Institute, Monoclonal Antibody Research Center (MARC)) was diluted by 1:150 and incubated for 3 hours at

Table 1 Association of CD44 and CD133 expression (intensity of staining, percentage of positive cells and *H*-score) with clinicopathological parameters in prostate cancer cases (*P*-value; Pearson $\chi 2$)

Patients and tumors characteristics	N (%)	Expression of CD44			Expression of CD133		
	Total no of cases:101	Intensity	Percentage of positive cells	*H*-score (cut-off = 68)	Intensity	Percentage of positive cells	*H*-score (cut-off = 201)
Age (years)		0.97	0.96	0.51	0.04	0.14	0.47
<66	59(58)						
>66	42(42)						
Gleason score		0.19	0.26	0.02	0.83	0.66	0.91
6	26(26)						
7	68(67)						
8 to 9	7(7)						
Tumor volume (%)		0.6	0.94	0.32	0.85	0.32	0.12
<34	61(60)						
>34	40(40)						
Serum PSA level (ng/ml)		0.48	0.9	0.81	0.42	0.57	0.12
<4	4(5)						
4–10	40(40)						
>10	23(23)						
unknown	34(32)						
TNM staging system		0.83	0.49	0.33	0.83	0.19	0.14
pT2	57(56)						
pT3	36(36)						
Unknown	8(8)						

room temperature. After washing with Tris-buffered saline (TBS), the sections were incubated with anti-rabbit/anti-mouse Envision (Dako, Denmark) as secondary antibody for one hour. The samples were developed with 3; 3′-diaminobenzidine (DAB, Dako) substrate as a chromogen for 15 min at room temperature and counterstained with hematoxylin. The slides were dehydrated following a standard procedure and mounted. The human tonsil tissue was used as a positive control for CD44 antibody, while the human normal kidney tissue was applied as a positive control for CD133. The omission of primary antibodies and their replacement with TBS (Tris Buffer Saline) were applied as the negative control.

Scoring System of TMA Slides

Initially, CD44 and CD133 expressions were evaluated semi-quantitatively by two observers after a series were examined on a double-headed microscope while being blinded to patients' outcome and other clinical finding as described previously [33–37]. The obtained results were also confirmed by the expert pathologist (M Asgari) to receive a comprehensive concept of staining in tumor cells. Final scoring assessment was done with reinvestigation of the overall distribution of the tumor cells at 10× magnification and positive cells were then assessed semi-quantitatively at higher magnifications and final scores were given an agreement.

CD44 and CD133 expression was defined with three scoring systems that the degree of staining was categorized based on the severity of staining with a comparative scale: intensity of the staining, percentage of positive cells, and Histochemical Score (*H*-score) or overall scoring which was assigned to each case by multiplying the intensity by the percentage of stained cells [38]. To compare all of the available data, the overall score was obtained by *H*-score and a final score of 0 to 300 was given [39]. The following scoring criteria were used for the assessment of immunohisochemistry results: in terms of intensity; score of 0 = no visible staining, +1 = faint staining, +2 = moderate staining and +3 = strong staining. The percentage of positive cells staining was graded as <25%, 25–50%, 50–75% and >75% of tumor cells. The median of *H*-score was selected as cut-off point (for CD44 *H*-score = 60 and CD133 *H*-score = 200) and specimens categorized in two groups, higher and lower expression in comparison to the cut-off point, as used in previous studies [20, 40, 41].

Statistical Analysis

Statistical analysis of data was performed using SPSS software version 16 (SPSS, Chicago, IL, USA). The Pearson's

Table 2 Association of CD44 and CD133 expression (intensity of staining, percentage of positive cells and *H*-score) with vascular invasion, perineural invasion, surgical margin, and involvement of adjacent tissues in prostate cancer cases (*P*-value; Pearson $\chi 2$)

Patients and tumors characteristics	N (%)	Expression of CD44			Expression of CD133		
	Total no of cases:101	Intensity	Percentage of positive cells	*H*-score (cut-off = 60)	Intensity	Percentage of positive cells	*H*-score (cut-off = 200)
Vascular invasion		0.2	0.8	0.74	0.96	0.6	0.95
Present	4(4)						
Absent	77(76)						
Unknown	20(20)						
Perineural invasion		0.11	0.26	0.34	0.93	0.62	0.47
Present	89(88)						
Absent	4(4)						
Unknown	8(8)						
Surgical margins		0.14	0.33	0.61	0.58	0.36	0.38
Present	32(32)						
Absent	60(59)						
Unknown	9(9)						
Regional lymph node involvement		0.12	0.72	0.31	0.45	0.43	0.57
Present	5(5)						
Absent	90(89)						
Unknown	6(6)						
Bladder neck involvement		0.11	0.66	0.81	0.08	0.34	0.92
Present	6(6)						
Absent	49(48)						
Unknown	46(46)						
Seminal vesicles deferentia involvement		0.14	0.13	0.31	0.89	0.65	0.38
Present	19(19)						
Absent	76(75)						
Unknown	6(6)						

$\chi 2$ and Pearson's R tests were used to analyze the association between CD44 and CD133 expression and clinicopathological parameters including age, tumor type, tumor size, pTNM staging, Gleason score, serum PSA level, vascular invasion, perineural invasion, surgical margins, bladder neck involvement, seminal vesicles deferentia involvement, and regional lymph node involvement. Also, the comparisons of CD44 and CD133 expression in PCa, HGPIN, and BPH samples were performed using Mann-Whitney U test. A difference of $P < 0.05$ between groups was considered significant.

Results

To gain insights into CD44 and CD133 expressions and their clinical relevance, the expression of these CSC markers in a series of prostate tissues, including 101(68%) PCa, 21(14%) HGPIN, and 26 (18%) BPH were investigated. The mean age of patients was 66 ± 7 years (range: 39–90), whereas 58% of patients were <66 years old. The Gleason scores for the 101 PCa specimens were classified into three subgroups, 26 (26%) samples showed a Gleason score of 6, 68 (67%) had a Gleason score of 7, whereas only 7 (7%) showed Gleason scores between: 8–9. In terms of pathologic tumor stage (pTNM staging) data out of 101 specimens, 57 PCa specimens (56%) were in stage pT2 and 36 PCa specimens (36%) were classified as stage pT3. Serum Prostate-specific antigen (PSA) levels were categorized as <4, 4–10 and >10 ng/ml: among PCa cases, 4 (5%) had serum PSA level < 4 ng/ml, 40 (40%) had serum PSA level 4–10 ng/ml, and 23(23%) had serum PSA level > 10 ng/ml. Tables 1 and 2 represents all clinical characteristics of the patients.

CD44 Expression in PCa, HGPIN, and BPH Specimens

Following IHC staining, CD44 expression was mainly localized in the cell membrane area of tumor cells. Forty

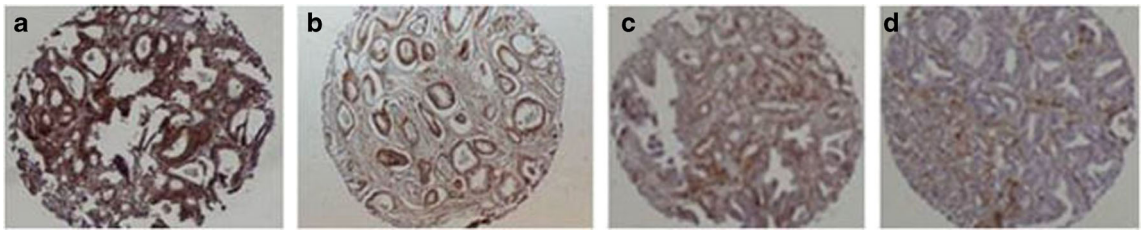

Fig. 1 Immunohistochemical analysis of CD44 expression in adenocarcinoma prostate tissues. From left to right; **a** strong negative, **b** moderate, **c** weak, and **d** negative (All figures are shown with a magnification of × 200)

two out of 101 PCa cases (42%), 12 out of 21 HGPIN samples (57%), and 11 out of 26 BPH samples (42%) showed high CD44 expression level and 58% of PCa, 43% of HGPIN, and 58% of BPH cases showed lower immunoreactivity of CD44 (Fig. 1). Based on the Mann–Whitney U test, no significant difference was detected between the expression of CD44 in three patients 'groups; PCa, HPIN, and BPH patients (Fig. 2a).

Association of CD44 Expression and Clinicopathologic Parameters

Univariate analysis showed an inverse significant correlation between CD44 expression and the Gleason score in PCa cases (P = 0.02). No significant correlation was found between CD44 expression and tumor type, pTNM staging, serum PSA level, and regional lymph node involvement. These findings are summarized in Tables 1 and 2.

The Mann-Whitney U Test was used to compare differences between the expression of CD44 within three the Gleason score subgroups (Gleason scores of 6, 7, and 8–9), indicating a significant difference between Gleason score 6, a Gleason score 7 (P = 0.02) and a Gleason score of 8 to 9 (P = 0.02) (Fig. 2b).

CD133 Expression in PCa, HGPIN, and BPH Specimens

Upon immunohistochemical analysis, CD133 expression was detected mainly in the cytoplasm and partially in the cell membrane of tumor cells. Of 101 PCa cases 47 (46%) showed higher expression of CD133 and 54(54%) showed a lower CD133 expression level (Fig. 3). Among 21 HGPIN samples, 9(43%) displayed higher expression and 12 (57%) showed lower expression levels. A higher immunireactivity of CD133 was also observed in 11 (42%) BPH cases versus the 15 (58%) cases with lower immunoreactivity. No significant correlation was found between the CD133 expression and clinicopathological features of the specimens (Tables 1 and 2). Moreover, the Mann–Whitney U test did not show any significant difference between expression levels of CD133 among PCa, HGPIN, and BPH patients (Fig. 4).

Combined Analysis of CD44/CD133 Expression

Comparing the expression patterns of both CD44 and CD133 markers, a reciprocal significant correlation was found

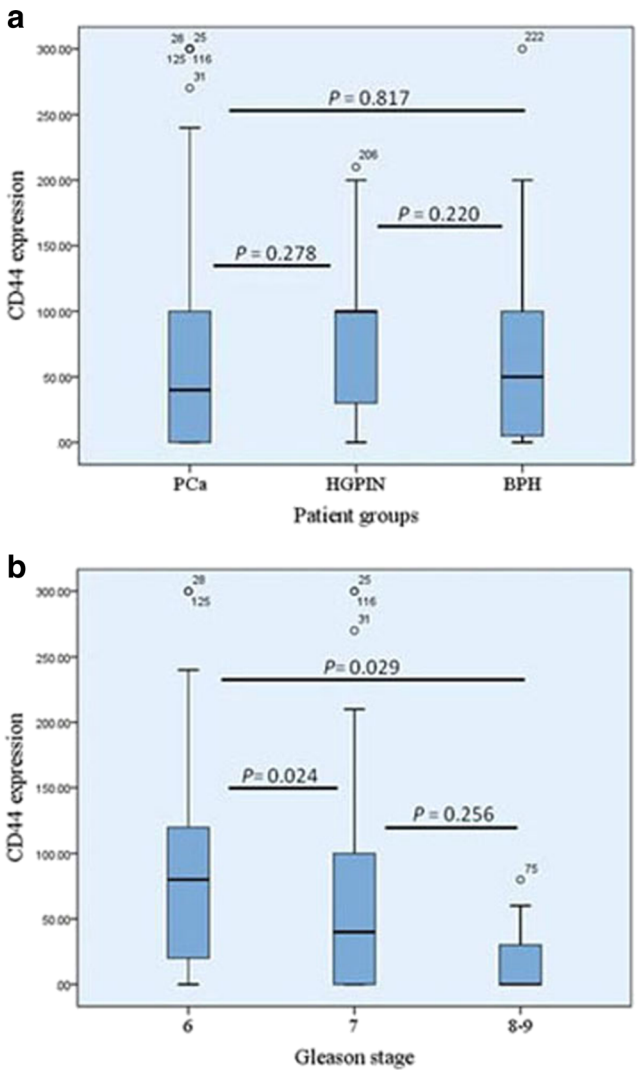

Fig. 2 Box-Plot analysis of the CD44 expression with clinicopathological parameters. In each panel, the vertical axis gives the CD44 immunostaining score, obtained as described in the Methods section. The correlations between CD44 expression with (**a**) Patient groups and (**b**) Gleason score using Mann-Whitney U Test. Based on the standard definitions, Box plot is showing median (*bold line*), interquartile line (*box*), outliers (*circle*), and extreme observations (*star*)

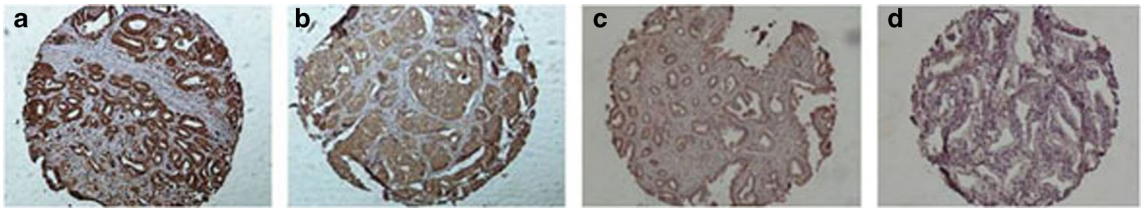

Fig. 3 Immunohistochemical analysis of CD133 expression in adenocarcinoma prostate tissues. From left to right; **a** strong negative, **b** moderate, **c** weak, and **d** negative (All figures are shown with a magnification of × 200)

between the two markers in the same series of prostate samples ($P < 0.04$). The combined analysis was performed to examine the correlation between the expression of CD44/CD133 with clinicopathological parameters in PCa (Table 3).

Among 101 PCa samples, 23 (23%) cases showed CD44+/CD133+ phenotype, 19 (19%) cases had CD44+/CD133- phenotype, 24(23%) cases had CD44−/CD133+ phenotype, and 35(35%) cases showed CD44−/CD133- phenotype. Of 21 HGPIN and 26 BPH samples, 6 (29%) and 6 (24%) cases showed the double positive phenotype, 6(29%) and 5(19%) cases showed CD44+/CD133- phenotype, 3 (13%) and 5 (19%) cases had CD44−/CD133+ phenotype, and double negative phenotype was showed in 6 (29%) and 10 (38%) cases (Fig. 5).

Discussion

Cancer stem cells (CSCs) are a subpopulation of tumor cells that possess the principal properties of self-renewal, tumor initiation, metastasis, recurrence, and drug resistance potential [42, 43]. CSCs have been identified in a wide variety of the solid tumors including prostate cancer with the utilization of surface markers which makes them potential targets for specific therapeutic applications [44]. Previous experiments have focused on the characterization and analysis of these CSC markers, separately and in combinations; however, there has been no reliable outcome due to the genetic heterogeneity of the tumors [13, 45, 46]. CD44 as a cell adhesion molecule with crucial function in the structure maintenance of basal cells in prostate gland has been introduced as a promising prostate CSC marker [12, 13, 47], although the immunohistochemical expression pattern of CD44 in human prostate carcinoma remains controversial [8, 15–17, 48, 49].

We observed a higher level of CD44 expression in 42% of PCa, 57% of HGPIN, and 42% BPH tissue samples. Our findings showed lower expression of CD44 to be more frequent in higher Gleason score prostate carcinomas. Ugolkov et al. showed higher levels of CD44 expression, in terms of intensity, in almost 60% of PCa cases, 94% of BPH and 88% of HGPIN cases [8]. In a study by Nagabhushan and co-workers, increased

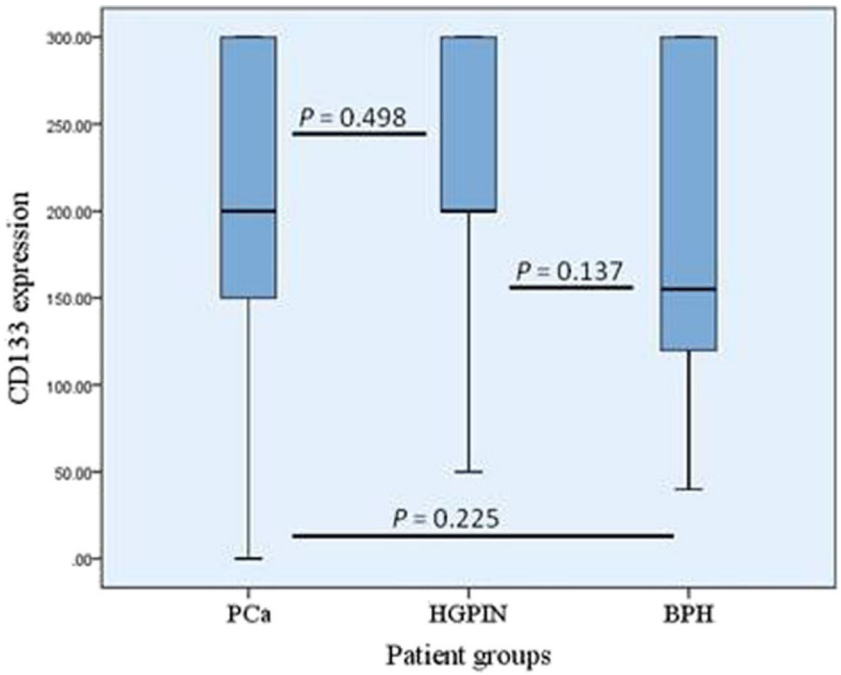

Fig. 4 Analysis of CD133 expressions in different prostate tissues including; prostate cancer (PCa), high-grade prostatic intraepithelial neoplasia (HGPIN), and benign prostate tissues (BPH) using Mann-Whitney U Test. Based on the standard definitions, Box plot is showing median (*bold line*), interquartile line (*box*), outliers (circle), and extreme observations (*star*)

Table 3 Association between CD44/CD133 phenotypes with clinicopathological parameters in prostate cancer tissues (*p*-value; Pearson $\chi 2$)

Tumors characteristics	Phenotypes of CD44/CD133 expression, N (%)				*P*-value
	CD44+/CD133+	CD44+/CD133-	CD44−/CD133+	CD44−/CD133-	
Age (years)					0.89
<66	13(22)	12(20.3)	15(25.4)	19(32.2)	
>66	10(23.8)	7(16.7)	9(21.4)	16(38.1)	
Gleason score					0.11
6	7(26.9)	9(34.6)	6(23.1)	4(15.4)	
7	15(22.1)	10(14.7)	16(23.5)	27(39.7)	
8 to 9	1(14.3)	0(0)	2(28.6)	4(57.1)	
Tumor size (%)					0.43
<34	13(21.3)	14(23)	12(19.7)	22(36.1)	
>34	10(25)	5(12.5)	12(30)	13(32.5)	
Serum PSA level (ng/ml)					0.34
<4	1(25)	0(0)	3(75)	0(0)	
4–10	12(30)	7(17.5)	9(22.5)	12(30)	
>10	6(26.1)	3(13)	5(21.7)	9(39.1)	
pTNM staging system					0.4
pT2	12(21.1)	14(24.6)	12(21.1)	19(33.3)	
pT3	10(27.8)	41(11.1)	10(27.8)	12(33.3)	
Vascular invasion					0.83
Present	1(25)	0(0)	1(25)	2(50)	
Absent	17(22.1)	17(22.1)	17(22.1)	26(33.8)	
Unknown	5(25)	2(10)	6(30)	7(35)	
Perineural invasion					0.1
Present	19(21.3)	17(19.1)	20(22.5)	33(37.1)	
Absent	0(0)	1(25)	3(75)	0(0)	
Unknown	4(50)	1(12.5)	1(12.5)	2(25)	
Surgical margins					0.9
Present	6(18.8)	6(18.8)	6(18.8)	14(43.8)	
Absent	15(25)	11(18.3)	16(26.7)	18(30)	
Unknown	2(22.2)	2(22.2)	2(22.2)	3(33.3)	
Regional lymph node involvement					0.59
Present	0(0)	1(20)	2(40)	2(40)	
Absent	22(24.4)	16(17.8)	20(22.2)	32(35.6)	
Bladder neck involvement					0.7
Present	2(33.3)	1(16.7)	1(16.7)	2(33.3)	
Absent	14(28.6)	9(18.4)	9(18.4)	17(34.7)	
Unknown	7(15.2)	9(16.9)	14(30.4)	16(36.8)	
Seminal vesicles deferentia involvement					0.24
Present	5(26.3)	1(5.3)	6(31.6)	7(36.8)	
Absent	15(19.7)	17(22.4)	16(21.1)	28(36.8)	
Unknown	3(50)	1(16.7)	2(33.3)	0(0)	

intensity of CD44 expression, was detected in 38% of PCa specimens [15]. They also reported an inverse significant correlation between CD44 expression and Gleason score [15]. In line with our study, De Marzo et al. demonstrated higher level of CD44 expression, applying *H*-score scoring, in 40% PCa samples as well as low expression of CD44 in advanced Gleason score cases [49]. In contrast, other group of studies reported higher

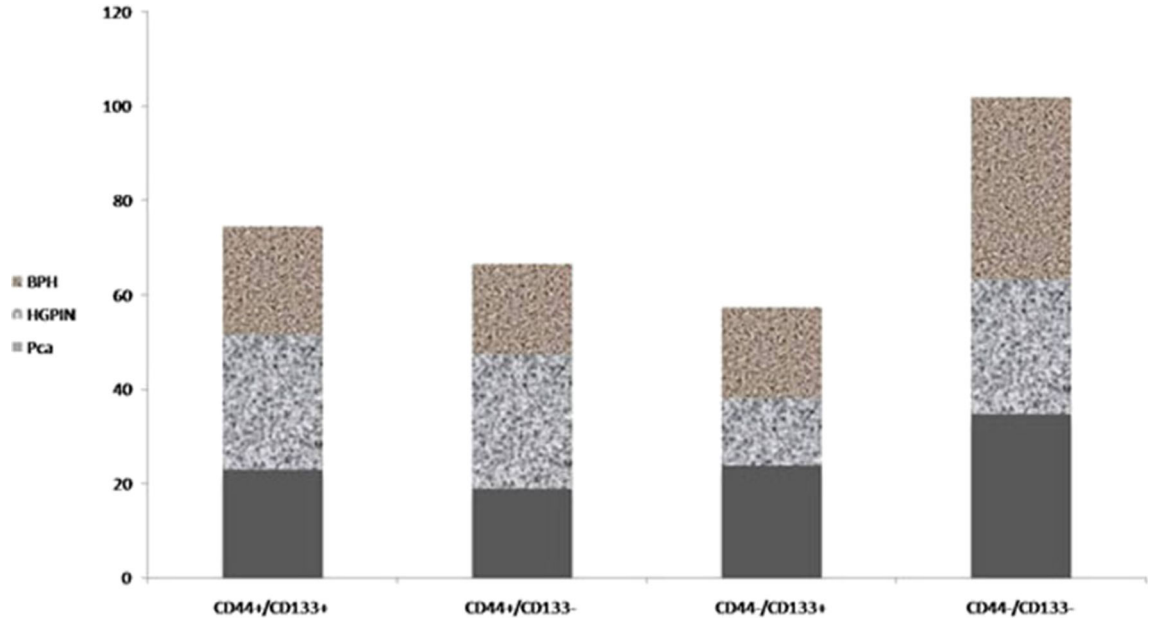

Fig. 5 Distribution of CD44/CD133 phenotypes in different patient groups including PCa, HGPIN, and BPH tissues

immunoraectivity of CD44 only in a small fraction (2–5%) of PCa cases [16, 17]. The discrepancy found in these findings could stem from the diversity of tumor histotypes and their heterogeneity [13, 50], although applying various isoforms of CD44 and their different pattern of expression in prostate tumor cells could be counted as another potential factor [51]. While earlier studies primarily use intensity in their analysis of immunohistochemical results, newer studies tend to use the more comprehensive score (*H*-score), however they primarily describe similar characteristics. Another known putative CSC marker, CD133, has been detected in a number of tumors including prostate cancer [23, 28, 52–54]. Previous in vitro studies have pointed that CD133 positive cells possess more tumorigenic potential, proliferation, metastasis, and chemotherapy resistance than CD133 negative cells [55–57], however a small portion of CD133 positive cells could be identified from PCa sample [13, 23]. Richardson et al., for the first time, showed that CD133 is expressed in approximately 1% of normal prostate basal cells [23]. In the current study using overall scoring we found higher expression of CD133 in approximately 45% of all PCa, HGPIN, and BPH specimens. Our results did not show any significant correlation between CD133 expression and clinicopathological variables. In a TMA-based immunohistochemical study, Ugolkov et al. showed higher intensity of CD133 expression in a small fraction (6%) of PCa cases [8]. Moreover, increased immunoreactivity of CD133 expression was found only a subset of PCa specimens [24]. Vatansever group reported a positive significant correlation between CD133 expression and Gleason score in PCa tissues [58].

Different expression patterns of CD133 in clinical tissues could be caused by promoter activity mutation, alternative splicing, and transcriptional regulation of the gene [59, 60]. It is believed that the current difficulty in detecting CSCs in clinical experiments using immunohistochemistry analysis could be addressed by incorporating sufficiently larger study populations. The present controversy in the clinical findings could stem from the increased sample size in our study compared to similar previous studies, which on one hand should reduce the chance of random errors and strengthen our study while on the other hand it has opened up a wide array of possible outcomes that may have been overlooked in smaller study samples.

Conclusion

To summarize, our results showed higher expression of both putative cancer stem cell markers, CD44 and CD133, in almost 50% of the PCa cases, while CD44 expression was observed to be significantly higher in PCa cases with a lower Gleason score. However, there is still some controversy in the clinical significance of observed CD44 and CD133 expression levels. Thus utilization of these markers as potential avenues of progression in new therapeutic strategies for human prostate cancer may only be achieved by further investigation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent "Informed consent was obtained from all individual participants included in the study."

Ethical Approval "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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